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CORRECTIONS

Page 19, line 6; for *"Amphioplus abditus"* read *"Amphiodea occidentalis."* Lines 10-11; for *"brevispina"* read *"lutkenii."* Lines 19-20; for *"japonica"* read *"miniata."* Line 22; for *"C. lubrica* and *C. pulcherrima"* read *"C. piperata* and *C. populifera."*

Page 21, Fig. 1; for "Orthasterias forreri forcipulata" read "Pisaster papulosus." Fig 11; for "brevispina" read "lutkenii."

Page 22, plate 8; in the description of the figures, for "Orthasterias forreri forcipulata" read "Pisaster papulosus."

Page 25, line 9 from bottom; for "very scarce" read "rather numerous."

Page 68; the last two lines on the page should be omitted.

Page 123, line 14; for "distinegration" read "disintegration."

Page 145, in table in column headed "4th week"; for "holdfast" read "sporeling."

Page 154, 12th line from bottom; for "manillae" read "maxillae."

Page 158, 4th line from bottom; for "nusclei" read "nuclei."

Page 196, line 2; for "trial" read "trail." Line 9; *"or sundew"* should be in Roman type.

Page 211, last line on page; for "pressure" read "presence."

Page 274, line 12; before "della" insert "della reg. freg. Magenta durante gli anni 1865-1867. Mem." Omit line 13.

Page 302; third line from bottom of page should be moved to the bottom.

Page 307, 3rd line from the bottom; for "or" read "of."

Page 367; the middle letter on the right side of the page is "g."

Preliminary Observations on the Development of Leptasterias hexactis

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Leptasterias is a genus of small starfishes common on the Pacific Coast from northern Alaska to California. Some species extend in their distribution into Puget Sound and abound on the shores of the San Juan Islands and adjacent territory; but to the best of the writer's knowledge, they are confined to this northern region of the Sound and are not recorded from regions south of Port Townsend. Of the species given by Verrill (1914), 3 appear to be found in the San Juan Island region, *Lepasterias hexactis, L. aequalis,* and *L. epichlora.* It is indeed difficult in the region of Friday Harbor to find specimens that answer well to the specific descriptions of these starfishes. There are apparently so many local varieties, and intergradation is so close, possibly through extensive hybridization, that the distinction of species with certainty, at least by the non-specialist, becomes very difficult or quite impossible. The adult Leptasterias may be readily found under rocks at low tide. These animals are grayish to brownish-green in color, closely similating the color of the rocks to which they cling; occasionally they may be reddish-brown. Some reach the maximum size of 120 mm. in diameter, while the average of those collected for this study was about 54 mm. Specimens measuring 24 mm. have been found sexually mature and reproducing. The Leptasterias of the San Juan Island region is normally 6-rayed (Fig. 1) tho rarely a 5- or a 7-rayed specimen is collected. The females of the genus brood their young, which never assume a free swimming stage, but pass through their entire metamorphosis protected by the mother. The young are therefore very easily obtained. All the young of a given female are, as would be expected, approximately in the same stage of development.

Material for the study of the development of Leptasterias was collected in 3 visits to the Puget Sound Biological Station, in April, 1912, March, 1913, and February, 1914. On account of other duties it has not been possible at these times of the year to remain at the station for a period sufficiently extended to follow the development in the living condition; and on account of the brevity of the visits the series of stages secured is still incomplete in the early development. The attempt was made to transfer the animals to Seattle and there rear the young, but with disastrous results, the young dying very quickly. It seems advisable, however, to make known the general character of the development and the

availability of the animals, which should furnish an abundance of easily accessible material, particularly favorable for some lines of experimental research.

It has been the past experience that Leptasterias enters its reproductive season in the latter part of February or first of March. At the end of the first week of April, 1912, many of the young had completed their metamorphosis and were escaping from their mothers, while the youngest stages then available had approximately half finished their metamorphosis. It seems likely that the entire season extends over a period of 6 to 8 weeks, but the exact period of development of the individual cannot yet be given.

As is to be expected in an animal possessing the brooding habit, the number of young produced by a single female is relatively small, while the eggs are very large and heavily laden with yolk, which suffices to carry the development through the metamorphosis. The largest number of young taken from a single female was 1160, but young females in their first season have only a few dozen. The eggs are large, from 0.6 to 1.1 mm., varying with the size of the female producing them. They are brightly orange-colored. The eggs are extruded from ventrally located gonopores. On account of their gelatinous envelope, they adhere in a mass which the female gathers into the oral region of her pursed-up body and holds securely with her tube-feet (Fig. 2). The eggs adhere so strongly to each other in the early stages that it is nearly impossible to isolate them in the living condition without injury. The fertilized egg has a distinct membrane surrounding it within which the egg floats freely (Fig. 3). In the early stages of brooding the female doubtlessly secures no food, but in later stages small gastropods are to be found in her mouth. These are sometimes entirely hidden by young starfish. Under these conditions the young starfish are usually in their last stages of metamorphosis or even fully developed, their distinctly white color betraying the exhaustion of their yolk supply. At this stage the young have protrusible stomachs and it does not seem impossible that they may thus share in the mother's food.

The cleavage stages were not obtained, but there is no reason for believing the segmentation in Leptasterias essentially different from that described for Asterina, Cribrella or Solaster. Neither is it possible to describe the formation of the blastula or gastrula. The form of the larva is first betrayed in a spherical embryo in which there is no superficially visible sign of blastopore. This embryo cannot be superficially distinguished from an egg, since it is still enclosed by the membrane. At one pole of this spherical embryo the 3 "arms" of the preoral lobe appear. They are at first very much depressed and only faintly visible (Fig. 4).

Gradually these arms lift from the surface, grow rapidly, and markedly increase the size of the embryo, bursting the enclosing membrane (Figs. 4, 5, 6). The larval arms are at first immotile and nicely symmetrical; 1 median, ventral, and unpaired; the other 2 forming a dorsal pair. Very early in their development the tips of the larval arms become adhesive knobs, enabling the larvae to attach themselves to each other and to the mother. At the upper pole of the preoral lobe between the larval arms an apical elevation is produced, homologous with the fixing disk of Asterina. This likewise forms adhesive cells, develops slowly into a terminal knob, and finally becomes the sole larval organ of attachment. The perfect bilateral symmetry which at first obtains is soon disturbed when the muscular development of the larval arms permits their movement. The larvae then become very active and the distortion of the preoral lobe may become very pronounced; so much so that it becomes difficult to recognize which of the arms is which and consequently which is right or left side. No larval mouth is ever developed. This feature as well as the general form remind one of the larvae of Cribrella and Solaster.

The first indication of the approaching metamorphosis is given by the appearance of a flattened disk. This arises on the left side of the larva. It is not situated exactly laterally but somewhat asymmetrically, occupying an obliquely latero-ventral position (Fig. 7). This disk is at first small, but as it grows it spreads out over the body of the larva. At its margin 5 lobes of the hydrocoele become superficially evident (Fig. 8). In a sinistral view these 5 lobes or pouches may be numbered consecutively in a clockwise direction as indicated in Figure 8. It is then seen that pouches 1 and 5 are separated by a wide interval occupied by the broadly attached preoral lobe. The body of the larva soon begins to show positive signs of transformation into the body of the future starfish. It becomes compressed from left to right side and expanded correspondingly in other directions, revealing what are to become the oral and aboral sides of the starfish. At the margins of this compressed body outgrowths appear situated aboral to the hydrocoele pouches. These outgrowths are the apices of the starfish rays of which only 5 are at first distinctly visible. From figures 10 and 11 it will be seen that a plane passed horizontally through the body fundament of the starfish does not correspond to a sagittal plane of the larva; but looking at the larvae in the direction of its postero-anterior axis, there is an anti-clockwise torsion of the body of the larva on the preoral lobe.

Before any indication of a 6th hydrocoele pouch appears, the first 5 pouches develop fundaments of the tube-feet (Fig. 12). By the time 2 pairs of tube-feet are distinctly developed in each of the 5 rays, the first superficially visible rudiment of the 6th hydrocoele pouch comes

partially into view, its development evidently interfered with by the preoral lobe. The oral aspect of the starfish therefore shows a definitely developed oral disk and a circumoral ring, but no mouth as yet. It also shows external evidence of the ring canal, from which the radial canals extend outward in each ray between the 2 pairs of tube-feet fundaments. Five apices of the hexagonal starfish are now distinctly visible. The further development of the 6th ray is dependent upon the retrogression of the preoral lobe (Fig. 13). This organ has by this time assumed a very variable shape, the larval arms being in various stages of resorption into the main body of the preoral lobe. New tube-feet are added so that generally when the 4th pair of tube-feet fundaments appear in the first 5 rays the second pair may have appeared in the 6th ray. There is, of course, considerable variation in different individuals of the same brood and still greater variation in different broods. The ring and radial canals become more prominent, and indications of a mouth can be made out.

The 3 arms of the preoral lobe gradually shrink and completely merge into the main portion of this organ, which assumes a form as shown in figures 14 and 15. The fixing disk becomes constricted into a terminal knob, which for a time still serves as an organ for attachment. It finally becomes so nearly separated from the rest of the preoral lobe as to suggest its final discard. It is possible that it may persist attached until the preoral lobe is all gone, but the writer has looked in vain for positive evidence of its final resorption. As the preoral lobe shrinks the interval between the first and 6th rays more and more approximates that between any of the other rays, giving more and more freedom for the development of the 6th ray, which appears to be making up for lost time and to be gaining perceptibly on the other rays. However, even after the preoral lobe has entirely disappeared there may be detected for a short time a slight but rapidly waning inferiority of the 6th ray. The position of the madrepore and the anus cannot be made out by superficial scrutiny alone, even after the metamorphosis is complete; but in stained specimens these organs can be located as shown in figure 14. The Madrepore therefore lies in the interray between rays 1 and 2, which agrees exactly with Gemmill's (1912) observations on Solaster. Before the larval organs of attachment entirely disappear the tube-feet become functional. Their capacities for extension and retraction and for coordinate movement are very striking. The mouth is also open at this stage as proved by the protrusibility of the stomach. Terminal and aboral spines develop in regular fashion, later to be followed by ambulacral spines also. All these arise from previously formed skeletal plates that may be readily demonstrated in cleared specimens. They may even be seen in the latest stages of metamorphosis in the living animal when the disappearance of the yolk supply renders the individual whiter in color and more translucent.

The appearance of the young starfish when it leaves the mother is shown in figures 16 and 17. The need of self-protection is met by the very rapid growth of numerous and very prominent spines and of the skeleton. After the young escape they disappear very quickly from the habitat of their mothers. In spite of their prolonged protection and advanced development the mortality must be very great. A few specimens can usually be found on the eel grass (Zostera) near by, where, by July first, they have reached a size of 3 to 4 mm.

The interest in this starfish is mainly in connection with the 6th ray, in the development of which, at least as far as superficial results are concerned, it corresponds nicely with what Gemmill (1912) found in *Solaster endeca*. The writer does not believe that the delay in the development of the 6th ray is due entirely to interference by the preoral lobe, altho its development after it does begin is undoubtedly retarded by this organ. But the initial appearance of 5 similarly developed hydrocoele pouches is interpreted as due to the persistence of a fundamental racial pentamerism, upon which the later hexamerism is superimposed. In other words, Leptasterias exhibits the first complete step in the addition of a whole new unit of structure, which Gemmill has so well shown, is carried further in a similar manner in *Solaster endeca*. It represents the individuation of a new part from the embryonic tissues. The fact that a 5-rayed or a 7-rayed form is occasionally found would lead one to surmise that it may be possible to control this individuation experimentally. It is hoped that the opportunity will present itself to some one to make a test of the possibilities along this line; also that the internal details of development may some day be checked with those of other starfishes.

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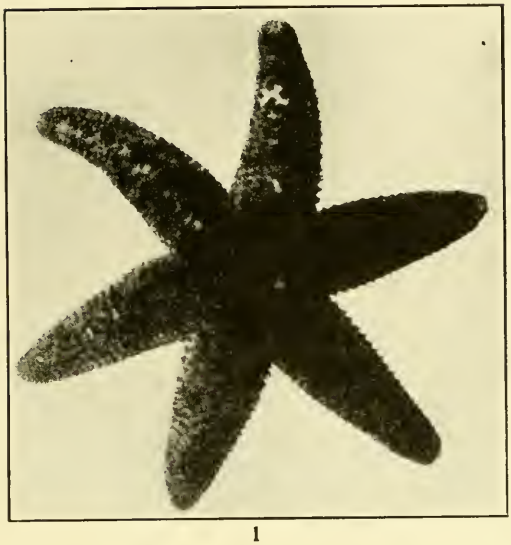
PLATE 1

Fig. 1. Photograph of living specimen of *Leptasterias hexactis*. The specimen is typical of those collected, but agrees with description of the type only in part. × 1.

Fig. 2. Photograph of females showing the manner in which the eggs are carried and the purse-like form which the body assumes during the brooding period. × 1.



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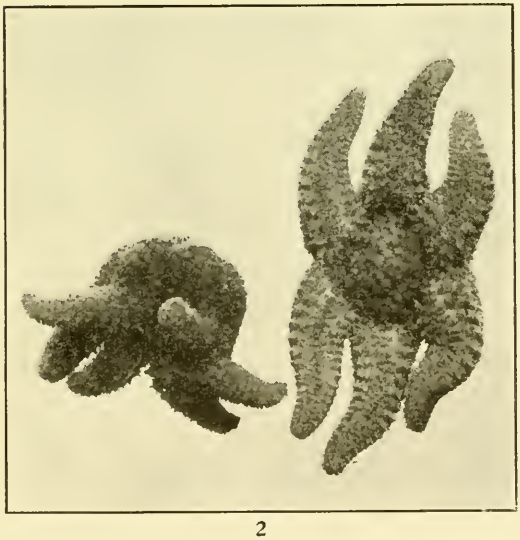


2

H. L. Osterud PLATE 1

PLATE 2

v. l., Ventral unpaired arm of the preoral lobe.

d. l., Dorsal paired arms of the preoral lobe.

f. d., Fixing disk.

s. d., Sinistral disk.

h. p. 1, *h. p.* 2, *h. p.* 3, *h. p.* 4, *h. p.* 5, Hydrocoele pouches Nos. 1 to 5.

Fig. 3. Egg with egg membrane for comparison of size with later stages. × 44.

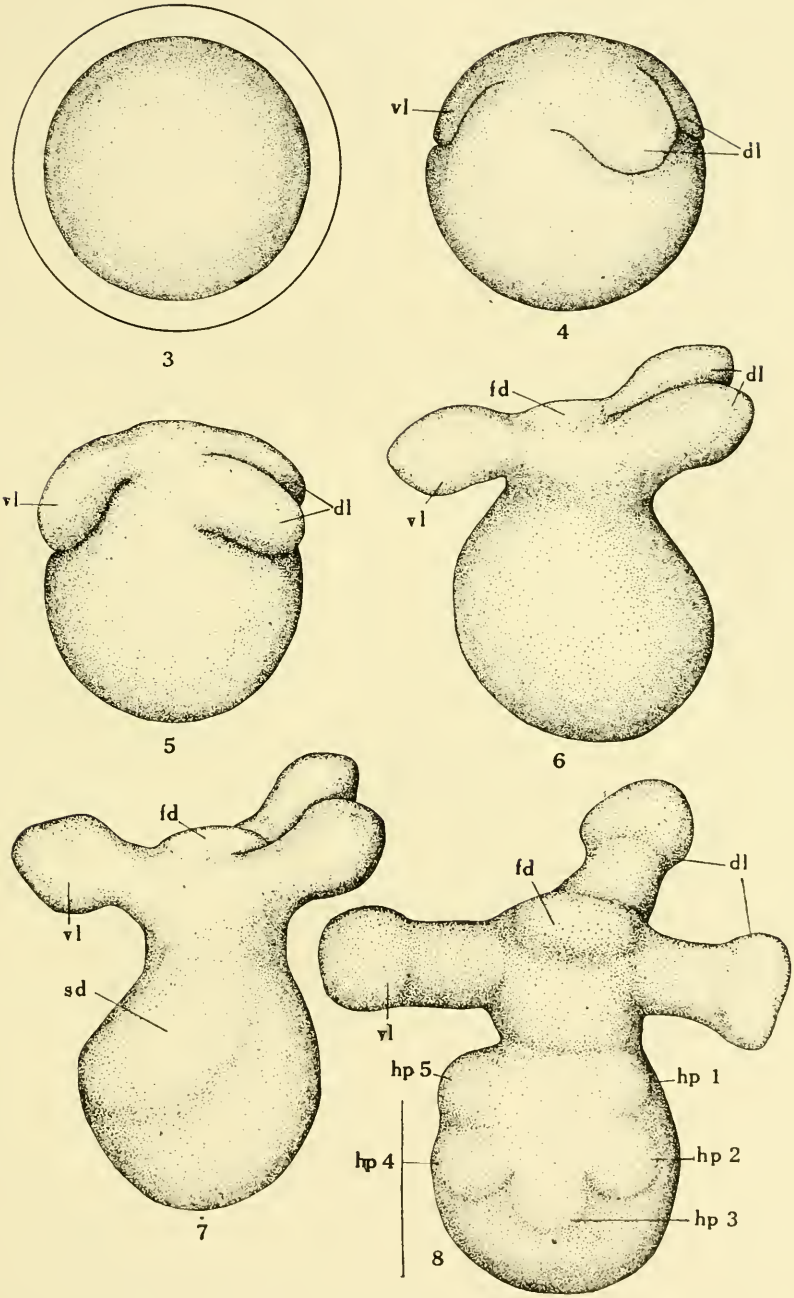
Fig. 4. Young larva with membrane removed to show the arms of the preoral lobe just appearing; sinistral view. × 44.

Fig. 5. Slightly later stage than figure 4, showing growth of the preoral lobe; sinistral view. Membrane removed. × 44.

Fig. 6. Typical symmetrical larva before any external evidence of metamorphosis appears; sinistral view. Membrane ruptured before removal. Preoral lobe distinct from the yolk-filled body of the larva. × 44.

Fig. 7. Larva showing first signs of metamorphosis in the appearance of the sinistral disk in oblique position on the left side of the larva. × 44.

Fig. 8. Larva in which the margins of the sinistral disk betray the appearance of the first 5 hydrocoele pouches. × 44.



H. L. Osterud PLATE 2

PLATE 3

a. 1, *a.* 2, *a.* 3, *a.* 4, *a.* 5, Apices of the early fundaments of the starfish rays, numbered with reference to corresponding hydrocoele pouches.

a. d., Aboral disk.

h. p. 1, *h. p.* 2, *h. p.* 3, *h. p.* 4, *h. p.* 5, *h. p.* 6, Hydrocoele pouches Nos. 1 to 6.

l. d. l., Left dorsal arm of preoral lobe.

o. d., Oral disk.

r. d. l., Right dorsal arm of preoral lobe.

t. f., Fundaments of first and second pairs of tube-feet.

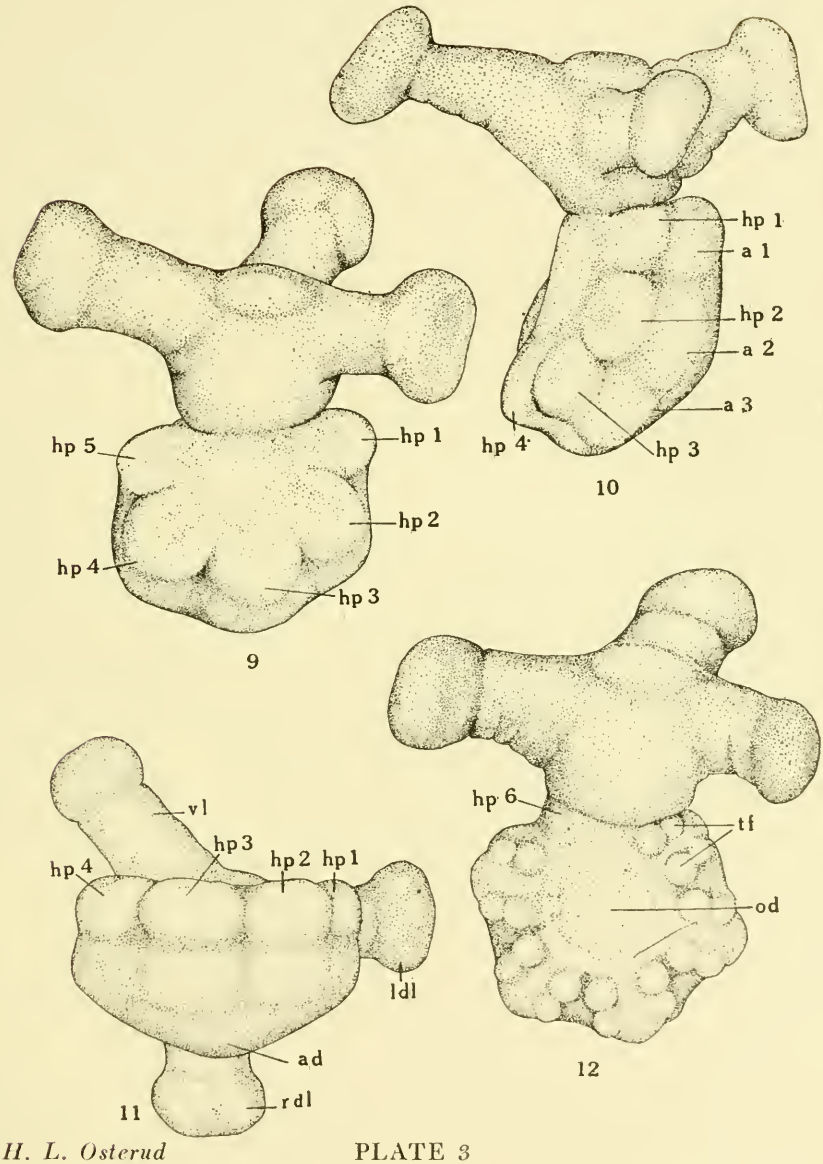
v. l., Ventral arm of preoral lobe.

Fig. 9. Larva with 5 well developed hydrocoele pouches. The body of the larva has sufficiently transformed to reveal the oral and aboral surfaces of the future starfish and the apices of the first 5 arms. × 44.

Fig. 10. Oblique view of a larva of a stage similar to figure 9, to show the transformation of the larval body into that of the starfish. × 44.

Fig. 11. Another larva with 5 prominent hydrocoele pouches; seen from the posterior pole to illustrate the angular relations between the larval and starfish bodies. ×44.

Fig. 12. Larva with 2 pairs of fundaments of the tube-feet in each of the first 5 rays, the belated 6th hydrocoele pouch just coming into evidence. Oral and aboral disks well developed. × 44.



H. L. Osterud PLATE 3

PLATE 4

a., Anus.

a. t., Azygos tentacle.

h. p. 6, Sixth hydrocoele pouch.

m., Mouth.

m. p., Madrepore.

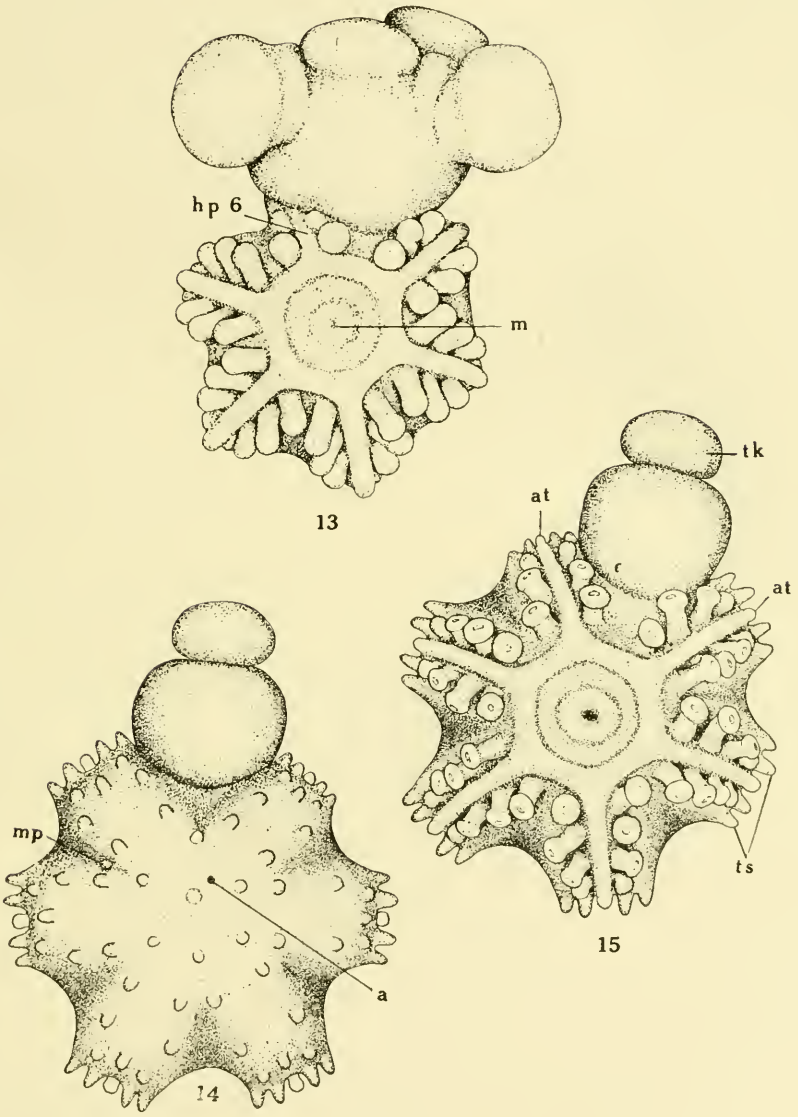
t. k., Ternimal knob.

t. s., Terminal spines.

Fig. 13. Larva showing the retrogressively developing preoral lobe, distinct mouth, ring and radial canals, 3 pairs of tube-feet in the first 5 rays, the 6th ray lagging behind with only 2 pair. × 44.

Fig. 14. Aboral view of a stage in which the 3 arms of the preoral lobe have been resorbed and the preoral lobe much diminished in size. The fixing disk, now the sole larval organ of attachment, is nearly separated as the terminal knob. The position of the madrepore and anus, the appearance of the first spines, and the still inferiorly developed 6th ray also is shown. × 44.

Fig. 15. Oral view of the same specimen as in figure 14, showing the open mouth, the functional tube-feet, prominent ring and radial canals, and azygos tentacle, the 6th ray still inferior with 1 pair of tube-feet less than the other rays. × 44.



H. L. Osterud. PLATE 4

PLATE 5

a., Anus.

a. t., Azygos tentacle.

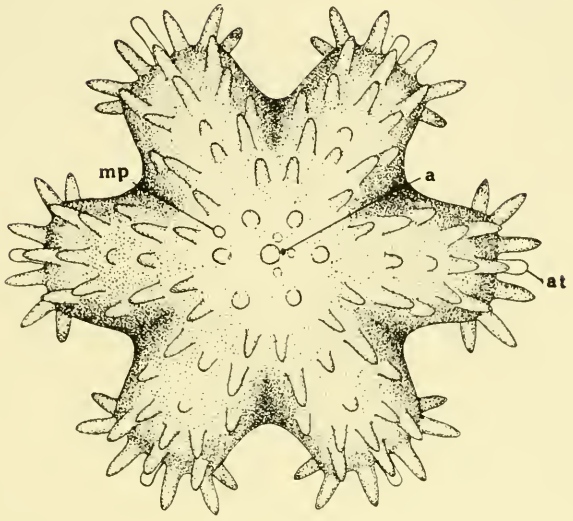
m., Mouth.

m. p., Madrepore.

s., Stomach.

Fig. 16. Aboral view of young starfish after complete resorbtion of the preoral lobe. × 36.

Fig. 17. Oral view of the same specimen as figure 16. Stomach partially protruded; 6th ray indistinguishable from the others; terminal, ambulacral, preoral and epioral spines; 5 pairs of tube-feet in each ray. × 36.



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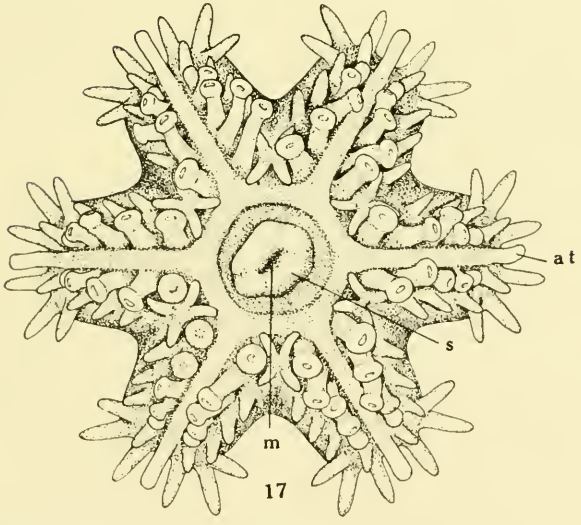

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H. L. Osterud. PLATE 5

Key to the Echinoderms of Friday Harbor, Washington*

Mildred Bush,
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The area included in this paper is roughly San Juan County, Washington. All the material was secured by means of a trawl and by shore collecting at low tide. The aim is to make it easier to determine the species of echinoderms in the vicinity of the Puget Sound Biological Station.

The starfish range in size from the small 6-rayed Leptasterias, which are from ½ to 3 inches in diameter, to the large Evasterias and Pycnopodia, which are sometimes 2 feet or more in diameter. They vary in number of rays from 5 or 6 to 20 or more (plate 7).

Hybrids are common among the starfishes. Specimens may show the general characteristics of *Pisaster confertus* but have some large capitate spines like those of *Pisaster ochraceus*. The larger spines may be somewhat clustered, with a large capitate one in the center, as in *Evasterias subnodosa*. The species of Leptasterias are particularly hard to classify because many young of larger 5-rayed species have 6 rays and a similar arrangement of spines. They are specially hard to distinguish from some Asterias types because the dorsal spines are similar, and each may have 1 or no peractinal row of spines. There are evidences of crossing among Evasterias species, especially of *E. troschelii rudis* and *E. troschelii subnodosa*. In these the size and form may be like the former while the dorsal spines may have the nodular arrangement of the latter. Some specimens show the feebly developed dorsal skeleton and few scattered spines of Orthasterias, and the many rows of interactinal spines of Evasterias. The dominant type may be determined by the presence or absence of the peculiar Orthasterias pedicellariae which in profile look like blunt hooks (Fig. 30).

In the classification of starfish, the form, number of rays, form and arrangement of spines, and the form of pedicellariae are the most evident dependable characteristics. The form and arrangement of the spines vary in the different species, and these will be described in each. Figure 34 illustrates in general the position of the groups of spines mentioned in the

*EDITOR'S NOTE—Taxonomic articles bringing together the species of larger groups so far as those species are found in the vicinity of the Puget Sound Biological Station are encouraged on account of their helpfulness in research in almost every other field of zoology. With descriptions scattered thruout numerous journals an ecologist, for example, might find it necessary to spend most of his first summer locating taxonomically the animals with which he wishes to experiment.

descriptions. Pedicellariae are generally of 2 sorts, major and minor. The major ones have jaws which are nearly straight. These jaws articulate at the base by a simple joint to a thick basal piece, so that they open and close like forceps. The minor ones are smaller; their blades curve

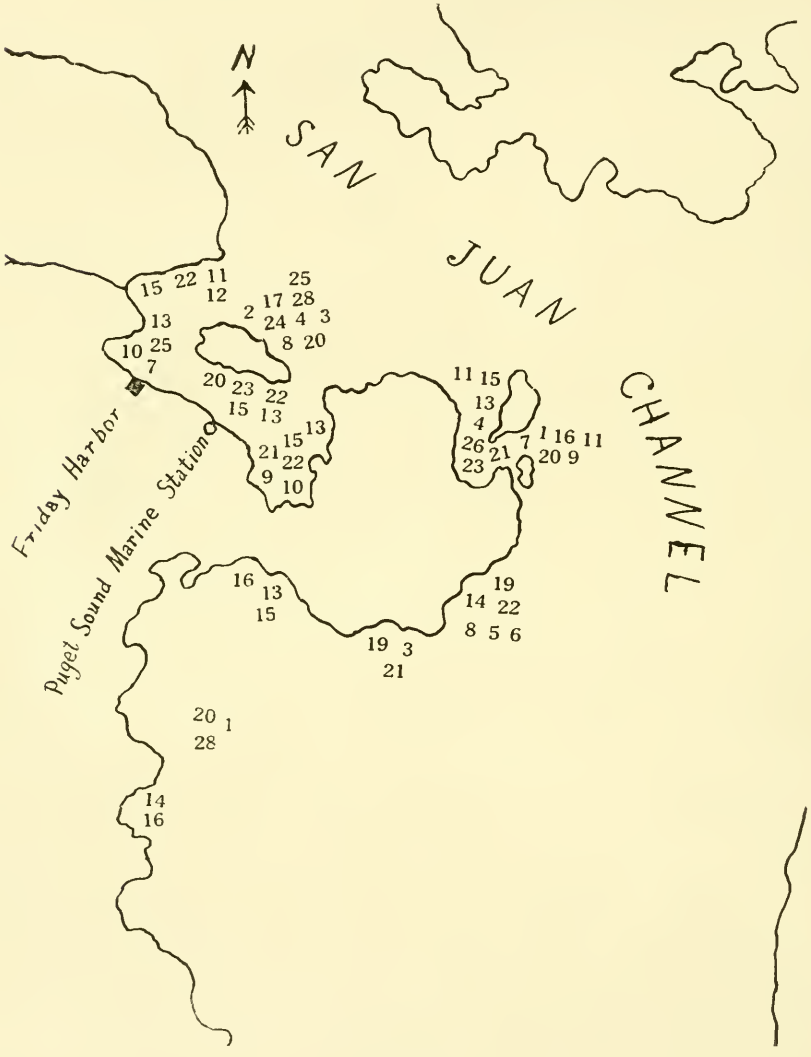


PLATE 6

Map of Friday Harbor region showing where most of the collections were made. The numbers correspond to those on plate 7.

and articulate like those of scissors. Pedicellariae with several valves may also occur; these are described with the species to which they belong.

The Ophiuroidea (brittle stars) are not numerous in the region included. They are usually found by trawling. *Ophiura aculeata* is the most common species. It is often found on the holdfasts of the common kelp (Nereocystis). *Amphioplus abditus,* a very delicate one with very long rays, was found along the tide line of Brown Island and Minnesota Reef. *Amphiodia periercta* was found only on the beach at Olga. It lies buried in sand about 15 inches deep with only the tips of the rays exposed, and these are drawn down when the animal is disturbed. *Ophiura brevispina* and *Gorgonocephalus eucnemis* (basket star), were found only in deep water.

Only 4 species of Echinoidea (sea urchins) were found. They are not difficult to distinguish. *Strongylocentrotus franciscanus* and *S. drobachiensis* are the most common. Only a few specimens of *S. purpuratus* were found. *Echinarachnius excentricus* was abundant in the sand at False Bay on the southwestern side of San Juan Island.

Holothuroidea (sea cucumbers) were very numerous in nearly all parts of the territory over which material was collected. *Cucumaria japonica* was the most abundant along the tide line, and specially noticeable on account of its brilliant color. *C. chronhjelmi* was found at the tide line as well as in deep water. *C. lubrica* and *C. pulcherrima* are not common and were not found except by trawling. *Stichopus californicus* was abundant below low tide, specially in some bays. It is commonly much noticed on account of its large size.

Acknowledgments are due to Dr. N. Fasten, Dr. T. C. Frye, Prof. T. C. D. Kincaid, Dr. S. C. Langdon and Prof. H. L. Osterud, all of the University of Washington.

Phylum Echinodermata

KEY TO CLASSES

A. With radial appendages or arms.
  B. Arms not sharply marked off from the disk; ambulacral grooves containing tube-feet present. ASTEROIDEA (p. 22)
  BB. Arms sharply marked off from the disk; ambulacral grooves and tube-feet lacking. OPHIUROIDEA (p. 33)
AA. Without radial appendages or arms.
  C. With hard calcareous shell. ECHINOIDEA (p. 35)
  CC. Without hard calcareous shell. HOLOTHUROIDEA (p. 36)

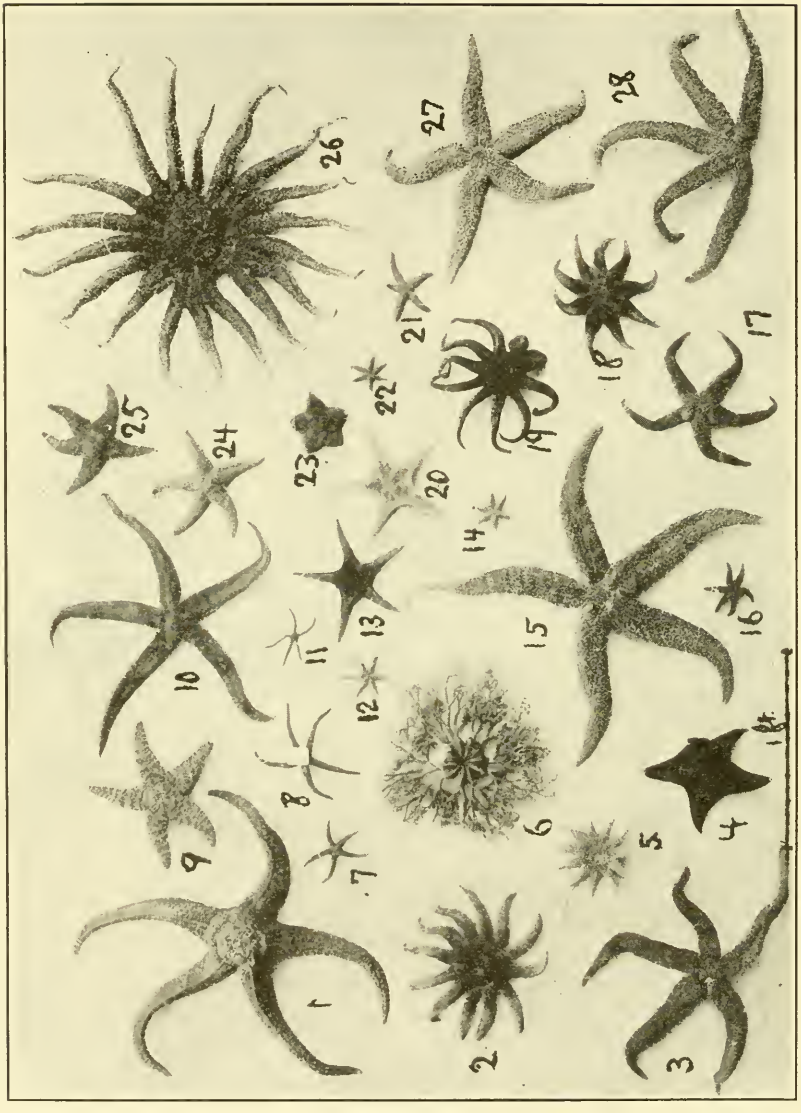


PLATE 7

PLATE 7

Fig. 1. Orthasterias forreri forcipulata.
Fig. 2. Solaster dawsoni.
Fig. 3. Evasterias acanthostoma.
Fig. 4. Dermasterias imbricata.
Fig. 5. Crossaster papposus.
Fig. 6. Gorgonocephalus eucnemis.
Fig. 7. Henricia leviuscula.
Fig. 8. Henricia leviuscula lunula.
Fig. 9. Pisaster orchraceus.
Fig. 10. Luidia foliolata.
Fig. 11. Ophiura brvispina.
Fig. 12. Leptasterias aequalis.
Fig. 13. Mediaster aequalis.
Fig. 14. Leptasterias alaskensis.
Fig. 15. Evasterias troschelii rudis.
Fig. 16. Leptasterias hexactis.
Fig. 17. Hybrid of Evasterias.
Fig. 18. Solaster galaxides.
Fig. 19. Solaster stimpsoni.
Fig. 20. Mediaster aequalis.
Fig. 21. Asterias victoriana.
Fig. 22. Leptasterias aequalis.
Fig. 23. Pteraster tesselatus.
Fig. 24. Pisaster confertus.
Fig. 25. Pisaster ochraceus.
Fig. 26. Pycnopodia helianthoides.
Fig. 27. Evasterias troschelii.
Fig. 28. Hybrid of Evasterias troschelii rudis and E. troschelii subnodosa.

Class *Asteroidea*

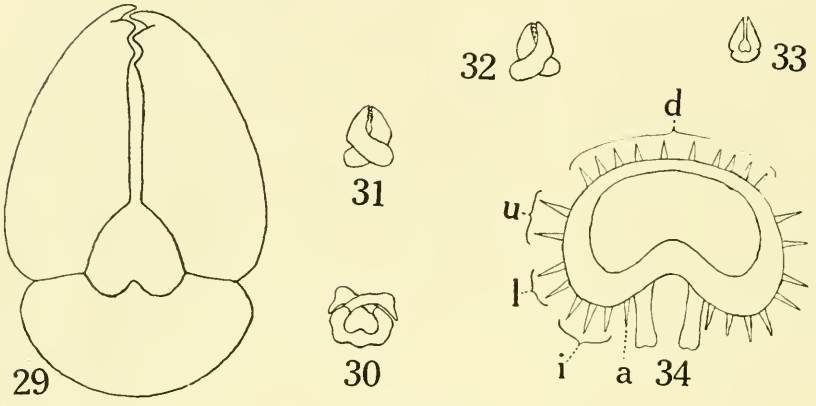
KEY TO ORDERS

A. Ambulacral feet usually in 4 rows; pedicellariae either forceps-like or shears-like. FORCIPULATA (p. 22)

AA. Ambulacral feet in 2 rows; pedicellariae not as above.

B. Marginal plates small, sometimes indistinct; margin of animal never rigid. SPINULOSA (p. 28)

BB. Marginal plates large; margin of animal rigid. PHANEROZONA (p. 31)



M. Bush. PLATE 8

Fig. 29. Major pedicellaria of Pisaster ochraceus. × 36.
Fig. 30. Minor pedicellaria of Orthasterias forreri forciplata. × 36.
Fig. 31. Minor pedicellaria of Pisaster ochraceus. × 36.
Fig. 32. Minor pedicellaria of Orthasterias forreri forcipulata. × 36.
Fig. 33. Small major pedicellaria of Orthasterias forreri forcipulata. ×36.
Fig. 34. Diagram showing the groups of spines used in classification; (d) dorsal region; (u) upper marginal region; (l) lower marginal region; (i) interactinal region; (a) ambulacral region. × 36.

ORDER FORCIPULATA (Forcipulosa)

Family ASTERIIDAE

KEY TO GENERA

A. Rays 5 (rarely 6).

B. Dorsal spines forming a network or clustered.

C. Interactinal spines in 3 or 5 or more rows.

D. Adambulacral spines 1 row, 1 spine to a plate. PISASTER (p. 23)

DD. Adambulacral spines alternating 1 and 2 to a plate.
EVASTERIAS (p. 23)

CC. Interactinal spines usually in 1 row with 1 or 2 short rows near disk. ASTERIAS (p. 25)

BB. Dorsal spines in 3 to 7 rather regular rows.
ORTHASTERIAS (p. 25)

AA. Rays 6 (rarely 5). LEPTASTERIAS (p. 26)

AAA. Rays 20 to 24. PYCNOPODIA (p. 28)

Genus Pisaster

Disk thick, rather large; rays 5 or 6, stout. Dorsal ossicles strong, numerous, not confined to 3 or 5 rows; dorsal spines forming a net, or irregular; upper marginals distinct or not; lower marginal and actinal plates and spines numerous, crowded; adambulacral spines in 1 row, 1 to a plate; peculiar major pedicellariae sessile, large, thick, ovoid or wedge-shaped in appearance specially laterally and ventrally. Ambulacral feet usually in 6 rows.

Hybrids between the 2 species mentioned below are common. In some the principal characteristics of *P. confertus* may occur, but spines of the large *P. ochraceus* type may also appear. The arrangement of the spines may also vary somewhat.

KEY TO SPECIES

A. Dorsal spines unequal, large, strongly capitate. *1. P. ochraceus*

AA. Dorsal spines equal, small, not strongly capitate. *2. P. confertus*

1. Pisaster ochraceus (Brandt) A. Agassiz.

Asterias ochraceus Brandt; *Asterias janthina* Brandt; *Asteracanthion margaritifer* Müller & Troschel.

Dorsal spines arranged net-like but sometimes with wide areas and sometimes with groups where the lines intersect, forming a conspicuous central pentagon and sometimes partial median rows; spines coarse, stout, strongly capitate, very unequal.—Abundant, along the tide line.

2. Pisaster confertus (Stimpson) Verrill.

Dorsal spines small, numerous, not strongly capitate, acute or clavate; arranged in a close network with or without median radial rows and central pentagon, without noticeable groups.

Genus Evasterias

Forms large; rays long, tapering gradually; disk small. Dorsal skeleton usually firm; ossicles small, lobed; dorsal spines numerous, arranged net-like or irregularly or in short transverse rows; marginal and

interactinal plates stout, lobed, joined firmly in longitudinal rows, each plate bearing 1 or more long spines.

Hybrids in this genus are common. Perhaps the most common cross is one with the dorsal spines like the slender *E. acanthostoma* type and the ventral spines like the *E. troschelii*. The dorsal spines may be partly of the thicker *E. troschelii* type and partly of the slender *E. acanthostoma* type. Some specimens are found which look like Evasterias but which have a feebly developed dorsal skeleton and unusually scattered spines. These seem to be a cross between some species of Evasterias and of Orthasterias.

KEY TO SPECIES

A. Dorsal spines arranged net-like or clustered; interactinal spines 4 to 6 rows. *1. E. troschelii*

B. Dorsal spines arranged net-like.

C. Dorsal spines in a rather large-meshed net-work. *1a. E. troschelii rudis*

CC. Dorsal spines in a small-meshed net-work. *1b. troschelii densa*

BB. Dorsal spines arranged not net-like but conspicuously clustered. *1c. E. troschelii subnodosa*

AA. Dorsal spines not arranged net-like but often in transverse rows; interactinal spines more than 6 rows. *2. E. acanthostoma*

1. Evasterias troschelii (Stimpson) Verrill.

Asterias troschelii Stimpson; *Asterias branchiata* Perrier; *Asterias* (*Diplasterias*) *epichlora* De Loriol.

Rays 5. Dorsal spines numerous, unequal, arranged net-like or areolate or clustered; larger spines capitate or truncated, smaller ones acute or club-like; marginals in regular rows, longer than dorsals; interactinals still longer, forming 4 to 6 regular close rows, spines curving upward; mouth spines elongated; pedicellariae numerous.—Numerous, in deep water and along shore.

1a. Evasterias troschelii rudis Verrill.

Size large; dorsal spines not clustered; spines medium or small, numercus, not grouped, mostly club-like, capitate or rather sharp, arranged in an open net-work with large papular areas; papulae numerous; interactinal spines crowded.—Numerous, in deep water and along shore.

1b. Evasterias troschelii densa Verrill.

True to type in form. Dorsal spines somewhat capitate, nearly uniform in size, forming a close net-work, in single lines or somewhat irregular but not clustered.—Not numerous, along tide line.

1c. **Evasterias troschelii subnodosa** Verrill.

Dorsal skeleton very firm; dorsal spines clustered in nodular groups, with large central capitate spines and smaller outer ones, often a median row; spines large, numerous.—Numerous, in deep water and along tide line.

2. **Evasterias acanthostoma** Verrill.

Forms large; rays long, acute at tips. Dorsal spines numerous, small, almost equal, columnar, club-like or tapering, tips rough, not arranged in evident net-work nor longitudinal rows but often in short transverse rows; marginal and interactinal spines from 5 to 6 mostly double and crowded rows with about 8 or 10 in each transverse row; pedicellariae numerous; major pedicellariae small. Adambulacral spines elongated, 2 to a plate.—Not numerous, in deep water.

Genus Asterias

Size usually large; rays 5, rarely 6. Dorsal ossicles and spines variously arranged, most often openly reticulate; dorso-lateral plates rather small, usually reticulate or irregularly arranged, united by their lobes or by smaller transverse ossicles; upper marginal spines form a distinct row with a channel between it and the lower marginal row; interactinal spines usually form 1 long row like the lower marginals and 1 or 2 short proximal rows, but these may be rudimentary or lacking, specially in the young, so that only 2 rows of ventrals are present; lower marginals and actinals may stand 2 or more to a plate, making 4 or 5 rows; major pedicellariae usually small, ovate or lanceolate, sometimes unguiculate. Larvae free swimming. Genital pores dorsal.

1. **Asterias victoriana** Verrill.

Rays 5, rather long, rounded. Dorsal spines few, short, capitate, forming a median row, a few irregularly scattered ones of similar size and form on each side; 2 marginal and 2 interactinal rows regular and mostly simple, their spines short, stout, blunt; ambulacral spines 1 or 2 to a plate. —Very scarce, in deep water.

Genus Orthasterias

Size usually large; rays 5; disk small. Dorsal skeletal plates lobed, arranged in rather evident longitudinal rows; dorsal spines very large, 1 or rarely 2 to a plate, forming 3 to 5 or more rather evident rows, smaller spines may occur irregularly between the rows; marginal spines more like dorsals and forming regular rows; actinal spines 1 or 2 rows or lacking; major pedicellariae large, 2 or more kinds, larger ones toothed; minor pedicellariae numerous; adambulacrals 2 to a plate or alternating 1 and 2.

KEY TO SPECIES

A. Dorsal spines in 5 or more indistinct radial rows. *1. O. columbiana*

AA. Dorsal spines in 1 to 3 radial rows with others scattered or reticulate.

B. Dorsal spines in 3 indistinct rows with a few scattered ones; no pedicellariae hook-like. *2. O. forreri*

BB. Dorsal spines in 1 median radial row, others reticulated; some minor pedicellariae hook-like. *2a. O. forreri forcipulata*

1. Orthasterias columbiana Verrill.

Dorsal spines 5 rows with intermediate ones scattered between; papular areas small; dorsal plates large, thick, firmly united by lobes; dorsal spines long, columnar, rough-fluted with wreaths of minor pedicellariae above the middle of the spines; lower marginal spines in 2 rows; actinals in 1 row; adambulacral spines flattened, those near mouth longer and more slender. Color usually yellow with bright red markings.—Rather numerous, in deep water and along tide line.

2. Orthasterias forreri (De Loriol) Verrill.

Asterias forreri De Loriol.

Dorsal spines long, in 3 indistinct longitudinal rows, with some scattered intermediate ones; upper marginals large, in 1 row, 2 to a plate; lower marginals in 2 rows, flattened or gouge-shaped; actinal plates and spines small; adambulacral spines long, flattened; minor pedicellariae large, toothed at tips, about half as long as major pedicellariae, forming large loose basal circumspinal wreaths; larger major pedicellariae nearly as stout as the spines but shorter, usually of 2 or more kinds. Type not found in our locality.

2a. Orthasterias forreri forcipulata Verrill.

Asterias (*Urasterias*) *forcipulata* Verrill.

Dorso-lateral spines long, rather slender, arranged in median radial rows with open net-work on each side of radial rows; papular areas large; ambulacral plates and spines crowded, spines slender. Minor pedicellariae large, abundant, of 2 kinds; some of the ordinary forceps-like sort; others arched widely, looking in profile like hooks (Fig.30); dorsal pedicellariae in large groups on the dermis and around the bases of spines.—Not numerous.

Genus Leptasterias

Size small; rays normally 6, rarely 5; disk medium. Dorsal plates usually lobed and overlapping; dorsal spines numerous, often club-like or capitate, equal or unequal, arranged net-like or in groups or in longitudinal or radial rows; papular areas small; marginal and interactinal spines longer than dorsals; interactinals usually 1 row, sometimes 2 rows near

disk; adambulacral spines alternately 1 and 2 to a plate or forming 2 rows; major pedicellariae ovate or lanceolate, and large serrate dermal ones may occur.

KEY TO SPECIES

A. Dorsal spines equal, arranged in 5 to 7 rows on each ray.
  B. Surface even; spines not capitate. *1. L. aequalis*
  BB. Surface not even; spines capitate. *2. L. hexactis*
AA. Dorsal spines unequal, not in rows except sometimes in median radial rows.
  C. Rays 5. *3. L. epichlora*
  CC. Rays 6. *3a. L. epichlora alaskensis*

1. Leptasterias aequalis (Stimson) Verrill.

Asterias aequalis Stimpson.

Rays tapering to acute tips; disk medium. Dorsal spines very numerous, mostly capitate, some club-like, very crowded, equal in height and thus presenting an even surface. Marginals and the 1 row of interactinals longer and sharper than dorsals and arranged in regular rows; adambulacral spines 2 to a plate; major pedicellariae few, large, rather long; minor pedicellariae numerous.—Numerous, along tide line.

2. Leptasterias hexactis (Stimpson) Verrill.

Asterias hexactis Stimpson.

Similar to *L. aequalis* in form. Dorsal spines numerous, mostly club-like, some slender, not very crowded, not equal in height, surrounded by wreaths of minor pedicellariae, sometimes arranged singly or in groups in not very definite rows; other spines and pedicellariae similar to those of *L. aequalis*.—Numerous, along tide line.

3. Leptasterias epichlora (Brandt) Verrill.

Asterias epichlora Brandt; (?) *Asterias camtchatica* Brandt; *Asterias saanichensis* De Loriol.

Rays 5, shorter than in *L. aequalis*, more blunt. Dorsal spines short, rather numerous, unequal, mostly conspicuously headed but some club-like, arranged net-like or in groups, median row distinct or not; marginal spines longer than dorsals and arranged in rows; interactinals similar to marginals, usually in 1 row; adambulacrals alternately 1 and 2 to a plate; minor pedicellariae numerous; large stout dermal major pedicellariae occur. —Type not found in our locality.

3a. Leptasterias epichlora alaskensis Verrill.

Much like *L. epichlora*, 6-rayed; dorsal spines more conspicuously headed, sometimes grouped, usually no median radial rows. 3 or 4 were

found with median rows formed by capitate spines so crowded that the heads almost touched. These correspond to the form *L. epichlora alaskensis siderea* of Verrill.—Not numerous, at tide line.

Genus **Pycnopodia**

Rays many, 20 or more in adult; disk large. Dorsal surface covered with a thick soft integument. Dorsal spines few, sharp, scattered; marginal spines distinct, ventral ones forming 2 distinct rows; adambulacral spines crowded, 1 to a plate; minor pedicellariae in large clusters on the dorsal surface and around the spines; major pedicellariae large, numerous.

1. **Pyncopodia helianthoides** (Brandt) Stimpson.

Asterias helianthoides Brandt.

Description same as for genus.—Numerous, in deep water and along tide line.

ORDER SPINULOSA

KEY TO FAMILIES

A. Surface rough, calcareous; spine-clusters on raised ossicles, not fan-shaped.

B. Disk small; rays long, rounded. (Figs. 7, 8).
ECHINASTERIDAE (p. 28)

BB. Disk broad; rays medium, somewhat flattened. (Figs. 2, 5, 18, 19).
SOLASTERIDAE (p. 29)

AA. Surface slippery; spine-clusters not on raised ossicles, fan-shaped.
PTERASTERIDAE (p. 31)

Family ECHINASTERIDAE

Genus **Henricia**

Rays long, terete, gradually tapering. Madreporite spinulose. Dorsal ossicles closely net-like, papular areas small; papulae few; dorsal spines minute, uniform, usually in crowded clusters; upper marginal spines like the dorsals; lower marginal spines distinct, larger than upper marginals; marginal rows of spines separated at base by a row of clustered spinules; actinal spines like lower marginals; adambulacral spines in transverse double rows or in clusters of graded spines; pedicellariae none. Color usually bright orange, sometimes yellowish.

KEY TO SPECIES

A. Dorsal ossicles rounded, elliptical or lunate. *1. H. leviuscula*

AA. Dorsal ossicles reniform, or curved, or crescent-shaped with blunt tips. *1a. H. leviuscula lunula*

1. **Henricia leviuscula** (Stimpson) Fisher.

Luidia leviuscula Stimpson; *Cribella leviuscula* Verrill; *Henricia leviuscula* Fisher.

Papular areas small; papulae few. Dorsal ossicles thick, rounded, elliptical or lunate, covered with clustered spinules; densely crowded, varying much in size and form; marginal rows of spines divergent at base; peractinal row distinct, regular, parallel to marginals; peractinal plates smallest of the 3 groups; lower marginals largest, all spinulose; adambulacral ossicles bearing 2 or 3 transverse groups of graded spines; these spines largest toward groove, 1 short one in groove; adorals short. Color of starfish usually deep orange.—Numerous, in deep water and along tide line.

1a. **Henricia leviuscula lunula** Verrill.

Similar to the typical *H. leviuscula*. Marginals and interactinals form 3 regular rows of plates that are longer and wider than the rest. The distinguishing characters are these: Dorsal ossicles reniform or curved, or crescent-shaped with blunt cusps, partially enclosing a papular pore on the concave side.—Numerous, in deep water.

Family SOLASTERIDAE

KEY TO GENERA

A. Dorsal spines short, blunt, equal in length; ossicles bearing spines crowded. SOLASTER (p. 29)

AA. Dorsal spines long, slender, unequal in length; ossicles bearing spines scattered. CROSSASTER (p. 30)

Genus **Solaster**

Rays 7 to 13; disk broad. Dorsal ossicles small, lobed, usually reticulated on disk and rays; bearing stellate clusters of slender movable spinules which are webbed together; upper marginal plates small, like the dorsals; lower marginals large, elevated, transversely oblong, forming evident rows; adambulacral plates with from 2 to 4 webbed groove spines, with a transverse row of longer webbed spines. Interradial areas small. Papulae numerous on dorsal surface, single or grouped.

KEY TO SPECIES

A. Rays 9 or 10.
  B. Rays short, flattened. *1. S. galaxides*
  BB. Rays long, rounded. *2. S. stimpsoni*
AA. Rays 12 or 13. *3. S. dawsoni*

1. **Solaster galaxides** Verrill.

Rays 9 or 10, rather short, tapered rapidly; disk broad. Dorsal ossicles small, closely united; papular areas small; dorsal surface covered with small crowded flat-topped pseudopaxillae with 12 to 15 spinules each; marginal plates present; lower marginal plates elongated transversely, with many spines; peractinal row one-third of proximal ray; interradial spaces

large, with many pseudopaxillae; adambulacral spines 2 in a groove, transverse rows of graded spines 6 to 8 and the 2 inner longer and stouter.—Not numerous, in deep water.

2. **Solaster stimpsoni** Verrill.

? *Asterias decemradiatus* Brandt; ? *Solaster decemradiatus* Stimpson; *Solaster vancouverensis* De Loriol.

Rays 9 or 10, rounded, rather long; disk medium. Dorsal ossicles closely united; pseudopaxillae composed of stellate clusters of small blunt divergent webbed spinules, larger than those of *S. galaxides;* upper marginals indistinct; lower marginals smaller and less prominent than in *S. galaxides,* spinules in transverse groups; adambulacral plates with 2 spines inside and a transverse simple row of 6 or 8 longer webbed spines.—Rather numerous, in deep water.

3. **Solaster dawsoni** Verrill.

Rays 12 or 13, tapering rapidly; disk rather broad. Dorsal plates lobed, closely over-lapping; dorsal pseudopaxillae larger than those of *S. galaxides* and *S. stimpsoni,* crowded with small slender spinules; spinules webbed together; papular areas small; papulae small; upper marginals well developed; pseudopaxillae rounded, larger than dorsals, bearing stellate groups of spinules; lower marginals large and prominent, transversely oblong, with many spinules; marginal rows close together; interradial spaces very narrow; interactinal rows very short; spines of adambulacral furrow 4 or 5, long, slender, webbed; transverse spines long, slender, subequal; oral spines long.—Rather numerous, in deep water.

Genus **Crossaster**

Rays variable in number, 10 to 12; disk broad. Dorsal skeleton feebly developed, flexible; ossicles slender, openly reticulated, leaving many large papular areas; dorsal and marginal plates bearing elongated pseudopaxillae with many spinules clustered in brush form, the central ones longest; upper marginal plates feebly developed; lower marginals much larger and with large pencils of spinules; actinal plates few, sometimes lacking; adambulacral spines form a furrow series of 3 to 5 to a plate and an exterior transverse comb of longer webbed spines.

1. **Crossaster papposus** (Linnaeus) Müller & Troschel. Rose Star.

Asterias papposa Linne; *Solaster papposus* Forbes; *Asterias affinis* Brandt.

Rays usually 11. As described in the genus. Conspicuous on account of large elevated conical tufts of slender elongated spinules which are more or less divergent. Actinal spinules mostly lacking; interradial spaces

very narrow. Color yellowish, with a red spot or circle on the disk and markings on the rays.—Numerous, in deep water.

Family PTERASTERIDAE

Genus Pteraster

Rays 5 to 8, usually 5. Ambulacral groove turned up distally to upper side; transverse fans of adambulacral spines varying in size, 5 or 6 to each fan. Ambulacral feet in 2 rows, orange in color.

1. **Pteraster tesselatus** Ives. Cushion Star

Pteraster reticulatus Verrill.

As described for the genus.—Rather numerous, in deep water.

ORDER PHANEROZONA

KEY TO FAMILIES

A. Surface rough; disk flat.
  B. Disk large; rays short. GONIASTERIDAE (p. 31)
  BB. Disk small; rays long. LUIDIIDAE (p. 32)
AA. Surface smooth; disk plump. ASTEROPIDAE (p. 33)

Family GONIASTERIDAE

KEY TO GENERA

A. Rays very short. CERAMASTER (p. 31)
AA. Rays longer, as long as the disk is broad. MEDIASTER (p. 32)

Genus Ceramaster

Form short-rayed, stellate. Dorsal plates numerous, closely united, roundish or hexagonal, granulated; marginal plates regular, corresponding closely, closely granulated, or the naked central areas with rows of granules around the margin, and the plates forming the margin of the disk and rays; interactinal plates like a mosaic, granulated; papular areas small; adambulacral spines numerous, small, crowded, grading into actinal granulations.

1. **Ceramaster granularis** (Retzius) Verrill.

Asterias granularis Retzius; *Astrogonium granulare* Müller & Trochel; *Goniaster granularis* Lütken; *Pentagonaster granularis* Perrier; *Tosia (Ceramaster) granularis* Verrill.

Rays 5, very short; disk pentagonal, flat. Dorsal plates hexagonal, closely united, leaving papular pores corresponding to the angles; plates covered with small granules; marginal plates prominent, covered with similar small granules; actinal plates crowded, polygonal, covered with spinules of various sizes; adambulacral spines forming a simple marginal row; actinal groups of spinules varying in size; oral spines many, short,

stout.—Very scarce, in deep water. Not found by the writer, but reported off Brown Island by Perry.*

Genus **Mediaster**

Rays 5, rather long, tapered; disk flat, broad. Dorsal plates rounded, spinulose; marginal plates well developed, rather numerous, large, higher than broad, paired series equal in size and number; actinal plates not close, longitudinally arranged, elevated, roundish, covered with spinules or granules; papulae single or clustered; adambulacral plates with 1 regular marginal row of splender spines, 2 exterior longitudinal groups of shorter spinules, similar to Ceramaster; pedicellariae sometimes lacking, sometimes the actinal plates with single broad, sessile valvular pedicellariae.

1. Mediaster aequalis Stimpson.

Rays 5, variable in length, regularly tapered, slender at tip; disk, medium; ratio 1:3. Dorsal ossicles and granules as for genus; marginal plates large, higher than wide, granulated; the plates of the 2 rows are similar in size and shape, alternate, and form the margin of the disk and rays; abactinal areas wide at base; plates in areas rounded, elevated, spinulose, separated by papular pores in groups; valvular pedicellariae often in center of plate; actinal plates closely united, varying in size; adambulacral plates, squarish, not large, with blunt spinules in marginal row and shorter ones externally; spinules varying in size. Madreporite small, sunken.—Rather numerous, in deep water northeast of Brown Island.

Family **LUIDIIDAE**

Genus **Luidia**

Rays 5 to 10, flat, flexible; disk small; paxillae columnar, lobate, articulated at base, quadrate at surface and crowded in rows, or stellate; upper marginal plates small, paxilliform, round; lower marginals spinose and spinulose, transversely elongated; adambulacral plates separated by grooves, bearing furrow-spines and ventral spines; pedicellariae if present forceps-like.

1. Luidia foliolata Grube.

Rays tapered to slender tips; disk small. Paxillae varying in form, crowded, forming longitudinal and transverse rows; papular pores large, alternating with paxillae; upper marginal plates similar to adjacent ones and joined to lower marginals; lower marginals large and prominent, each bearing 3 to 5 principal spines, varying in size; peractinal row continuous with lower marginals, each bearing a cluster of spinules; adambulacral plates with 1 inner spine and about 3 grouped on the actinal surface; a few pedicellariae on ventral surface.—Rather numerous, in deep water.

*Perry, Edna M. Distribution of certain invertebrates on a restricted area of sea bottom. Puget Sound Marine Sta. Pub 1:175-176. 1916.

Family ASTEROPIDAE

Genus Dermasterias

Rays 5, short, broad; disk broad, thick, not distinguished from rays; entire surface covered with thick soft lubricous skin. Dorsal ossicles concealed by skin in living specimens, showing in dried specimens, roundish, imbricated; pedicellariae small, valved, on dorsal surface.

1. **Dermasterias imbricata** (Grube) Perrier. Leather Star

Asteropsis imbricata Grube; *Dermasterias inermis* Perrier.

Rays 5, broad, tapering, usually short; disk large, plump; surface smooth and lubrious in life due to thick soft skin. Dorsal ossicles roundish, lobed, connected by other small ossicles; ossicles of interradial region imbricated; interactinal plates ovate, rows parallel to ambulacra and imbricated; adambulacral spines 2 to a plate, terete distally; small valved dermal pedicellariae may occur; large ventral pedicellariae often present and may take place of outer adambulacral spines.—Rather numerous, in deep water and along tide line.

Class *Ophiuroidea*

KEY TO ORDERS

A. Rays unbranched; lateral plates of rays spine-bearing; ambulacral ossicles articulating by means of processes and pits.
Zygophiura (p. 33)

AA. Rays branched or unbranched; lateral plates of rays not spine-bearing; ambulacral plates articulating by means of hour-glass-shaped surfaces. Cladophiura (p. 35)

ORDER ZYGOPHIURA

KEY TO GENERA

A. Ray-spines short, ¼ the width of the rays, parallel to ray-axis.
Ophiura (p. 34)

AA. Ray-spines long, ¾ the width of the rays, at right angles to ray-axis.

B. Median dorsal plates of rays surrounded by a series of smaller plates; length of arms less than 10 times the diameter of the disk.
Ophiopholis (p. 34)

BB. Median dorsal plates of rays not surrounded by a series of smaller plates; length of arms more than 10 times the diameter of the disk.

C. Oral papillae 3, equal, arranged in a regular series.
Amphiodia (p. 34)

CC. Oral papillae 4, unequal, arranged in a discontinuous series.
Amphioplus (p. 34)

Genus **Ophiura**

1. **Ophiura brevispina** Say.

Disk small, pentagonal, not thicker than rays; rays 5, long, slender, from corners of disk-pentagon, limited in movement. Spines on disk none; plates on upper and lower side of disk overlapping like fish-scales; scales small except a large one on each side of the base of each ray; mouth-shields very large, interradial. Ray plates well developed; lateral plates bearing 4 or 5 graded spines, the longest above, all parallel to the axis of the rays. Many mouth papillae and spines present. Color various.—Numerous, in deep water.

Genus **Ophiopholis**

1. **Ophiopholis aculeata** (Linnaeus) Gray.

Rays 5, long, slender, not covered with skin, movement limited. Disk small, covered with small conical spines and a series of 3 to 5 rounded plates on the upper side from the base of each ray almost to the center, 5-lobed; disk-lobes alternating with the rays, showing conspicuously on the lower side. Plates of rays well developed; the lateral plates each bearing 5 spines on each side; the spines varying in length, the middle one the longest. Usually red.—Rather numerous, in deep water, often on holdfasts of kelp (Nereocystis).

Genus **Amphiodia**

1. **Amphiodia periercta** Clark.

Disk pentagonal in form, 20 mm. in diameter; rays slender, 15 cm. or more in length. Surface of disk covered with overlapping scales; spines none. Oral papillae 3 on each side, thick, rounded and subequal. Radial shields small, somewhat pentagonal, with an inner angular and an outer curved margin. Side arm-plates small, with 3 sharp arm-spines of which the middle one is slightly the longest. Color in dried specimens pale yellowish brown.

Found buried in about 15 inches of sand at Olga, Washington, by Dean Engberg of the University of Nebraska.

Genus **Amphioplus**

1. **Amphioplus abditus** Verrill.

Small, delicate; rays slender, long, 12 to 18 times the diameter of the disk. Disk rather thick, not deeply lobed, lobes alternating with the rays; spines none; surface covered with overlapping plates, all small except 2 at the base of each ray on the upper side; these pairs large, longer than broad, not closely joined. Rays originating from shallow grooves on the under side of the disk; movement in a vertical plane and toward mouth. Dorsal plates of rays not surrounded by smaller plates; lateral plates each

bearing 3 equal spines forming longitudinal rows along the sides of the rays. Disk usually gray; rays white.—Not numerous, in mud at tide line.

ORDER CLADOPHIURA Gorgon-heads

Rays unbranched or repeatedly branched; those with unbranched rays having at the inner angle of each lower interradial space a mouth-shield, 1 mouth-shield serving as a madreporite; those with branched rays having no mouth-shields but with 1 to 5 madreporites in the interradial spaces. Entire surface covered with a granular deposit in a thick integument. Ambulacral ossicles articulating by means of hour-glass-shaped surfaces; lateral spines none; pedicellariae-like processes sometimes present.

1. **Gorgonocephalus eucnemis** Müller & Troschel. Basket-star

Rays 5, movable in a vertical plane and toward mouth, repeatedly divided into 2 equal branches, the first division near the base; outer branches very numerous and slender. Disk medium, flattened, marked by raised lines extending from each side of the bases of the rays almost to the center. Upper plates of rays indistinct, covered with a granular membrane; lower plates extending to the mouth, each bearing 6 equal spines, arranged 3 on each side and forming ventral marginal rows. Spines on upper surface of disk and rays none, on mouth-plates many.—Numerous, in deep water.

Class *Echinoidea*

KEY TO ORDERS

A. Oval; anus apical. CENTRECHINOIDA (p. 35)
AA. Disk-like; anus not apical. CLYPEASTROIDA (p. 36)

ORDER CENTRECHINOIDA Sea Urchins

Genus **Strongylocentrotus**

Size varying, about half as thick as broad; spines of 2 or 3 lengths; apical system with many plates; globiferous pedicellariae with end tooth but without lateral teeth.

KEY TO SPECIES

A. Mature test 12.5—15 cm. in diameter; color purple or red. *1. S. franciscanus*
AA. Mature test 6-10 cm. in diameter.
B. Color greenish; spines sharp. *2. S. drobachiensis*
BB. Color purple; spines blunt. *3. S. purpuratus*

1. **Strongylocentrotus franciscanus** A. Agassiz. Large purple urchin

Very large, purple but varying in shade; spines many, 4 to 60 mm. in length, delicately fluted, pointed at tips; test large, 12.5-16 cm. in diameter; apical system prominent; genital plates larger than radials; am-

bulacral pores in rows of 8 or 9 pairs, curving toward the oral region.—Numerous, below the tide line.

2. **Strongylocentrotus drobachiensis** O. F. Müller. Green sea urchin

Mediumly large, greenish, with tube feet whitish or violet; spines many, all over test, varying in length, 5 to 15 mm., the smallest ones very slender; the largest spines solid, fluted, pointed. Test somewhat flattened; apical plates many, all bearing minute protective spines; genital plates larger than radials. Pedicellariae many.—Numerous, below tide line.

3. **Strongylocentrotus purpuratus** Stimpson. Small purple urchin

Mediumly large, purple, with tube-feet also purple. Spines numerous all over test, varying in length, 4 to 16 mm., the smallest ones slender; the largest spines solid, fluted, with truncated tips, thicker than those of *S. drobachiensis*. Test flattened; apical plates many, all bearing spines; genital plates not noticeably larger than radials.—Not numerous, along shore.

ORDER CLYPEASTROIDA

Genus **Echinarachnius** Sea cakes, Sand dollars

Divisions of dorsal star petal-like with open ends; furrows on lower side bifurcated and branched; genital pores 4, none in the posterior interradius.

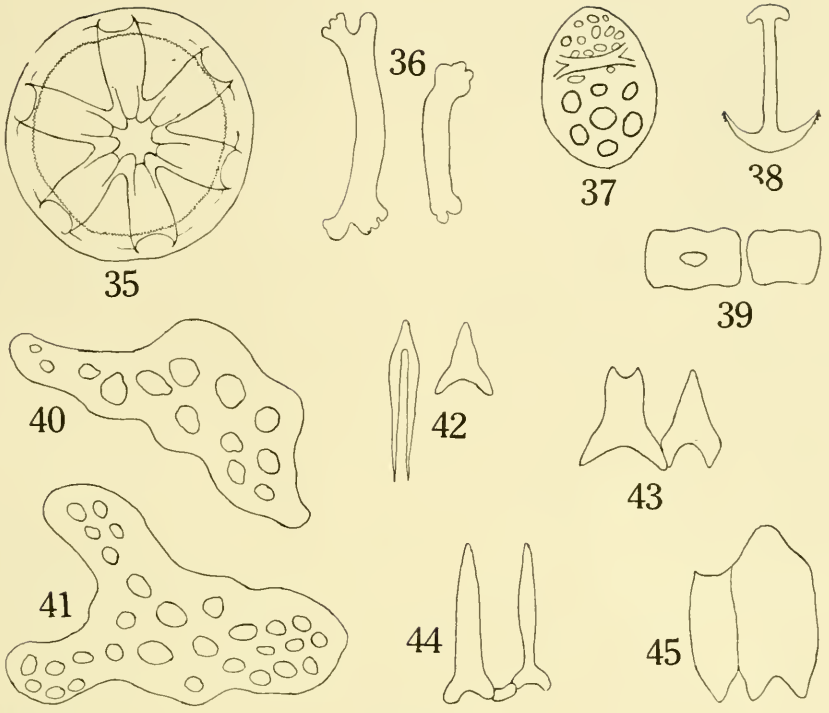
1. **Echinarachnius excentricus** Eschscholtz.

Disk somewhat straight across the posterior part; posterior ambulacral zones shorter than the other three.—Numerous, in sand at False Bay.

Class *Holothuroidea* Sea Cucumbers

KEY TO ORDERS

A. Tube-feet present. Pedata (p. 38)
AA. Tube feet none. Apoda (p. 41)



M. Bush. PLATE 9

Fig. 35. Calcareous deposit from body wall of Chiridota laevis. × 258.
Fig. 36. Calcareous deposit from tentacles of Chiridota laevis. × 258.
Fig. 37. Plate from body wall of Leptosynapta inhaerens. × 258.
Fig. 38. Anchor from body wall of Leptosynapta inhaerens. × 258.
Fig. 39. Two plates from calcareous ring of Leptosynapta. × 16.
Figs. 40, 41. Calcareous plates from body wall of Cucumaria pulcherrima. × 258.
Fig. 42. Two plates from calcareous ring of Cucumaria pulcherrima. × 4.
Fig. 43. Two plates from calcareous ring of Psolus chitonoides. × 4.
Fig. 44. Two plates from calcareous ring of Cucumaria japonica. × 4.
Fig. 45. Two plates from calcareous ring of Caudina obesacauda. × 4.

ORDER PEDATA

KEY TO GENERA

A. Tube-feet not confined to ventral surface but in radial rows. Cucumaria (p. 38)

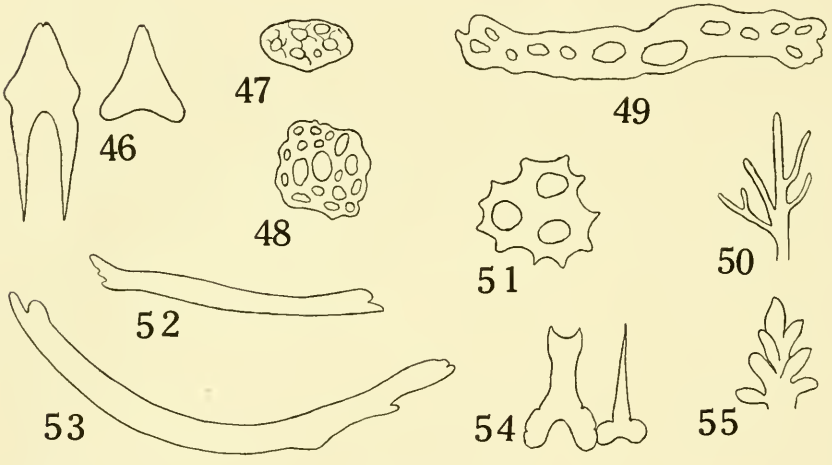
AA. Tube-feet confined to ventral surface.

B. Animals very large, 40 cm. or less; sole-like ventral surface not well developed. Stichopus (p. 40)

BB. Animals small, 5 cm. or less long; sole-like ventral surface well developed. Psolus (p. 41)

Genus **Cucumaria**

Body usually thick; tentacles 10; tube-feet on radii, a few often scattered over the interradii dorsally.



M. Bush. PLATE 10

Fig. 46. Two plates from calcareous ring of Cucumaria chronhjelmi. × 4.

Figs. 47, 48. Calcareous deposits from body wall of Cucumaria chronhjelmi. × 258.

Fig. 49. Deposit from tentacle of Cucumaria chronhjelmi. × 258.

Fig. 50. Branch of respiratory tree of Cucumaria chronhjelmi (Typical for Cucumaria). × 12.

Fig. 51. Calcareous deposit from body wall of Cucumaria lubrica. × 258.

Figs. 52,53. Calcareous deposits from tentacles of Cucumaria lubrica. × 258.

Fig. 54. Plates from calcareous ring of Cucumaria lubrica. × 4.

Fig. 55. Branch from respiratory tree of Cucumaria lubrica. × 12.

KEY TO SPECIES

A. Salmon colored to dark purplish red; animal up to 20 cm. long; tube-feet on each radius irregularly distributed in 1 band. *1. C. japonica*

AA. White, yellowish, or white with brown dots; not over 15 cm. long.

B. Tube-feet on each radius in 2 single rows with scattered ones between; animal up to 15 cm. long; usually along tide line. *2. C. chronhjelmi*

BB. Tube-feet on each radius in 2 definite rows; animal up to 10 cm. long; in deep water. *3. C. lubrica*

BBB. Tube-feet on each radius irregularly distributed in 2 narrow bands; animal up to 5 cm. long; in deep water. *4. C. pulcherrima*

1. Cucumaria japonica Semper.

Larger than *C. chronhjelmi,* sometimes 20 cm. long; tube-feet large, arranged in series along the radii, not in rows; tentacles 10, equal in size, much branched, rather long; calcareous deposits not numerous, rod-like in tentacles, irregular perforated plates in body wall; calcareous ring delicate for size of animal; radial pieces somewhat wider than the interradial, with deep notches in posterior margin (Fig. 44). Color red-salmon to dark brownish-purple.—Numerous, along shore, sometimes in deep water.

2. Cucumaria chronhjelmi Theil.

Medium in size, sometimes 15 cm. long; tube-feet very long, confined to radii, arranged in 2 rows with others scattered irregularly; tentacles 10, short, much branched, tuft-like, yellow; calcareous deposits many, various in form, rod-like in tentacles, plates-like in tube feet, basket- or cup-like in body wall; calcareous ring large, well developed; radial pieces with rather short pointed posterior prolongations (Figs. 46-50). Color white or yellowish.—Numerous along tide line, often found in deep water.

3. Cucumaria lubrica Clark.

Medium in size, 5 to 10 cm. long; cylindrical, blunt at both ends; surfaces smooth; tube feet short, confined to 2 simple rows on each radius; tentacles 10, short, branched, unequal, 4 dorsal ones longest. Calcareous deposits in the tube feet few, like curved rods; in the body wall numerous, resembling thick knobbed plates or buttons; in the tentacles a few large supporting rods; calcareous ring small, both radial and interradial pieces with short anterior prolongations, radial pieces notched posteriorly. Respiratory tree with short branches enlarged at the tips, differing from the long finger-like branches of other Cucumaria respiratory trees (Figs. 51-55). Color white with brown dots, dots sometimes almost covering the surface.—Rather numerous, in about 35 fathoms near Olga.

4. **Cucumaria pulcherrima** (Ayers) Lampert.

Pentamera pulcherrima Ayers; *Thyone pulcherrima* Semper

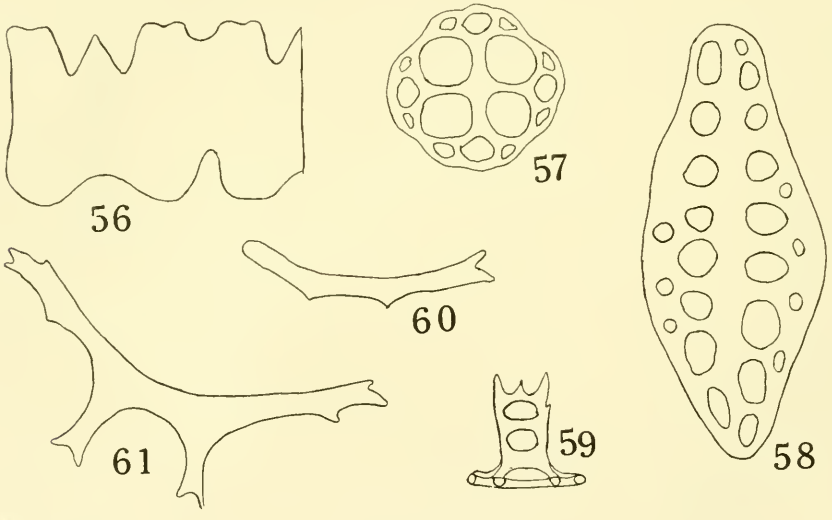
Very small, usually less than 5 cm. long; ends of body rather pointed, curved upward making ventral radii longer than dorsal; tube-feet many, arranged in 2 crowded rows along each radius; tentacles 10, much branched, 2 ventral ones smaller; calcareous plates symmetrical or irregular, in crowded groups in body walls, rod-like in tips of feet, small and irregular in tentacles; calcareous ring well developed, radial pieces with 2 long slender posterior prolongations (Figs. 40-42). Color white or pale gray; almost transparent.—Numerous, in about 35 fathoms, near Olga.

Genus **Stichopus**

Extremely large forms with large conical projections on the dorsal and lateral surfaces; freely moving.

1. **Stichopus californicus** (Stimpson) Edwards.

Forms very large, sometimes 50 cm. long, almost cylindrical; ventral sole slightly developed; tube-feet many, in longitudinal rows, on ventral



M. Bush. PLATE 11

Fig. 56. Plates from calcareous ring of Stichopus californicus. × 4.

Figs. 57, 58. Calcareous deposits from body wall of Stichopus californicus. × 258.

Fig. 59. Calcareous deposit from tube foot of Stichopus californicus. × 258.

Figs. 60, 61. Calcareous deposits from tentacles of Stichopus californicus. × 258.

sole only; upper surface marked by large conical dermal projections; mouth terminal; tentacles 15, much branched; color dark red.—Numerous, in shallow and deep water.

Genus Psolus

Small, chiton-like forms with a well-developed ventral creeping sole; dorsal surface without ambulacral appendages, often with calcareous scales.

1. Psolus chitonoides Clark.

Small, about 30 mm. long and 20 mm. wide, chiton-like; dorsal surface arched, covered with many firm granulated plates; these plates varying in size, largest ones on upper part; dorsal ambulacral appendages none; mouth dorsal, anterior, surrounded by 10 tentacles; ventral sole well developed, depressed below edge of shell; tube feet arranged in 2 to 4 rows around the margin of the sole, a fairly well defined double row down the middle of sole, and a single row of smaller imperfect tube feet close to the margin on the under side of the marginal dorsal plates. Color red.—Not numerous, at tide line.

ORDER APODA

KEY TO GENERA

A. Worm-like; semi-transparent.

B. Calcareous deposits in form of anchors and plates, not forming dots on skin. LEPTOSYNAPTA (p. 41)

BB. Calcareous deposits in form of wheels, forming dots on skin. CHIRIDOTA (p. 42)

AA. Not worm-like, body tapering to a tail; not transparent. CAUDINA (p. 42)

Genus Leptosynapta

Forms small, delicate, worm-like, skin almost transparent showing muscle bands and organs; tentacles 10 to 13, pinnate, with 3 to 7 digits on each side; calcareous deposits of various shapes in body-wall and tentacles. Burrowing in mud or sand at tide line and in deep water.

1. Leptosynapta inhaerens (O. F. Müller) Verrill.

Holothuria inhaerens O. F. Müller; *Chiridota pinnata* Grube; *Synapta inhaerens* Rathke; *Synapta duvernaea* Quatrefages; *Holothuria flava* Rathke; *Synapta henslowana* Gray; *Synapta tenuis* Ayres; *Synapta ayresii* Selenka; *Synapta gracilis* Selenka; *Synapta albicans* Selenka; *Letosynapta girardii* Verrill; *Leptosynapta tenuis* Verrill.

Small, 40 to 100 mm. long; white or yellowish with perhaps a reddish tinge; tentacles 12, with 5 to 7 pairs of digits pinnately arranged; calcareous deposits rod-like, dumb-bell-shaped or rounded in tentacles, anchors attached to perforated plates in body wall; calcareous ring well devel-

oped with no projections on either side, the radial plates with a central perforation for the radial nerves (Figs. 37-39.)—Numerous, in the sand at False Bay and at Argyle.

Genus **Chiridota**

Forms medium, about 10 to 12.5 cm. long, delicate, worm-like; skin almost transparent; tentacles 12, with 3 to 10 digits on each side, the terminal pair of digits longest; calcareous deposits in body wall in the form of 6-spoked wheels, in tentacles in the form of bent rods. Usually found in rather deep water.

1. **Chiridota laevis** Fabricius.

Holothuria pellucida Vahl; *Trochinus pallidus* Ayres; *Chiridota tigillum* Selenka; *Chiridota typica* Selenka; *Chiridota abyssicola* von Marenzeller.

Forms medium in size, 12 cm. or more long; tentacles 12, with 5 digits on each side; calcareous wheels forming papulae in rows on the 3 dorsal interradii, fewer scattered ones appearing on the ventral interradii (Figs. 35, 36). Color pink.—Few, at 35 fathoms, near Olga.

Genus **Caudina**

Cylindrical, tapering to a tail; tube feet none; tentacles 15, cylindrical or with paired digits; calcareous ring stout, radial pieces bifurcated posteriorly; calcareous deposits various. Burrowing in mud or sand, the caudal tip exposed.

1. **Caudina obesacauda** Clark.

Body stout, about 7 cm. long and 25 mm. wide; tail not abruptly narrowed, about a third the body-length; tentacles 15, each with 4 sharply-pointed digits; calcareous ring stout; radial pieces with short slightly bifurcated posterior prolongations (Fig. 45); calcareous deposits in form of tables or enclosed cups. Color purplish brown.—Not numerous, at 35 fathoms, near Olga.

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GLOSSARY

Abactinal. Upper; opposite actinal.
Acervate. Clustered; heaped.
Adambulacral. Close to ambulacral groove.
Areolate. Divided into large areas.
Brachiolaria. Larval form of starfish.
Capitate. With distinct head.
Carinate. Keel-shaped.
Clavate. Club-shaped.
Cryptozonate. Having hidden zones.
Diplacanthid. Having 2 spines to a plate.
the valves.
Forcipulate. Forceps-like.
Foraminate. Having sessile valvular pedicellariae with a pore between

Forficulate. Shears-like.

Fossate. Having valvular pedicellariae with pits or fossae into which the valves fit when wide open.

Gonocidium. Pouch for protection of young in genus Pteraster.

Imbricated. Overlapping like shingles.

Interactinals. Spines between the adambulacrals and lower marginals.

Monacanthid. Having 1 spine to a plate.

Osculum. Opening in gonocidial membrane thru which water is expelled.

Ossicles. Calcareous plates which make the skeleton of a starfish.

Papillae. Columnar or hour-glass-shaped ossicles with isolated, circular bases and bearing at summit a group of small divergent spines.

Papulae. Thin projections thru the body wall used in respiration.

Paxilliform. Like paxillae.

Pedicellariae. Modified spines; pincer-like calcareous structures consisting of 2 or more blades articulated to a plate in the dermis and capable of a snapping movement.

Peractinal. A row of spines next to the lower marginals.

Phanerozonate. Having a perpendicular margin formed by the junction of the 2 rows of marginal plates.

Protopaxillae. Conical plates covered with rounded granules.

Pseudopaxillae. Articulated plates with flattened, lobed base, and bearing spinules on a central elevation.

Reticulate. With lace-like meshes.

Sub. Almost or somewhat.

Sulcate. Furrowed.

Synactinal. A row of spines next the adambulacrals.

Tesselated. Mosaic-like.

Triplacanthid. 3 spines to a plate.

Truncate. As if cut off at tip.

Unguiculate. With a claw; as tipe of valves.

Early Development of Haminea

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INTRODUCTION

In this paper, which can be at best only a fragmentary account of some of the very early phases of development, my purpose has been to set forth some of the pertinent facts of early development as observed in *Haminea vesicula*, a species of opisthobranchiate mollusk; and to discover, if possible, wherein lay any significant similarities or differences in development between that and the other molluscs of the same class whose embryology has been studied.

Haminea vesicula is a marine Gasteropod of the order Opisthobranchiata. It is found in profusion in the waters of Puget Sound. The material used in this study was obtained at the Puget Sound Biological Station during July and August, 1916. The work was done entirely from preserved material. The eggs were killed in corrosive-acetic solution, stained in acidulated Delafield's haematoxylin according to Conklin's method, and mounted in balsam, under a cover-slip supported on glass rollers to permit of rolling the egg at will after mounting.

HABITS

The habits of *Haminea vesicula* during July and August are those characteristic of the breeding season, since these months cover a period of great reproductive activity. I have been unable to observe the habits during a period of reproductive quiescence. During July and August it is littoral, appearing on the eel-grass and *Ulva* just below the low water mark. The writer has gathered large numbers of the animals and of their characteristic egg-masses from the under side of the *Ulva* at all times of day. Eggs in the 1-celled stage and apparently freshly laid were obtained most plentifully between the hours of 5 A. M. and 9 A. M., and *Haminea* is frequently found in the act of laying between these hours. However, one of the animals was found laying as late as 11:40 A. M., and continued laying in the laboratory until 2:45 P. M.

Frequently an individual was found laying on a comparatively isolated piece of eel-grass or *Ulva* on which were as many as 7 other egg masses, all containing eggs in the 1-celled stage. No other adults were present in the immediate vicinity. The question arises whether these egg

masses were all laid by one individual. That a given individual may stop laying and begin a new mass of eggs on the same piece of eel-grass or *Ulva* is indicated by observations on a female found at 11:20 A. M. laying on a piece of *Ulva* bearing seven other egg-masses. She stopped laying when disturbed by being put into a pan. When brought into the laboratory she was again laying within 40 minutes, and on the same piece of *Ulva*. By 12:45 P. M. she had laid a band about 1½ cm. long; by 2:45 P. M. she had laid about 4½ cm. in all. Laying then ceased. What in the natural habitat might cause cessation of laying, and again renewal at a different point on the same *Ulva,* remains unanswered.

In the process of laying the eggs are extruded in a flat band about 1 cm. in width from beneath the right side of the mantle, and pushed back so as to extend posteriorly. During this process *Haminea* is moderately extended. At intervals definite contraction of the mantle on the right side occurs, at which time two or more strands of ribbon are pushed out. Due apparently to a slow spiral motion of the female while in the act of laying, the egg-masses are spirally coiled when of sufficient length.

Each egg is enclosed in a comparatively large capsule, containing a transparent fluid which does not become opaque upon fixation. These capsules are embedded in a clear, transparent, colorless or pale yellow jelly, which is in the form of a flat band about 1 cm. in width and varying from a fraction of a centimeter to 6 cm. or more in length. The bands are attached by their lower border to the substratum. The eggs vary in diameter from 50 to 90 microns and are embedded in the gelatinous band in a continuous spiral line. The eggs when collected in their masses will live and develop normally for several days under laboratory conditions.

THE UNSEGMENTED EGG

Fertilization is internal, hence the eggs when collected had already been fertilized. None were found at a stage previous to the formation of the first polar body, and none were dissected out of the oviduct to ascertain whether the first polar body is formed before extrusion. No unfertilized eggs were found, and it is a question whether any are laid.

In surface view the egg is bright yellow, with opaque yolk granules scattered thruout. After the egg is laid the second polar body is thrown off by the formation of a typical polar spindle (*Figs. 1 and 2*). The male and female pronuclei approach until closely pressed together (*Fig.* 3). No trace of the germ path was discovered in the sections at this stage, which show the deutoplasm spheres densely distributed thruout except for an increasing clear area surrounding the polar region. There follows a comparatively rapid shifting of the yolk material, so that by the forma-

tion of the first cleavage spindle the zonal arrangement has become decidedly marked.

FIRST CLEAVAGE

It was not observed how long after fertilization or extrusion the first cleavage occurs. The first cleavage spindle forms at right angles to the plane of the polar spindle, at a point a little more than ¼ the diameter from the animal pole (*Fig. 4*). The zonal distribution of the yolk material is marked even in the unstained specimen, the vegetal hemisphere being dense and opaque with the aggregation of deutoplasm spheres, the upper hemisphere clear except for a fine granular network which runs throughout the egg.

The cleavage of the cytoplasm begins as a broad depression at the animal pole, gradually extending around the entire egg and becoming deeper and narrower until separation is complete (*Fig. 5*). As cytoplasmic division begins, the spindle apparently rotates slightly so that the end which is to enter the smaller of the two daughter cells lies higher than the other, thus indicating a spiral trend of cleavage. Since my work has all been done on preserved material I have not been able to observe the process of this rotation, but preparations of the earlier and later stages of the first cleavage indicate clearly that such a rotation does take place after the formation of the first nuclear spindle (*Figs. 4, 5*). The cleavage is unequal, dividing the egg, in the nomenclature of Conklin (1897), into the two cells AB and CD (*Fig. 6*). Thruout this paper I shall use the nomenclature of Conklin as the clearest and the most applicable to the situation as found in *Haminea*.

Of the two cells produced by the first cleavage, the smaller one (CD) is the posterior; the larger (AB) the anterior. In the inequality of the first cleavage *Haminea* agrees with *Umbrella* (Heymons 1893) and *Philine* (Guiart 1901); *Crepidula* (Conklin 1897), *Fiona* (Casteel 1904), *Tethys* (Viguier 198), and *Planorbis* (Holmes 1900), have on the contrary an equal first cleavage. Following the cleavage the cells round into two nearly perfect spheres, and the two nuclei return to the resting condition, lying on a perpendicular bisector of the first cleavage furrow when the animal pole is directly up (*Fig. 6*). This position they appear to keep during the subsequent flattening against each other of the blastomeres, and in fact until the formation of the spindles for the next cleavage (*Fig. 7*). Conklin (1897) finds in *Crepidula* that "at the close of the first cleavage the nuclei, asters, and archoplasmic bodies lie opposite each other in the blastomeres; but as soon as the blastomeres begin to flatten against each other, and the whole egg assumes a more compact form, all these structures move in the direction of a clock's hands. This movement

of the nuclei and asters takes place invariably in the same direction, and it must therefore have been predetermined during, and perhaps before, the first cleavage. In *Crepidula* the first spindle does not seem to indicate any such rotation, though it is exceedingly significant to note that Warneck (1850) in the case of *Limax* and Fol (1875) in *Cymbulia* found that the first cleavage was oblique to the line of elongation of the egg. Kofoid (1895), however, in his recent careful work found no evidence in favor of Warneck's account."

In the preserved material in *Haminea* the writer finds no indication of any such rotation of the nuclear structures after the first cleavage is completed; it is, however, possible that in the living egg some such movement might be observed. There is no evidence of the occurrence of this phenomenon in *Fiona* Casteel (1904); Heymons (1893) and Viguier (1898) make no mention of its occurrence in *Umbrella* and *Tethys*. Definite rotation of the first nuclear spindle in *Haminea* gives further support to the conclusion reached by Conklin that "in all cases in which the second cleavage is laeotropic the first is dexiotropic, and that the initial cause of the spiral cleavages is not to be found in the direction of the nuclear spindles but rather in the structure of the unsegmented egg itself."

SECOND CLEAVAGE

As the spindles for the second cleavage form at right angles to the plane of the first, the two cells flatten against each other and each elongates (*Fig. 8*). The spindles do not lie in the same plane, but are slightly inclined, so that when seen from the side they appear to cross each other slightly. This cleavage consists in reality of two rapidly succeeding cleavages (*Fig. 8*), the division of the smaller, posterior blastomere (CD) slightly preceding that of the larger, anterior (AB). The furrows of the second cleavage, like those of the first, proceed from the animal to the vegetal pole, dividing each of the two cells equally. These furrows do not meet in a point at either pole, but at the upper pole each proceeds from a point a little to the left of the polar bodies when observed from the center of the egg; at the lower pole each furrow meets the first cleavage furrow at a point still further removed to the left (*Fig. 9*). Thus the cleavage is diagonal and not meridional, and clearly indicates laeotropic spirality, in that from B and D, two new cells, A and C, are cut off in an anti-clockwise spiral. As is the rule for all known cases of dexiotropic cleavage, the lower, or ventral, cross-furrow, formed between the points of juncture of the first and second cleavage, bends to the right when viewed from the animal pole along the first cleavage furrow. The upper cross furrow is parallel to the lower, but much shorter, at times being almost indistin-

guishable. Of the four blastomeres (A, B, C, D) formed by this cleavage, B and D meet in the cross-furrow at both poles; the other two (A and C) being entirely separated.

The 3-celled stage produced by the difference in time of cleavage of AB and CD, which is typical of *Umbrella* (Heymons 1893), and of *Tethys* (Viguier 1898), does not appear in *Haminea*. Heymons finds in *Umbrella* and Viguier in *Tethys* a typical cross-formation in the 4-celled stage, in that the two cells homologous with A and C (*Fig. 9*) characteristically lie above the other two cells B and D. Warneck (1850) reports the same phenomenon for *Limnea* and Lang (1884) for *Discocoelis tigrina*, in which the two smaller blastomeres lie above the two larger. This situation does not occur in *Haminea*, although it would at first appear so in viewing the egg from the animal pole, on account of the diagonal direction of the cleavage planes which cut the blastomeres essentially into an upper and a lower moiety, that is, so that the cells A and C overlap the cells B and D. That they do not actually lie above them as in *Umbrella* and *Tethys* is demonstrated in a side view of this stage (*Fig 10*), in which it may be clearly seen that all four cells touch the same plane at both upper and lower poles.

THIRD CLEAVAGE

The third cleavage is the first segregating cleavage, separating four protoplasmic micromeres from the four yolk-laden macromeres. The spindles lie with the inner ends higher than the outer, and vary from a nearly radial (*Fig. 11*) to a markedly spiral (*Fig. 12*) position. This is in close agreement with *Crepidula* (Conklin 1897) in which the spindles are "usually nearly radial in position, though frequently slightly inclined in a right spiral and occasionally even in a left spiral." Whether, as in *Crepidula*, there is a dexiotropic rotation of the upper poles of the spindles during division has not been determined. The most markedly arranged spindles were found in the earliest stages of division (*Fig. 12*). The posterior cells, C and D, tend to precede A and B slightly in the formation of the spindles (*Fig. 12*), altho cell division appears to be practically synchronous.

The cleavage is dexiotropic and unequal (*Fig. 13*). The four micromeres (*1a, 1b, 1c, 1d*) formed by it come to rest in the furrows between the macromeres (A, B, C, D), their contours becoming modified to fit more closely between the cells (*Fig. 14*). Whether the dexiotropic rotation of the micromeres or any part of it is accomplished after division is complete the writer has been unable to determine on the material at hand; but there is evidence of the rotation continuing after complete separation, since in no instance has a spindle been found having a dexiotropic inclina-

tion such that a cleavage plane passing perpendicularly to it would cut off a micromere in the position in which it would come to rest at this stage. That this might be the case seems probable if we consider the rotation as due to a seeking of equilibrium of position. Viguier (1898) takes this point of view concerning both the cause of rotation and the cause of the typical alternation of cleavages. He considers the spiral inclination of the nuclear spindles, however, to be the result of an intensification by heredity of the movements caused by the equilibrating forces. He says:

"On peut admettre que, primitivement, cette rotation des micromeres ne s'effectuait qu'apres la fin de la division. Devenue héréditaire, elle commence d'une façon plus précoce, ainsi que le montre le changement de direction de fuseau nucleaires; mais elle ne peut cependant s'achiver que lorsques la division est complete. . . . Me parait très compréhensible en admettant que tant cela résulte des changements de position détermines par les lois de l'equilibre, et qui, par hérédité accélérée, apparaissent avant la séparation complète des cellules."

A cross-furrow may appear in the upper quartet, but it is inconstant. The micromeres contain none of the dense yolk material and are almost clear when mounted; whereas the macromeres are dense with deutoplasm. Because there is a markedly smaller amount of yolk present in the eggs of *Haminea* than in those of *Crepidula* the contrast in the size of the macromeres and micromeres is less marked than in *Crepidula,* the situation being essentially the same as in *Tethys* and *Umbrella.*

FOURTH CLEAVAGE

The fourth cleavage consists of the laeotropic division of both quartets synchronously, giving rise to 16 cells, 12 micromeres and 4 macromeres (*Fig. 16*). From the upper or first micromere quartet arise four smaller cells ($1a^2$-$1d^2$) which Conklin has termed the turret cells. The four remaining micromeres of the first quartet are now termed $1a^1$-$1d^1$ in accordance with Conklin's nomenclature. From the four macromeres arise the second quartet of micromeres (2a-2d), smaller than their parent macromeres but larger than the micromeres of the first quartet (*Fig. 16*).

In *Nereis* the first quartet divides once at the time the second is being formed, and before the third is formed it divides again. In *Crepidula, Tethys, Planorbis,* and *Fiona* it divides once before the third is formed, and in *Umbrella* it does not divide at all before the formation of the third. Of this Conklin (1897) says: "In general, the rate of development of the upper hemisphere is indicated by these facts; in *Nereis* the development of the upper hemisphere is very precocious; it is very tardy in *Umbrella;* while *Crepidula* occupies an intermediate position in this re-

speet." If this comparison is applied to *Haminea,* in which the first quartet divides onee simultaneously with the formation of the second and again between the stages of 24 and 29 cells, as it does in *Crepidula,* we may expeet it also to be intermediate in the rate of development of the upper hemisphere.

The second quartet of mieromeres come to rest in the furrows between the macromeres produeing them, each in the furrow to the right of the macromere produeing it, thus pushing the cells of the first quartet baek to a position directly above the macromeres which produeed them. The turret cells lie in the furrows between the cells of the second quartet and thus almost directly over the maeromeres (*Figs. 16, 17*). Conklin says: "In this case as in every other which I have observed, the spiral eharaeter of the eleavage is much more pronounced after the nuclear division than during that division. It seems to be a phenomenon belonging to and caused by the eytoplasm rather than the nueleus."

FIFTH CLEAVAGE

The two divisions whieh eonstitute the fifth eleavage divide the macromeres, A-D, and the second quartet of micromeres, 2a-2d, dexiotropieally (*Figs. 17, 18*). The division of both quartets is essentially equal, the mieromere quartet giving rise to a lower moiety, $2a^2$-$2d^2$, and an upper moiety, $2a^1$-$2d^1$; the maeromeres giving off the third quartet of mieromeres, 3a-3d. Division in the micromere quartet tends to precede slightly that in the macromeres (*Fig. 17*).

At the close of this eleavage the egg consist of 24 eells: 8 cells ($1a^1$-$1d^1$ and $1a^2$-$2d^2$) products of the first quartet; 8 cells ($2a^1$-$2d^1$ and $2a^2$-$2d^2$) products of the seeond quartet; the 4 cells of the third quartet; and 4 maeromeres. The cells of the third quartet, which are about equal in size to those of the seeond, lie in the furrows between the macromeres, eaeh to the right of the maeromere produeing it. The lower moiety of the seeond quartet lies just to the lift of the mid-line of the macromere beneath, the upper moiety lies just to the right. At this stage, as in the earlier stages, the cells of the *Haminea* egg do not flatten against eaeh other as do those of *Planorbis* (Holmes 1900) or of *Crepidula* (Conklin 1897), although this phenomenon is less marked in *Crepidula* than in *Planorbis.* A side view of the *Haminea* egg at this stage shows eaeh of the clear protoplasmie eells fully rounded on the free surfaees and flattened only where in actual eontact with an adjoining eell.

SIXTH CLEAVAGE

Following the division resulting in the 24-eelled egg the cells of the first quartet again divide dexiotropieally, giving rise to 4 small eells below,

which lie between the cells of the second quartet, these latter 8 cells having now come to lie nearly on a level.

Either just preceding or just subsequent to this division the posterior macromere (D) divides laeotropically giving rise to a cell (4D) which is apparently homologus to that found in *Crepidula* (Conklin 1897) and *Umbrella* (Heymons 1893). A 29-celled stage thus occurs, but the writer has not been able thus far to follow accurately the steps in its formation, nor to determine the time relation between the formation of 4D and the second division of the first quartet; hence the cell 4D is not included in Fig 20 in which 28 cells appear.

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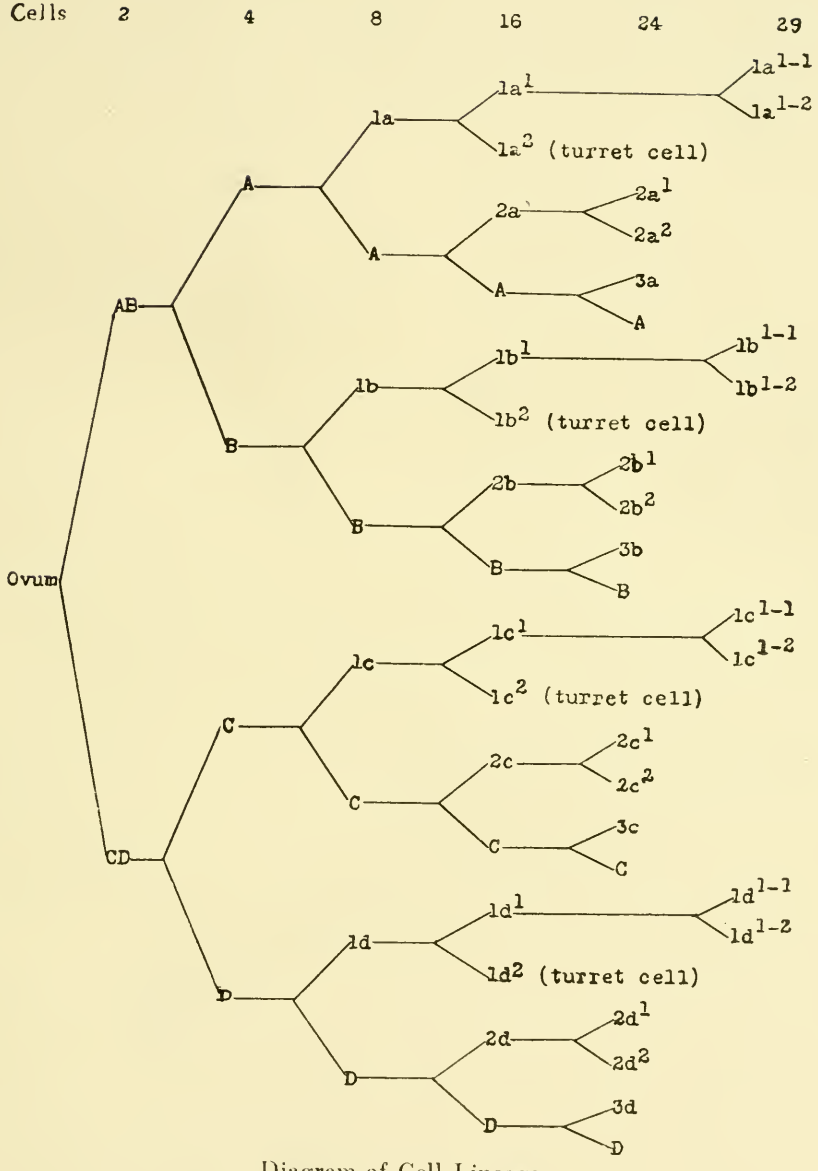


Diagram of Cell Lineage.

The figures have all been drawn with a camera lucida from preserved material. Figures 1 and 2 are from sections stained in iron haematoxylin; the rest from whole mounts of which figures 6, 9, 13, 17 and 20 are from tissue stained in acidulated Delafield's haematoxylin, the others from unstained tissue. The drawings are all × 515. The lettering of the plates is mostly explained by the diagram of cell lineage.

PLATE 12

cap., egg capsule.

egg n., egg nucleus.

f. p. n., female pronucleus.

m. p. n., male pronucleus.

p. b., polar body

p. n., pronucleus.

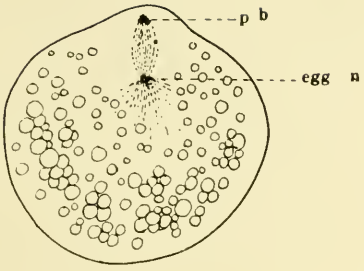
Fig. 1. Late anaphase in polar body formation on *Haminea*.

Fig. 2. Telophase in formation of second polar body. Male and female pronuclei have not yet approached. The long astral rays are characteristic. The arrangement of the yolk spheres indicates a cone-shaped clear protoplasmic area below the polar bodies.

Fig. 3. The resting 1-celled stage. Male and female pronuclei closely appressed. The large egg capsule is shown surrounding the egg. Zonal distribution of the yolk is beginning.

Fig. 4. The first cleavage spindle lying at right angles to the polar axis. Zonal distribution marked.

Fig. 5. Beginning of cytoplasmic cleavage. The spindle has rotated to a diagonal position.



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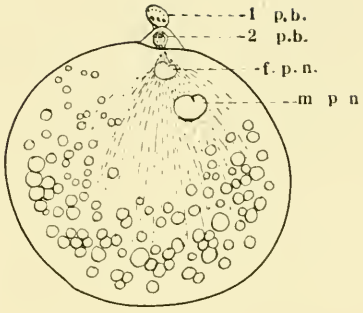

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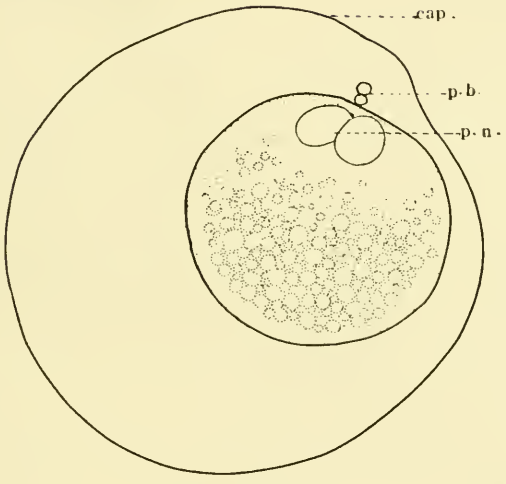

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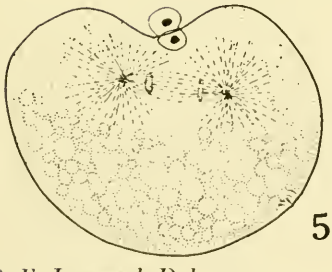

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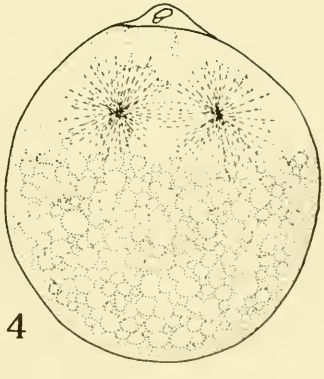

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R. E. Leonard, Del. PLATE 12

PLATE 13

For abbreviations see table of cell lineage.

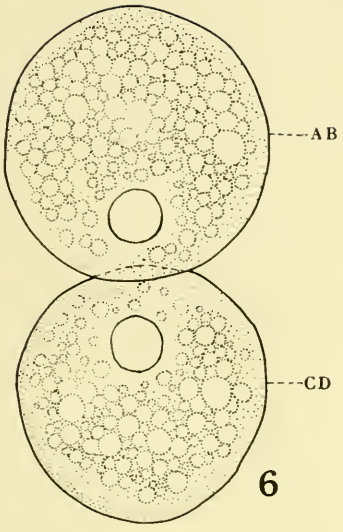
Fig. 6. Resting 2-celled stage immediately following cleavage.

Fig. 7. Resting 2-celled stage a little later than that in figure 6. The cells have begun to flatten against each other and the whole egg to become more compact.

Fig. 8. Formation of the spindles for the second cleavage showing the slight lead of CD. The left end of each spindle lies higher than the right, indicating laeotropic cleavage.

Fig. 9. Completed 4-celled stage, showing the overlapping of A and C over B and D.

Fig. 10. Same stage as Fig. 9 seen from the side. All four cells touching the same plane at their highest and their lowest points.



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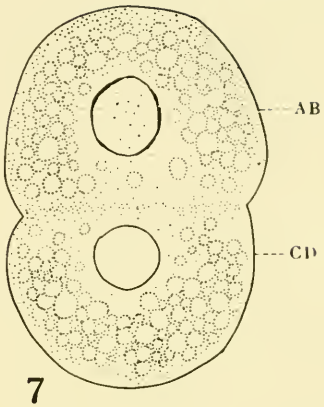

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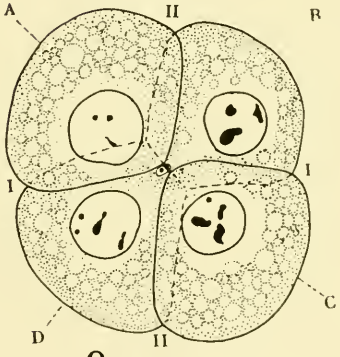

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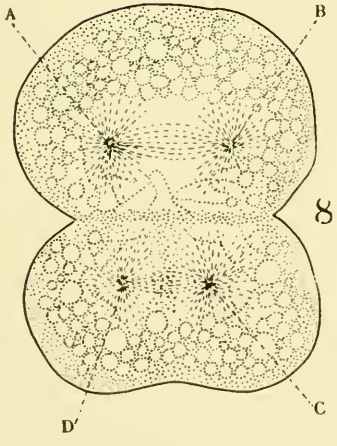

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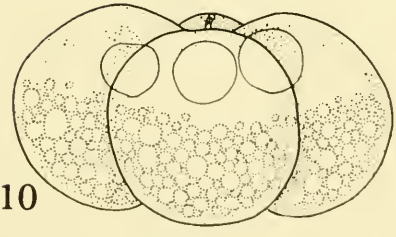

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R. E. Leonard, Del. PLATE 13

PLATE 14

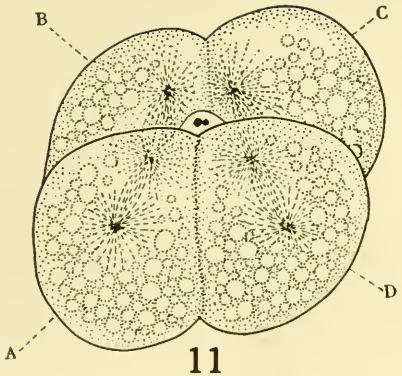
For abbreviations see table of cell lineage.

Fig. 11. Radial formation of spindles for third cleavage.

Fig. 12. Formation of third cleavage spindles in dexiotropic spiral; C and D preceding A and B.

Fig. 13. Resting 8-celled stage.

Fig. 14. Similar stage as Fig. 13 seen from the side showing modification of contour of cells in increased compactness of egg.



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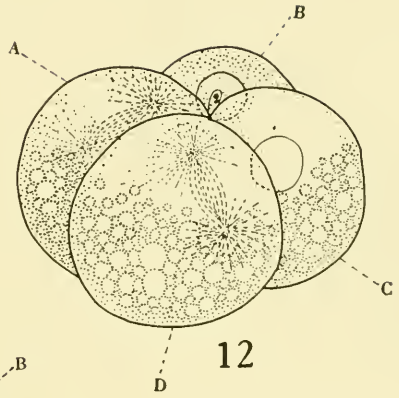

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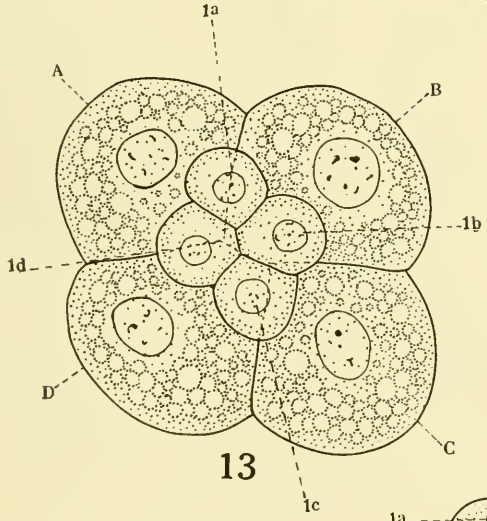

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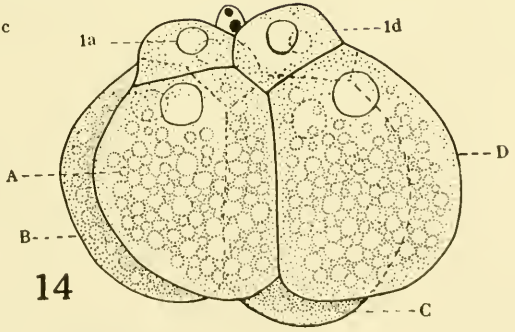

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R. E. Leonard, Del. PLATE 14

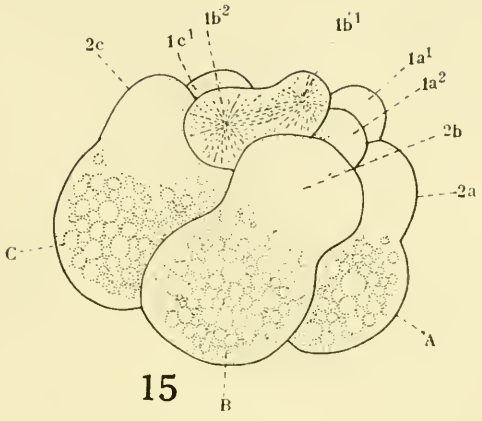
PLATE 15

For abbreviations see table of cell lineage.

Fig. 15. Simultaneous laeotropic division of macromeres and first quartet; 8 to 16 cells.

Fig. 16. Sixteen cells completed. Division of first quartet not quite synchronous.

Fig. 17. Formation of dexiotropic spindles for fifth cleavage* 16 to 24 cells. The cleavage in the second quartet is slightly in advance of that in the macromeres.



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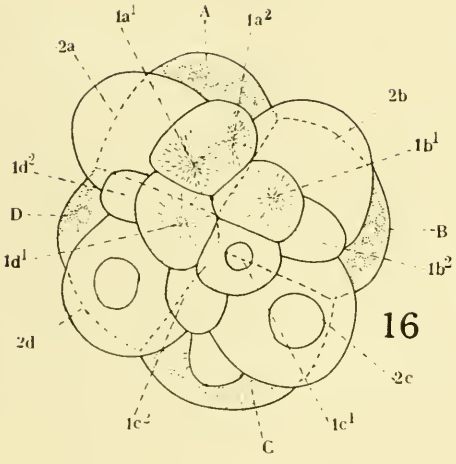

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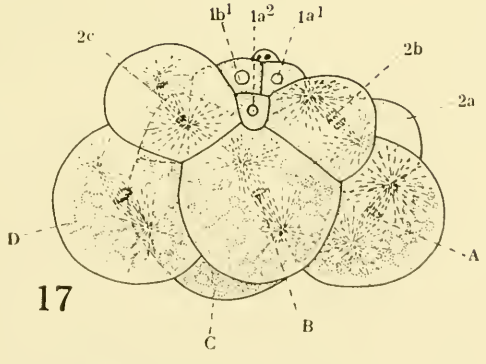

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R. E. Leonard, Del. PLATE 15

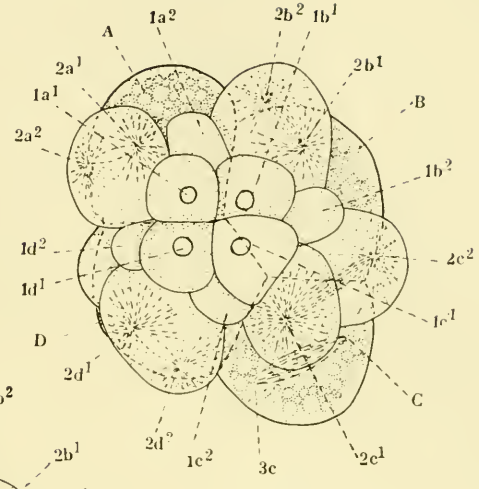
PLATE 16

For abbreviations see table of cell lineage.

Fig. 18. Similar stage as Fig. 17 seen from the top.

Fig. 19. Twenty-four cells completed.

Fig. 20. Resting stage following the second division of the first quartet; 28 cells.



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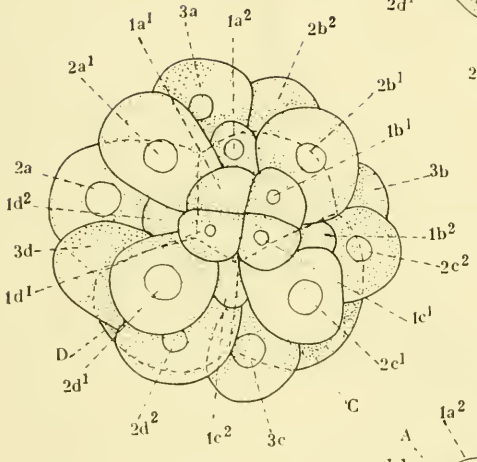

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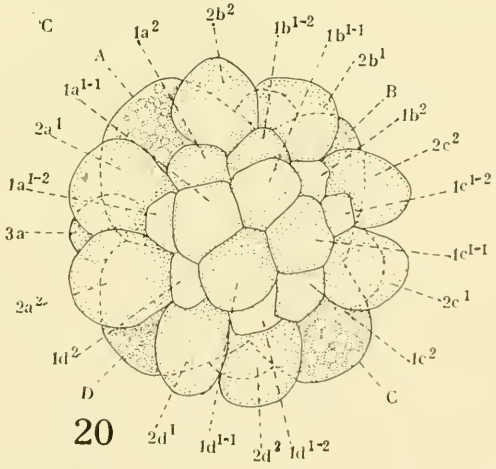

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R. E. Leonard, Del. PLATE 16

The Age of Pterygophora californica

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University of Washington, Seattle

The growth rings of *Pterygophora californica* have been known for 70 years. Ruprecht (1848) first called attention to them. Areschoug (1884) calls attention to the fact that they are not easily counted since some of them are often faint. MacMillan (1902) has gone rather fully into their structure. Setchell (1912) refers to similar rings in Laminaria as "annual(?)" rings; but whether each ring represents an annual growth or not has not been settled. The question becomes the more interesting when one considers that the work of Sauvageau (1915-1917) and of Kylin (1916) seems to indicate that at least in some of the Laminariaceae the conspicuous plant body is a sporophyte generation and therefore homologous with the sporophyte of the pteridophytes and spermatophytes.

Pterygophora californica is one of the Puget Sound algae showing the rings about as clearly as any in the region. Thus, with a view to determining their significance, material was gathered at Blakeley Island and at Cape Flattery, both in Washington. Since MacMillan (1902) has already pointed out that the rings are partly due to different material and partly to cell form, the structure of the rings does not form a part of this paper, except that a section of a ring in a haptere is shown (Fig. 5).

So far as the writer has been able to find there is no literature on the age of perennial algae. If the rings of Pterygophora are annual growth-rings similar to those of trees, we have a means of estimating the age of the plants fairly accurately; but the method would help only in a few genera of kelps.

Winter plants of Pterygophora in Washington are mostly bladeless stalks, even the terminal blade dying back to near its base. Occasionally, however, leaves persist thru the winter. The summer plants are again leafy. Thus the number of leaves present in the fall indicates roughly the number grown that year. Sometimes blades are lost thru injury, but their scars remain. Sometimes leaves persist throuout the winter, but familiarity with the new ones in the early summer enables one to recognize the old ones even in the fall of their second year.

Each leaf when it disappears leaves a trace on the stalk (Fig. 9, l). On quite old stalks the basal traces are often obliterated, thus making it

impossible to count them. Often individuals only 6 or 7 years old are found on which some of the leaf-traces are no longer discernible. But some individuals show the marks with a dependable degree of certainty.

If the plant grew roughly the same number of leaves per year after the first year, one could count the years back along the stem by the leaf-traces, perhaps adding one for a first year with only 1 blade. Just how old the plants are when the first lateral leaves appear is uncertain. However, on the first of August there was still quite an abundant crop of small plants without lateral leaves, altho a few showed signs of them (Figs. 3 and 4, b). Table 1 gives the results of observations made in the manner described.

Table 1. *Showing number of rings, and the estimated age by the number of leaves and leaf-traces.*

Leaves	Leaf-traces and Leaves	Estimated age by number of leaves	Rings at 25 mm.
20	230	13+	13
11	84	10—	8
18	81	6+	6
13	72	8—	6
13	117	11	11
17	142	10+	11
11	40	6—	5

Thus the age figured by the leaves and leaf-traces roughly corresponds with the number of rings at 25 mm. from the base.

The number of rings figured from the leaves is more on the average than the number of rings counted. This may arise: (a) Thru indistinct rings; some might have been missed in the counting. (b) Thru the number of leaves not being constant; in fact it is not likely that the number of leaves per year is constant. (c) Thru the counting of the leaves in July, when probably not all had yet been formed. The fractional years figured and shown as + or — in table 1, are well within the limit of possible error, and are likely due largely to variation in the number of leaves annually formed. The number of rings corresponds closely enough with the age figured by the number of leaves, to suggest that the rings are annual ones like those of trees, and not successive during 1 year, like those of beets. The evidence is not conclusive, however.

Further, cross sections through the upper part of the stalk show that there is only 1 ring among the lateral leaves of the current year. The second growth ring begins very near the point between the upper old lateral leaves and the lower new lateral ones. The faintness of the rings makes the exact spot difficult to determine. A longitudinal section

shows the same thing, but the exact spot is even more difficult to fix in such a section. This could only signify that the rings are formed 1 per year.

A third evidence is the fact that in June and July the outer ring is thin and evidently incomplete (Fig. 6), but not so in November. This might be true of 1 kelp or of several but hardly of all, unless the ring is conceived to be only partially completed. The ocean is much more uniform in growing conditions than the land. There are no marine dry years to affect kelp, as there are on land to affect trees.

Thus it appears that 3 different lines of observation all point in the same direction; viz., to annual growth rings in *Pterygophora californica.*

How old these kelps get is not yet certainly known. The writer has found none over 5 cm. in diameter at 2.5 cm. from the base. The maximum number of rings counted was 21. MacMillan (1902) reports some stalks 7.5 cm. in diameter, without stating the number of rings. The maximum number of rings reported in Pterygophora by MacMillan (1902) is 24, in a kelp stalk 5 cm. in diameter. We have thus fairly good evidence that certain kelps live for nearly a quarter of a century.

A peculiarity, tho unrelated to the facts above, should be noted. One plant was found which had its central blade split through the growing region about 3 or 4 years before examination and one of the parts again 1 year before examination (Fig. 9). Half the plant, from the original split, formed blades on both sides after a time. The other half did not. The region of blade formation is evidently not fixed at the original margins. The half which formed blades on 1 side only had its rib near one side (Fig. 9, right side); the other half split again, but one of the parts had a central rib.

Also a plant was found growing upon another (Fig. 7). The holdfast of the epiphyte (Fig. 7, e), evidently induced the formation of hapteres in the other at this point about 6 cm. up the stalk (Fig. 8). Possibly contact induces the formation of holdfasts in abnormal positions. The writer observed a similar thing in *Nereocystis luetkeana* in 1913 (Frye, 1915). In that case, which was photographed but not explained, the holdfasts were believed to be due to a wrapping of filamentous red algae.

Certain other kelps show growth rings. *Alaria tenuifolia* grows abundantly at Friday Harbor, Washington. Old stalks show growth rings. Some of these were cursorily examined in connection with this work. *A. tenuifolia* on the rocks near low tide showed in the oldest looking specimens 1–4 rings near the base; but gradually fewer upward on the stalk, until there was only 1 at the top. Near by, a floating dock had fine large *A. tenuifolia* on it, but not one among a dozen tried con-

tained more than 1 ring. However, the dock had been in the water only 1 year. From this is appears that the rings in *A. tenuifolia* are also annual.

Nereocystis luetkeana showed no rings in the second year of growth. It has never been found older than that with certainty.

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PLATE 17

Fig. 1. Young plant. x 1.2.

Fig. 2. Young plant. x 1.2.

Fig. 3. Young plant; *b,* beginning of first lateral blade. x 0.6.

Fig. 4. Young plant; *b,* beginning of first 2 lateral blades. x 0.6.

Fig. 5. Cross section thru annual ring of haptere; *r,* the region of narrower flattish cells. x 330.

Fig. 6. Diagram of cross section of a stalk near its base showing the narrower outer ring, July 10. x 1.2.

Fig. 7. Plant, *a,* with top cut off, and a second plant, *c,* growing upon it; *s,* stunted branch. x 1.

Fig. 8. Longitudinal section thru the connection between the two shown in Fig. 7; both have formed hapteres, but there is no union. x 0.4.

Fig. 9. Split plant; right half forming blades on one side only; left half forming blades on both sides and continuing to do so after splitting again; *l,* leaf-traces. x 0.4.

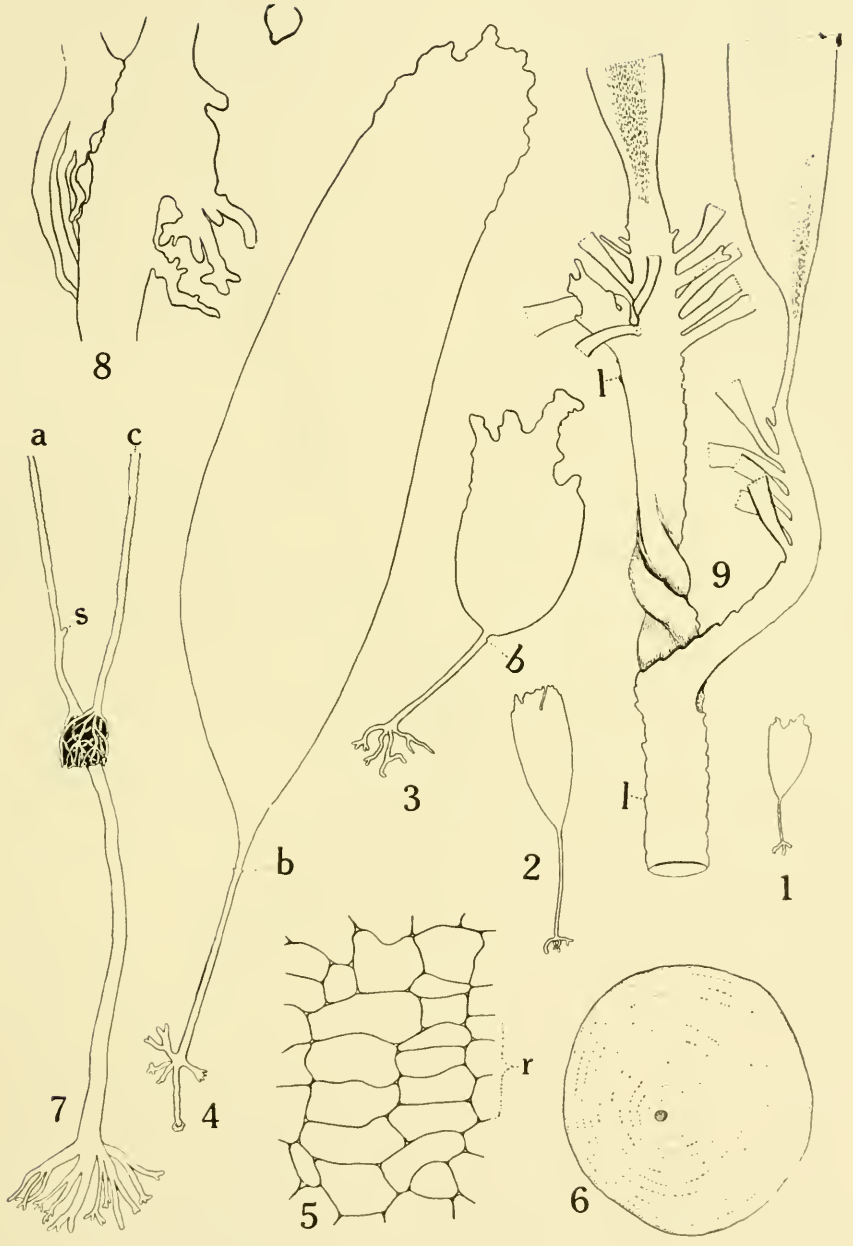

PLATE 17

Trout and Fish Lice

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Trout are attacked by a great many parasites. Those often met with in our streams and hatchery ponds are commonly known as fish-lice, and technically as copepods. These parasites often become so serious a pest in hatchery ponds that they kill thousands of fish. A knowledge of the life history of these animals is therefore not only desirable, but highly important from the standpoint of fish culture.

The fish-lice which attack the trout belong to the family Lernaeopodidae, the most highly specialized group of copepods. They spend nearly their entire life on the host, attacking such delicate structures as the gills (Figs. 1 and 2), fins (Fig. 5), and the membranes of the mouth (Fig. 3). They have a particular fondness for regions which are richly supplied with blood vessels, for the blood is their sole food. The adult females (Fig. 4) are found in greatest abundance on the fish and may be easily recognized. They are quite large, yellowish-white, and a few millimetres in length; they possess two cigar-shaped egg-sacs which dangle from the posterior end of the body. Within these egg-sacs the embryos mature and complete their life-cycle.

For a number of years the writer (Fasten, 1912-1916) has concerned himself with a fish-louse which attacks the brook-trout (*Salvelinus fontinalis*) found so commonly in the middle western states. Technically this parasite goes under the name of *Salmincola edwardsii* Olsson, and it has proved a serious stumbling block to trout culturists through many regions of the United States. In our own state of Washington the author has discovered a similar copepod (*Salmincola falculata* Wilson), which infests the steelhead trout (Figs. 3-5). This fish louse has been previously recorded by Wilson (1908, 1915) on the salmon of Washington and the trout of California, but was not known to exist on the trout of this state. The copepod was observed on some Washington steelhead trout (*Salmo gairdneri*) from Lake Samish, which were on exhibition in one of the aquaria in the state hatchery building at Seattle during the fall of 1914. It has since been seen on steelhead trout exhibited in various restaurants in Seattle. From all appearances the life histories of *Salmincola edwardsii* and *Salmincola falculata* are similar and will be briefly sketched.

The embryos found within the egg-sacs of the adult females hatch into perfect larvae, which are capable of swimming about freely in the water. These larvae are minute, about 1/35th of an inch in length, and

are very active. They swim about with a snappy, spiral dart. In this condition they may exist for about two days, constantly searching for a host to which to attach themselves. If the larva cannot find a host it soon dies; if it comes in contact with one it attaches itself and undergoes further development.

Each larva possesses powerful mouth parts and a peculiar attachment filament. When the louse comes in contact with a desirable portion of the fish, it first rasps a hole by means of its mouth parts. Then it brings the attachment filament in contact with this cavity and injects the mushroom-like knob into it. The attachment filament is supplied with a glue-like substance, which makes the organ adhere to the flesh of the host. Furthermore, the regenerating tissue of the host fastens the parasite more securely. The organism then begins to feed, increases enormously in size and undergoes degeneration.

In about two weeks after attachment, the copepod is ready for fertilization. The male is only about one-third the size of the female. When mature the male releases his hold on the fish, seeks out the female and attaches himself to the posterior extremity of her abdomen, near the reproductive organs. Here the male pastes a pair of elliptical pouches known as spermatophores. These are filled with spermatozoa, which become stored within the female's body and are later used for fertilizing the mature eggs. At the time of the ripening of the eggs two long cigar-like sacs are developed from the posterior margin of the female's abdomen. These are the egg-sacs; and it is here that the embryos undergo their complete development. It takes about a month for the young free-swimming larvae to hatch from these structures.

After fertilization the male dies, while the female lives on to complete the life-cycle. The female increases enormously in size, becomes very degenerate and produces a great many young. In *Salmincola edwardsii* two sets of young are developed, one lot containing about 120 embryos, whereas the second one is somewhat smaller in number. The female dies soon after all the eggs are liberated.

The naturally crowded conditions of the fish within the hatchery ponds makes it very easy for the fish-lice to find their hosts and the loss is therefore enormous. The young fish (Fig. 1) as well as the adults are here attacked, but it is mainly the adults which are parasitized. The adult fish are attacked by such numbers of the pests that they are ultimately killed. As many as 250 copepods may be picked off one trout. The injury produced in such cases is very great. The blood of the host is removed in enormous quantities; thus the fish is literally starved. The injury produced to the host when the parasite attaches itself causes the

flesh to swell and develop a large amount of scar tissue (Fig. 2), which interferes considerably with respiration. Bacteria and other organisms may make their way into the injured portions of the trout and cause infections of a serious nature. Taking all these facts into consideration, there is little wonder that fish succumb under the attacks of these parasites, particularly in hatchery ponds where conditions are just right for parasitism. In one Wisconsin hatchery the author found that in a single year about 12,000 adult trout out of 14,000 kept in outdoor ponds died from the attacks of these copepods.

Many states have had this trouble for years, with very serious losses. The writer has devoted considerable attention to the control of these parasites, and has recommended the following remedies in the state of Wisconsin. These may also be found of use in our own hatcheries.

1. When the water supply is polluted, sand filters should be installed at the mouth of the water stream as it makes its way into the hatchery ponds. The sand catches most of the free copepods before they enter.

2. The young fry should be given salt baths quite often. This kills the adult copepods during the early stages of attachment. At the same time the salt makes the fish more resistant.

3. Since the adult trout are the ones most heavily parasitized, it is better to keep only the younger fish for spawning purposes.

4. Since the free-swimming stages of the copepods are strongly attracted by intense light, powerful arc lights should be erected at various points over the fish ponds. By means of fine gauze bags towed over the illuminated regions, the copepods can be gathered and removed.

5. The introduction of certain types of minnows into the hatchery ponds tends to keep the parasites down. These minnows feed on the free-living larvae of the copepods and thereby destroy many of them before they have the opportunity of coming in contact with the proper host.

Although these remedies are not absolute, they may nevertheless tend to reduce the loss from the parasitic organisms enormously. There is no absolute cure known. A most desirable remedy would be one which would destroy the adult copepods while they are attached to the fish, without in any way harming the host; but all attempts in this direction have thus far been without success. Trout are so delicately constituted that they can withstand only a very slight change in their environmental medium. The adult copepods, on the other hand, can resist powerful chemical solutions by virtue of their resistant body walls. One must catch the organisms as they break out of the egg cases of the mother, and kill them before

they come in contact with their hosts. "Prevention before parasitism occurs," should be our motto, rather than "cure after parasitism."

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PLATE 18

Fig. 1. Young brook trout about 5 months old showing a copepod attached to the gills. × about 1.

Fig. 2. Portion of the gill of a brook trout showing numerous *Salmincola edwardsii* attached. × about 1½.

Fig. 3. Floor of the mouth of a steelhead trout showing attached adult females of *Salmincola falculata*. × about 2.

Fig. 4. Adult females of *Salmincola falculata* found on steelhead trout. × about 2.

Fig. 5. Fin of steelhead trout with copepod attached. × about 2.

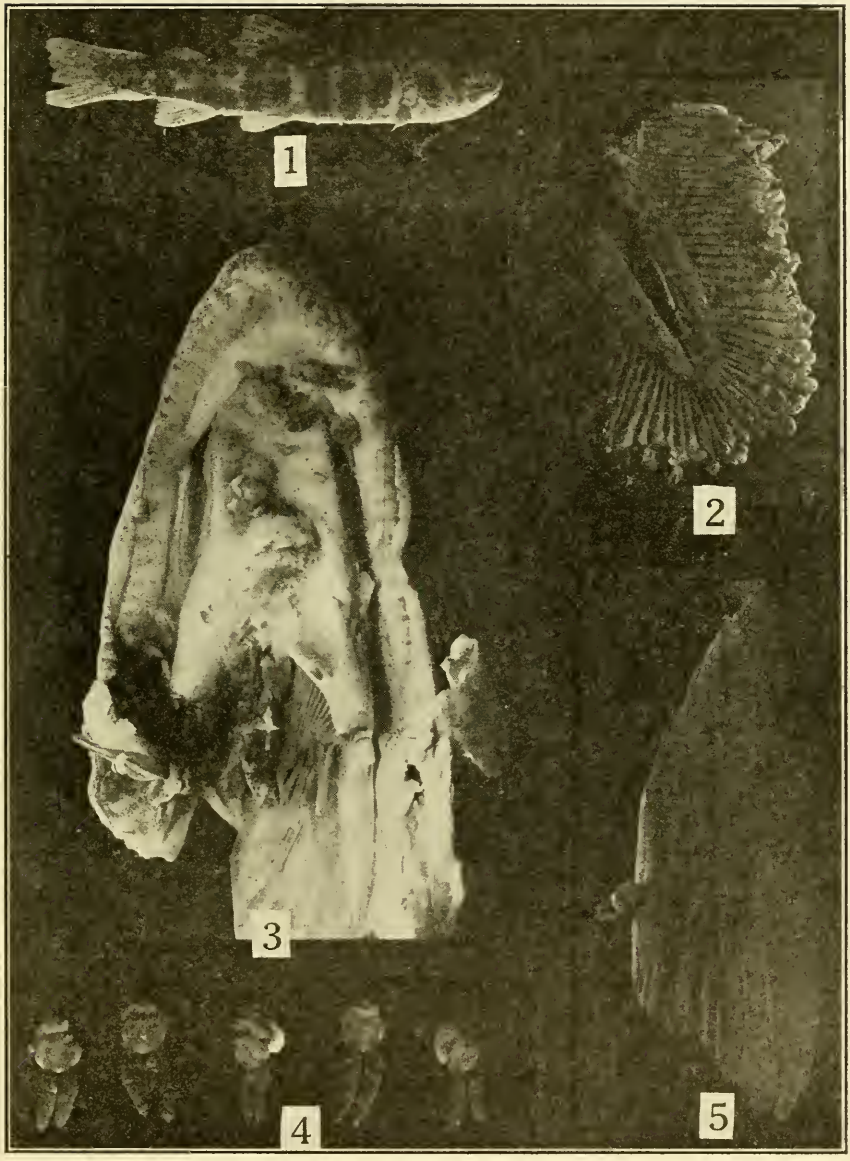

PLATE 18

Experiments on the Behavior of Some Puget Sound Shore Fishes (*Blenniidae*)

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The fish, their habitat and their general behavior

The blennies are a group of elongated more or less cylindrical fishes often without scales. The Blenniidae group, composed of several genera, includes many shore forms. They are known to be represented in Puget Sound by 13 species (Starks, 1911), several of which were found among the San Juan Islands during this study. Many of the blennies are often found entirely out of water.

Interest in the relation of behavior to shore existence led to this investigation. Only the distinctly shore species were used in the experiments. All of the important data refer to *Xiphidion mucosum* and *Anoplarchus atropurpureus,* although occasional specimens of *Pholis ornatus* were included with the latter.

The shore species are found chiefly along rocky, exposed shores where the water approaches saturation with oxygen. They are sometimes found in beds of Zostera (eelgrass) in vigorous Ulva, or in the water on sandy shores, but not upon depositing shores where the rate of decomposition is high.

The three principal species studied were found in the upper Laminarian belt, between the upper limit (1 meter above average tide) and 1 meter below the lowest tide line. Those found above low water level were confined to rocky shores, where they remained under loose stones. There were often as many as 5 or 6 individuals under a stone covering 1 sq. ft., and a lone blenny was rare rather than common.

Those individuals taken at points more than 1 meter below lowest tide were usually of a different species from those found higher up. Of those mentioned, occasional large individuals of *Xiphidion mucosum* ranged higher. After an unusually high tide they appeared above the Laminarian belt. At times of small tidal difference the zone of greater abundance was lower. In cases of exhaustive collection over a given area, the next high tide virtually reestablished uniform distribution. In one instance, reported upon good authority, the same individual (*Pholis ornatus?*), seemingly was repeatedly found upon a piece of kelp under experimental observation. The kelp was anchored upon a floating raft and submerged at a constant depth. The blennies on the shore line seem to maintain no

fixed abode under the stones, but shift with the tide, thus regulating the depth of submergence.

Since blennies were not found dead on shore their means of leaving critically untenable positions was investigated. In a series of trials, in which stones were turned and the blennies exposed to the sun, there was a definite tendency, as the heat became severe, to seek new covering with some accuracy of movement. This was the rule, although exceptions were found in which death occurred without any coordinated attempts to seek cover. Jordan reports cases in which the fins were used to make short hops in getting over the ground, but this was not observed in these blennies.

Methods and materials

Most of the experimental work was confined to individuals collected on the rocky shore of Brown Island, a small wooded island opposite the Puget Sound Biological Station. The collections were made by hand from under stones at low tide. This was rendered doubly difficult by the eel-like shape and the mucous secretion from the surface of the body of the specimens when under excitement. These blennies were transferred, in water of regulated temperature, to the laboratory directly or to a live-box made partly of wire netting and floated near the laboratory. Those fish obtained from the water were taken with the Puget Sound trawl furnished by the Station.

No marked difference was recorded for the reactions of the 3 species which were most abundant in this common habitat and which were most used. In fact, specific differences often intergrade making superficial identification quite difficult. Since a comparison of species, as such, seemed to promise little of value for this study, separate records were soon abandoned when only reactions to existing conditions were sought. A few tests with *Xiphistes chirus,* which is found perhaps farther off shore than those named before, are not considered.

The blenny as a rule is not very active under experimental observation but gives a positive reaction to the corners and bottom of the vessel. In many instances this necessitates a strong stimulation to produce an observable reaction.

In most of the experiments the receptacles used were graniteware pans of a rectangular shape (10x35 cm.). Heavy tin pans of the same shape but a little larger were provided as covers where needed. For the experiments in reaction to light, a section of the cover of the experimental pan was cut out and an adjustable tin slide cover fitted into the space. In this way the pan could be divided into approximately 3 equal light-areas: bright sunlight, intermediate, and deep shadow. A second pan, completely

covered, was used as a control. For experiments in color reaction, a removable frame was provided which held 3 squares of glass, respectively, red, green and blue.

A device for studying the reaction to the color of the background was a floating live-box containing 5 colored compartments connected by square openings about 10 cm. in width. The box was designed by Professor E. Victor Smith of the University of Washington. It was about 15 dm. long, with ends approximately 3 dm. square. The bottom, sides and partitions were of wood. The sides contained holes covered with wire screen. The top was open to the sun. The central section was painted white; the end ones red and orange, respectively. A fish placed in the white compartment was forced to pass thru a blue one to reach the orange one, and thru a green compartment to reach the red one. The bottoms of the compartments were strewn with a few shells and stones.

A gradient tank used in experiments in reactions to temperature changes and chemicals was the same as that described by Shelford and Powers (1915). It was principally of metal, had a glass front, was rectangular in shape and measured 15x122.3 cm. with a depth of 13 cm. In order to insure uniform lighting the tank was covered with a black, hooded frame with openings for observation. Light was furnished by 2 candles placed at equal distances from the ends at the rear of the tank.

Response to contact (thigmotaxis)

It was observed that few of the species used were taken in nets even near shore at average tide, although hundreds were found under stones at low tide. They were found under stones in the water, and in live-boxes they sought shelter below sea lettuce (Ulva) or stones. Where shelter could not be found they adapted the shape of the body as nearly as possible to the surface of some submerged object and remained motionless. They appeared to be positive to the bottom rather than free swimming above it.

In more definite experiments half a pan was laid with flat stones and the other half left bare. The experiments were performed in the dark., *i.e.,* with covers over the pans. After several trials, with different individuals a positive reaction to stones was recorded as shown by positive 82.2%, negative 17.8%. The corresponding ends of the control gave positive 54.8%, negative 45.2%.

Sea lettuce (Ulva), on the other hand, produced a negative reaction in the dark, as shown by positive 26.6%, negative 73.4%. The control was recorded as positive 48.8% and negative 51.2%. As noted, the reaction was positive in the live-box in sunlight. If we assume that light

was the only factor changed, we must consider the relative amounts of carbon dioxide given off as a factor in the negative reaction as well as direct reaction to light. The lowest tides on Brown Island occurred, for the most part, during the night or early in the morning, which corresponds to a period of inactivity in photosynthetic work by the sea lettuce and a corresponding decrease in the liberation of oxygen. This was the most common time of investigation, and blennies were found high on shore.

Response to current (rheotaxis)

Attempts to determine the reaction to current were made in a rheotaxis tank, but no quantitative work was attempted. Blennies do not seem to be as sensitive to currents as many other fishes. Of the individuals used, very few turned to breast the current. The reaction was usually in the direction headed. If this opposed the current the effort was increased. The result of a current stimulus was the seeking of a sheltered object or corner without reference to direction. However, resistance to being carried out of their position by the current was always offered. In a tub, circular motion and heavy washing, resembling wave motion, were not noticeably resisted.

Reactions to light and color

The fact that blennies live under stones while out of water suggested a study of their reactions to light. These were obtained by use of the pans described. Since the fish reacted positively to the ends and corners, the glasses in the color experiments were interchanged so that each occupied the three sections at different times during the experiment. The ends of the pans were occasionally reversed to serve as a control on other conditions. Readings of the number of blennies in each third of the pan were taken every 5 minutes, usually for an hour, and recorded under the proper head. At the end of each experiment all of the readings for 1 compartment (or color) during the series of 5-minute periods were added. The ratio of any one such sum to the grand sum for all the compartments is taken to indicate the reaction to a definite light-condition or color and is expressed as percentage. For the sake of comparison, percentages rather than numbers of individuals are given in table 1.

10 blennies of various species from various habitats were placed into the white compartment of the color box. The individuals included were (*a*) dark olive-blue; (*b*) gray; (*c*) mottled, with light fins; (*d*) light grass-green; while one had bright red-orange fins and markings. No permanent selective reaction was shown which could be due to individual color, and all location seemed to be a matter of chance.

TABLE 1. *Reactions of blennies to light and color; data in per cent*

Exp. No.	Reaction to light			Reaction to color		
	Sun light	Inter-mediate	Deep shadow	Blue	Green	Red
1	3.6+	60	36.3+	16.7—	40	43.3+
2	1.8+	52.7—	45.4+	28.2—	34.5+	37.3+
3	2.9—	68.6—	28.6—			
4	8.6—	60	31.5—			
Av.	4.2+	60.3+	35.5—	22.5—	37.2+	40.3—

In all of the experiments the blennies exhibited marked periods of activity and rest to such a degree as to suggest a physiological cause. In this instance the individuals stopped in first one compartment and then in another. In 2 individuals the periods of rest lasted about 3 minutes in each.

Later about 20 individuals were added and time given to become accustomed to the box. After a period of 3 days, while the color of the individuals seemed in no way to affect the reaction, a common reaction could be noticed. Although scattered, there were more blennies in the red section than in any 2 others excepting the orange, and the orange contained fewer than the red. The fact that the red and the orange were the end sections probably affected the results. It should be noted, however, that it was much more difficult to see dark objects over the red background in water than in the other sections.

From the above data blennies give a positive reaction to red light and red background, but it is possible that such reaction is merely to greater obscurity. In fact it would seem that selective reaction to background color, merely as such, is very weak or wanting, although there is a definite positive reaction to obscure positions and subdued light. When total darkness is approached the reaction is reversed. These facts, it should be noted, show positive reaction to conditions found in the normal habitat.

Relation between color of background and pigmentation

In the collection from various localities, the close correspondence between the coloring of blennies and that of the surrounding vegetation continually presents itself. Gamble (3) found that fish tend to become the color of their surroundings but that they respond to the color of the background when exposed to light rather than to the color of the rays themselves. Starks (1911) reported finding red and green blennies in exactly

the same surroundings, but this was not found by the writer. Those few cases which seemed to be exceptions, or to be on the borderline, were considered as unusual or as "strays." Blennies, bright green in color, were taken from eelgrass (Zostera) almost exclusively; while rare, decidedly red forms were taken only from water around red algae beds, where little red light penetrates. Here crabs and other forms were also red.

During several weeks no change was noted in differently colored individuals confined in the same live-box with common food and background. If adaptive color-changes occur in blennies they would seem to be extremely slow in the adult forms.

Resistance and reactions to temperature changes

Blennies are sometimes subjected to greater ranges in temperature than other marine fishes. In determining comparative resistance, "rat-tail" (*Xeneretmus alaskanus?*) and cottid (*Icelinus borealis*) fishes, from a depth of 60 to 100 meters, were compared with blennies from the shore belt. Several individuals were placed in pans and the temperature raised 1° C. at the end of each 10-minute period by pouring in hot water. A control pan with water at only a little above sea temperature was used to check other conditions.

The results were striking. The fish from 60-100 meters in no case survived above 23.5° and died between 22.5° and 23.5°. The blennies died between 26° and 29°. In 2 experiments most of the blennies died near and below 27°; in 1 experiment, in which the temperature was raised gradually at the rate of 1° C. every 3 minutes, half the individuals were alive at 28°, and 1 individual after remaining for some time at 29° revived when the water cooled.

During these experiments the movements and general state were carefully noted and recorded. Ordinarily the activity of the fishes decreases as the temperature rises above the optimum. This is true in general of the blennies but with modifications worthy of mention. These fish normally lie motionless much of the time, against the bottom of some submerged object. These periods are followed by other periods of rapid movement abruptly terminated. Like other fishes, they are more actively responsive to stimuli at low temperatures, and as the temperature rises are more sluggish in individual movements. However, when the optimum is much exceeded the periods of rest and activity disappear and the total amount of movement is increased. The fish, now in constant motion, listlessly visits every corner and object possible. The movement becomes slower, with increasing temperature, until complete torpor precedes death.

Some attempts were made to determine the reaction and degree of

sensitiveness to temperature changes but without any definite results, due in part to the inactivity of blennies. When a gradient pan, giving a difference of 3° in 35 cm., was used, no results differing from the control pan could be obtained. It seemed unwise to use much of the limited time upon these experiments.

Equal quantities of cold and warmed water were run into the respective ends of the gradient tank and the corresponding temperature kept at 15° and 16° respectively. The individuals were used one at a time and the movements charted to scale, considering the time and direction of movement. Of 4 individuals, 2 came to rest at the cool end, 1 in the center, and 1 at the warm end. Those which once reached an end remained there. Cottid fish showed more freedom of movement and reacted positively to the cooler temperature. It is probably true that blennies are not readily sensitive to small differences in temperature.

Chemical methods

The chemical conditions in the gradient tank, in which much of the work was done, were controlled by means of the water introduced into the tank from faucets through tubes placed at each end and enclosed by screens. Sea water in good condition was on tap from a storage tank used to supply the laboratory. The inlets to the gradient tank were fitted with "tee" tubes with several perforations which distributed the flow uniformly over the width of the tank. The drain tubes, 4 in number, were situated at the surface and bottom respectively, on each side. They were controlled by 4 pinch cocks. This arrangement, with the added facility of regulating the depth of the tee bars at the inlet, made possible a gradient from 1 end of the tank to the other which was reasonably homogeneous in cross section. The usual method was to regulate the inflow at 300 cc. per minute at each end, and introduce the chemical to be used into the right hand inflow, regulating the concentration of ions as desired.

The methods used in the analysis of the samples are standard and give fairly accurate results.* Chlorine was determined by neutralizing with sodium carbonate and titrating with silver nitrate, using potassium dichromate as the indicator. From this method total salinity was easily computed from standard tables, the relative proportion of the chief salts in sea water being about constant. The oxygen was determined by the Winkler method. For carbon dioxide the sample was titrated with N/20

* The reliability of the silver nitrate solution used in the salinity tests may be questioned since the results obtained show a higher degree of salinity than others have found in this locality, but this fact has little significance for the comparative results sought.

sodium carbonate using phenolphthalein as the indicator. The hydrogen sulphide was determined with iodine.

Reaction to carbon dioxide

The gas used was supplied by releasing CO_2 from the carbonates in the sea water with dilute hydrochloric acid. This was dropped at a calculated rate from a siphon tube and mixed with the right hand inflow current, as described. The temperature was a little above the sea temperature. In the graphical records kept, the minutes were represented by vertical distance, and position in the tank by horizontal distance. Each individual was introduced at the center of the tank.

Some of the results are shown in chart 1. In column 1 the water at the right contained 28 cc. of CO_2 per liter; that at the left, coming from the water-pipe, contained about 11.2 cc., causing a difference of 16.8 cc. per liter in the length of the tank. The water flow caused the CO_2 content of the entire tank to be high. The reactions were very marked. All of the blennies soon reached the left inlet and remained with heads pressed against the screen. The tank clearly contained above the optimum of CO_2.

As a means of comparison a small fish * taken from between 70 and 90 fathoms was introduced into the tank, and its very significant movements recorded. After several minutes of rhythmic movement from end to end the departure from the center became less, until after 20 minutes the fish came to rest at the center of the tank. An explanation may be that the deeper sea water, to which the fish was probably accustomed, has a high CO_2 content.

To find the degree of sensitiveness and the optimum CO_2 for blennies the CO_2 content was lowered until the ends differed by 7.29 cc. per liter, containing 8.96 cc. and 16.25 cc. per liter respectively. Of 5 individuals used, 1 went to the right as it was introduced and remained; 2 came to rest near the center with little activity; while the 2 remaining, after some exploration, came to rest at the extreme left. A negative reaction was clearly indicated, although thigmotaxis and faint shadows seemed to play some part. The results suggest that these shore blennies are not only negative to CO_2, even in very small quantities, but they are more sensitive than deeper water fish. These results are specially suggestive when considered with the fact that in the water from the habitat of blennies no CO_2 was found as a rule, and the amount was never more than a trace.

* The specimen has since been identified as *Oligocottus maculosus* and probably entered the dredge much nearer the surface than was at first supposed. However, having a different environment from the distinctly shore species, its reactions are of value in comparison.

The amount is no doubt affected by the action of H_2S which was always present.

Reaction to hydrogen sulphide

In the water samples taken from the habitat of blennies the H_2S content varied from .296 cc. per liter at the surface to 1.39 cc. just above the Ulva. In no case was it wanting where blennies were found, so far as tested. In fact, the Laminarian belt would normally be expected to produce H_2S in some quantity.

Despite the fact that sea fish are said to be sensitive to H_2S, blennies did not prove to be sensitive to any marked degree. In a gradient between 1.67 cc. and 3.34 cc. of H_2S per liter, the individuals, while not at all intoxicated, usually remained near the center. In no case was there definite negative reaction. While this may not indicate an optimum H_2S, it should be noted that it is slightly in excess of the normal H_2S content of the environment of the blennies. Shelford and Powers (1915) noted a sharp negative reaction in herring.

Reaction to acidity and alkalinity

The experiments with the reaction to alkalinity and acidity were carried out with sea water. Great difference in density was avoided in this way, as well as a difference in solutes which might affect the reaction. It is true that the optimum on either side of neutrality could not be determined.

When Na_2CO_3 and HCl were introduced at the 2 ends of the gradient tank, a decided reaction was observed in the blennies. In one case the turn was sharp at each trial. In all cases the final place of rest was the extreme alkaline end.

With an excess of HCl at one end and strong Na_2CO_3 at the other, the blennies still sought one end or the other, but never the center. A positive reaction to alkalinity was not always indicated, but as the blennies soon became stupid, extreme concentration of ions at both the inlets did not force them away from the ends. When first put into the tank they usually gave a positive reaction to the alkaline side of neutrality but soon came to rest at one of the ends. The ends were lighted with a little less intensity this time, owing to the length of the candles. As it was found that the acid floated while the alkali settled, a set of siphons was substituted for the outlets in order to equalize the gradient. The reactions were not changed by this. However, a cottid fish (sp.?) came to rest on the acid side of neutrality.

In another experiment no acid was introduced, and sea water containing not more than a trace of CO_2 was used against the strong alkali.

In each of 2 cases the reaction was positive to the sea water end. While a positive reaction to mild alkaline conditions is shown above; no optimum could be determined, although it lies not far from neutrality. Reaction to the ends (thigmotaxis) is stronger than to differences in acidity.

Resistance to salinity

Several experiments were performed with reference to salinity. The first group dealt with resistance to fresh water. 2 of the rectangular pans described were filled to a depth of several centimeters with sea water and spring water respectively and the temperature maintained the same in the 2. In these experiments 1 or 2 blennies died after 1 hour and 45 minutes, and the other after 2 hours and 15 minutes. Of 5 individuals 3 died at the end of 3 hours, the other 2 at the end of 1 hour and 59 minutes and 4 hours respectively. Practically all of the 7 lived 2 hours or more. Those in the control pans remained normal. In a comparative trial 4 fish secured at 30 to 60 fathoms (*Icelinus borealis* and *Xeneretmus alaskanus*) survived 1 hour and 59 minutes and 2 hours respectively. This is another instance in which the blennies are better fitted for a shore habitat than the deeper water fishes. Shore animals are often subjected to variations in salinity.

A still more striking experiment was carried out with differences in salinity. 10 blennies apparently in the same physical condition were used to determine resistance to salinity. 2 were placed in each of 5 shallow glass dishes containing water grading from half-evaporated sea water to spring water. This time scarcely enough water was used to submerge the blennies. Record was kept for 4 days, after which time the fish could not properly be considered as normal. In table 2 the number at the left refers to the dishes, containing 2 individuals each, while the time indicates the end of the period through which they were observed.

Table 2. *Resistance to degrees of salinity*

No.	Salinity	Time in hours	Condition
1	Sea water concentrated ½.....	33	dead
2	Equal portions, normal sea water + concentrated ½.....	96	about normal
3	Sea water, normal.....	96	about normal
4	Equal portions, sea + fresh water.....	96	about normal
5	Spring water, fresh.....	44	dead

The sea water used contained about 16.65 grams total chlorine per liter. Thus the waters of the region are less saline than average sea

water; and the above figures are high in comparison with those of any previous analysis. In the above experiment it seems probable that sufficient oxygen was supplied from the surface of the shallow water. Blennies seem to be adaptable to an extent; and in the limited amount of water, the diffusion through the body walls undoubtedly affected the degree of salinity. However, in the extremes, both increased and decreased salinity hasten death.

Resistance to desiccation

The fact that the blennies are often found entirely out of water, but rarely dead, leads to interest in the resistance to desiccation. 5 individuals were placed in a large pan with stones and shells and floated on a tub of cold water. The pan was placed where a very fine spray from a faucet kept the fish damp, but the water was drawn off as it collected. This gave much the same conditions as those beneath damp beach stones.

After ineffectual attempts, apparently, to reach a more suitable position the blennies lay on their sides and began gasping. Although very sensitive to being touched they were quite inactive otherwise, and little oxygen was used in movement. Later there was only occasional reaction to touch, but if there was reaction at all, vigorous activity was shown. After 30 hours they were removed to a large battery jar. As the air was quiet here it is probable that CO_2 increased somewhat at the bottom of the jar. The spray was stopped, but a mucous secretion from the skin prevented drying of the bodies, and all of the individuals were alive after 35 hours. After 36 hours 1 soon became about normal when placed in water. 1 lived 48 hours and recovered slowly in water. The general time for death was after 40 to 45 hours. In this experiment size seemed to play a more important part than in any of the others. The blennies weakened in an order the inverse of their size. The larger ones survived best but also remained damp longest.*

In the second experiment 5 blennies were floated as before but with caution to make them dry. The tendency was to lie quietly with the mouth somewhat open. Little response was given to prodding with a pencil, although this was in no wise due to lack of ability for action. All 5 lived 14 hours and 1 lived nearly 36. The average time for death was about 24 hours. 1 which was very weak died almost immediately when placed in water. It seems that the rate of drying of the mucous membranes affects the time of death directly. If these be moist death is delayed indefinitely.

* It should be stated that while a clear division did not appear between the species, the larger specimens were *Xiphidion mucosum*.

Relation to oxygen

Although blennies seem easily to withstand long confinement in a limited amount of shallow water, they are sensitive to the changed conditions in a deeper tub. At one time nearly all of a large number died before the laboratory was reached. The oxygen demand must be very high or diffusion slow. This, however, is compensated by the nature of the Ulva beds where the blenny feeds. The Ulva naturally releases great amounts of oxygen, especially in sunlight. In tests made from the beds on the north shore of Brown Island at low tide the oxygen content reached as high as 11.66 cc. per liter. One was 10.5 cc., and the surface near the shore gave 5.17 cc. per liter. This is much higher than the waters farther out. Shelford and Powers (1915) found in 1914 that the waters of the vicinity in general contained less than 5 cc. of oxygen per liter.

Conclusions

It has been made clear by these experiments that blennies differ much from deeper water fish in the response to certain stimuli which differ in the lower Laminarian and shore belts. It is apparent that this may explain in great degree at least why blennies are found in the shore environment. The species studied are negative to bright light and show a remarkably low degree of sensitivity, for marine fishes, to changes in temperature. They withstand living out of water for periods of time much greater than the intervals between high tides and have great resistance to fresh water.

There seems to be little reason for doubting that behavior, that is, reaction to definite environmental stimuli, is responsible for the existence of blennies in this unusual habitat. Of the several factors presenting themselves as possible causes, perhaps the varying amount of carbon dioxide in connection with the oxygen demand constitutes the chief one. The oxygen and carbon dioxide in turn depend upon other conditions. Carbon dioxide in the shore waters depends both upon the temperature of the water and the activity of the Ulva. An increasing amount of decomposition will increase the hydrogen sulphide, and this lowers the CO_2 content. While the oxygen is more or less constant at the surface, the activity of the Ulva controls the oxygen content of the water washing it. This activity naturally varies with day and night.

It seems tenable that these fish are most active among the Ulva while the tide is in or the light permits the green plants to work actively. Later they rest quietly beneath stones, thus avoiding a greater concentration of carbon dioxide in the deeper water as the tide recedes. In

many cases the water entirely withdraws, while the blennies secure ample oxygen from the air with little discomfort.

The experiments and observations recorded were begun at the suggestion of Prof. V. E. Shelford of the University of Illinois and were carried out under his direction during the summer of 1915 at the Puget Sound Biological Station, Friday Harbor, Washington. The methods of attack are those which have been developed by him, and to his friendly suggestions must be ascribed any points of possible scientific value which may be here recorded. Prof. J. O. Snyder of Stanford University identified the somewhat puzzling collection of specimens.

CHART I.

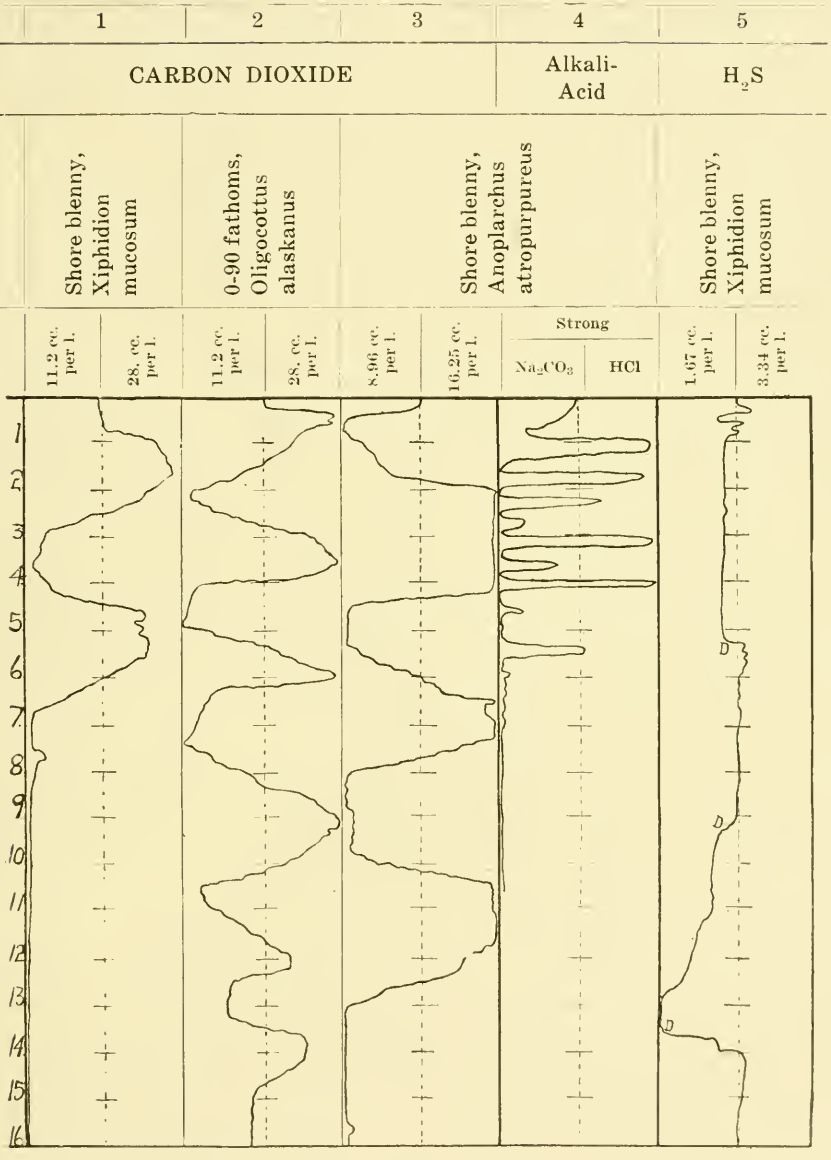
Graph 1 shows the reaction of a blenny to a difference of 16.2 cc. carbon dioxide per liter between ends of a tank 120 centimeters long. The greater concentration being to the right, the reaction is negative. Figures at the left represent the time in minutes.

Graph 2 shows the reaction of a fish (see foot-note page 86) from deeper water under the same conditions as in graph 1. This fish shows no observable response to the carbon dioxide.

Graph 3 shows the reaction of a blenny to a difference of 7.29 cc. of carbon dioxide per liter between the two ends. Although less marked the reaction is still negative.

Graph 4 shows the reaction of a blenny to a gradient from strong alkali on the left to strong acid on the right. Note the increased activity, the undoubted negative reaction to acid and the positive reaction to the end (possibly thigmotaxis).

Graph 5 shows the reaction of a blenny to a difference of 1.67 cc. hydrogen sulphide per liter in a distance of 120 centimeters. This case illustrates, better than the others shown, the general activity of a blenny under experimental conditions. *D* represents disturbance by touching with a pencil point.


1
2
3
4
5
CARBON DIOXIDE
Alkali-Acid
H_2S
Shore blenny, Xiphidion mucosum
0-90 fathoms, Oligocottus alaskanus
Shore blenny, Anoplarchus atropurpureus
Shore blenny, Xiphidion mucosum
11.2 cc. per l.
28. cc. per l.
11.2 cc. per l.
28. cc. per l.
8.96 cc. per l.
16.25 cc. per l.
Strong
Na_2CO_3
HCl
1.67 cc. per l.
3.34 cc. per l.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

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An Interesting Fungus from Friday Harbor, Washington

Sanford M. Zeller,
Missouri Botanical Garden, St. Louis, Mo.

On July 4, 1917, a collecting party from the Puget Sound Biological Station visited the fern cove just north of Point Caution. While collecting maiden hair ferns (*Adiantum pedatum*) there was found among the fern rhizomes and in the crevices under the overhanging rocky ledge a quantity of sporophores of a fungus which proved to be an undescribed species of Rhizopogon. This species has previously [1] been treated taxonomically under the name *Rhizopogon diplophloeus* Zeller & Dodge, but it was considered advisable to publish the collector's note here.

Rhizopogon belongs to the family Hymenogastraceae, the genera of which, for the most part, are hypogean. Species of Rhizopogon, however, are either subterranean, emergent or totally superficial.

Rhizopogon diplophloeus has puffball-like fructifications which are globose to irregular, 1—2.5 cm. in diameter when dry, clay colored when fresh, becoming darker when bruised, Verona brown to nearly black when dry; fibrils over the surface scanty, innate-appressed, black when dry and leading to rhizomorphs at the base; peridium 400—480 μ thick, duplex, the outer layer dark tawny under the microscope, about 140—180 μ thick, composed of irregularly swollen hyphae, loosely interwoven, the inner layer honey-colored, about 260—300 μ thick, composed of closely woven hyphae; gleba from Isabella-color to brown or almost black on drying, bony hard when dry; the cavities of the gleba subglobose to somewhat irregular, empty when young, filled with spores at maturity; partitions between the cavities 30—40 μ thick, composed of compact, subhyaline hyphae, not split through the trama; basidia clavate, 25—30$\times$12—18 μ, hyaline, 2—8-spored (mostly 8-spored); sterigmata 6—10 μ long; spores acrogenous, dilute cream-colored under the microscope, ellipsoid, 5.3—7$\times$2—3.5 μ, smooth, often 2-guttulate.

The present range of distribution is western Washington, one recorded collection having been made by W. N. Suksdorf (811) at Bingen, Klickitat County.

A histological study of the development of the fructifications of different species of Rhizopogon should be made to ascertain the significance

[1] Zeller, S. M., and Dodge, C. W. Rhizopogon in North America. Ann. Mo. Bot. Gard. 5:1—36. pl. 1-3. 1918.

of the duplex character of the peridium exhibited by some species, and such a study should be made by one to whom fresh material in all stages of development is available.

The Relation of Marine Fishes to Acids with Particular Reference to the Miles Acid Process of Sewage Treatment

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1. Introduction

A number of new biological problems have arisen in connection with the war. The munition works have been throwing large quantities of acid into streams and into the sea. Along the coast of New Jersey certain tide pool fishes have been relied upon to keep down mosquitoes, under a system of ditching. The acids thrown into the tide waters by the munition factories repelled the fishes so it was feared the mosquito work would be vitiated. About the same time that this difficulty attracted attention, a group of scientists asked Congress to appropriate a sum of money to install a plant for the treatment of the sewage of Boston by the Miles Acid Process. This process recovers fertilizer, ammonia, grease and glycerine (Weston, 1916). The plant was asked for as a war measure, but the appropriation was not granted because it was argued that the process would not yield sufficient profits. The scientists in charge argued that it would supply a sterile medium for the oyster beds so seriously infected with typhoid. It was estimated that the sewage of 97 cities of more than 50,000 inhabitants, treated by the Miles process, would yield per year as follows (Oil Paint and Drug Reporter, 1918):

	Tons
Fertilizer	97,393,680
Ammonia	4,869,684
Grease	23,780,684
Glycerine	1,289,039

Under such a plan the cities of Seattle, Tacoma, Olympia, Bellingham, Spokane and many smaller towns in Washington could make use of the process.

The residual effluent of the Miles Process is from 35 to 50 parts per million acid. Either sulphuric or sulphurous acid may be used. The question as to which is most dangerous or beneficial to fisheries and to public health, and as to the condition which would be produced by the introduction of this acid effluent as compared with the introduction of raw sewage, all become of great importance. This is true because the sewage disposal question, particularly the recovery of the nitrogen and grease, is one of the most important confronting modern civilization. So that whether or not the Miles Process is introduced, it is reasonable to

suppose that some similar form of recovery and treatment will be installed for all of our cities and towns, large and small, in the near future.

Following a series of experiments on herring performed at the Puget Sound Biological Station in 1914, the writer and Dr. Powers (Shelford & Powers, 1915) concluded that the historical migration of the European herring which had such important economic results as the decline of the Baltic towns of the Hanseatic League, may have been due to the introduction of sewage and refuse which caused an increase in the hydrogen ion concentration of sea-water. It was found at that time that the movements of these fishes were governed largely by differences in hydrogen ion concentration; slight increases in hydrogen ion concentrations as compared with normal sea-water being uniformly avoided by both herring and salmon. Sewage contamination of the sea results in the development of an increase in free carbon dioxide and hydrogen sulphide and the consequent increase in hydrogen ion concentration. This has undoubtedly not only kept important fishes from running on our shores but has also probably decreased the number of many useful animals. The question as to whether this new process with an acid residue will be beneficial or detrimental is one to be determined by a study of the effects of acids on useful fishes and other organisms particularly during the more sensitive periods. In taking up such a study it is not possible to investigate all the various organisms involved or to investigate many of them perhaps during the most sensitive stage. The following points must be kept in mind in such an investigation (Shelford, 1918b):

1. Animals differ in their resistance to poisons;—is the animal used in making tests one of representative sensitiveness?

2. Different stages in the life history of a given species differ in their resistance to poisons;—what is the most sensitive stage in the life histories of important animals?

3. The condition of the sea-water differs at different times of the year;—at what period will the addition of poisonous substances have greatest effect?

4. Many animals, particularly fishes, recognize dangerous substances and turn back, thus avoiding localities where such substances are found;—what is the reaction of important food animals to the poisons in question and what concentrations can they distinguish?

5. Effluents often may easily be treated so as greatly to reduce the toxicity;—what methods of treatment will render acids added to sea-water harmless?

It is not readily possible to conduct an investigation in an ideal manner with reference to all these questions, but they have been kept in mind throughout the work herein described.

2. Materials and methods

The material used in the experiments was collected by seining in the vicinity of the Biological Station. Four species of fish, the surf smelt (*Hypomesus pretiosus* [Gir.]), herring (*Clupea pallasi* C. & V., viviparous perch (*Cymatogaster aggregatus* Gib.) and flat fish (*Psettichthys melanostictus* Gir.) were the principal ones used. After seining, these were kept in a float with a screen bottom where an abundance of plankton organisms supplied them with food. In addition to the work by the writer, Miss Hall (1918) undertook the study of the effects of acids on the eggs of the large red sea-urchins, while Dr. Powers undertook a survey of the hydrogen ion concentration of the water in the vicinity of the Biological Station, and also a careful study of the differences in hydrogen ion concentrations the fishes could recognize, when produced by the addition of carbon dioxide. Work was done with both sulphuric and sulphurous acids.

Experiments were performed to determine the resistance and reactions of the fishes to these acids. They were performd in two ways: (1) In standing water, to which the acids were added and which was kept cool by running water surrounding the containers. (b) In running water. Most of the standing water experiments, of which there were a large number, were performed in section dishes with a capacity of about 3 liters, and a few experiments in bottles holding from 1½ to 3 liters. These experiments were finally used, however, only as a general guide and the more accurate work was done with running water. In the running water experiments a flow of sea-water was introduced into a bottle through a 3-hole rubber stopper. A flow of acid was introduced into the same bottle through another tube. The general arrangement was as shown in Figure 1. Here a 1 cc. pipette projected into the large chamber of a 50 cc. pipette. The 1 cc. pipette was connected with the siphon in the acid bottle and by means of a pinch-cock the flow was adjusted to any desired number of drops per minute. Thus the acid and water were introduced into the bottom of a 200 cc. mixing bottle and left at the top through a tube in the third hole of the stopper. With the water comparatively free from floating particles, the flow of water and of acid could be maintained for 24 hours or more with little or no adjustment. The tests of fish resistance were made in a large bottle (Fig. 1, D) connected with the mixing bottle and at a temperature only 1° C. or less above that of sea-water.

The reaction experiments were performed with water to which the acids were added by the same method. The gradient tank used is shown in Figure 2. Water of two kinds were introduced, one at one end and one at the other, and flowed out at the middle so that a gradient in

concentration of acids occurred in the long axis of the tank (Fig. 3). The fishes swim back and forth in such a long narrow tank and on encountering differences, frequently indicate that they sense them by turning back. The sulphuric acid used in the running water experiments was 40 liters of slightly more than a tenth normal solution made up in the Friday Harbor city water. The H_2SO_3 was a 20-liter bottle made up to about a twentieth normal, but which did not keep for any length of time. Solutions of calcium sulphite and calcium hydrogen sulphite were made up and used in the same manner.

The amount of H_2SO_3 in the water was determined by titration with N/100 iodine; a quantity of iodine was measured into a flask and a sample of water, usually 25 cc., was introduced under the surface of the iodine and the excess iodine titrated with N/100 sodium thio-sulfate. The flow of H_2SO_3 in the water when set at any desired point could be maintained for a day at a time at any desired concentration as shown by the iodine titrations. A correction for the iodine absorption by the sea-water was made. This varies from .15 to .125 cc. of N/100 iodine per 100 cc. of water. The calcium hydrgen sulphite was determined by the same method (Sutton, 1911). The H_2SO_4 was determined by taking account of the amount of the N/100 solution added to the sea-water to make 1 liter, and the hydrogen concentration of the resulting mixture was used as a general guide. This was determined in terms of the pH with buffer solutions and indicators as given by Clark and Lubs (1917); see also Hass (1916) and McClendon (1916). A pH of 7.0 is neutrality; higher numbers indicate alkalinity, lower numbers acidity. These buffer solutions were made up by Dr. Powers and checked against a Hynson, Wescott and Dunning phenol-red colorimeter loaned to us by Prof. C. M. Child. Mixtures of sea-water and N/100 sulphuric acid gave the same pH for the same mixture quite consistently thruout. The variation was only that of the sea-water supply itself which ranged one or two points either side of 8.0. The pH of mixtures of sea-water and sulphurous acid were not consistent, as the pH of a given concentration of acid added to a definite amount of sea-water appears to differ with the length of time the mixture has stood, and the results, except with fresh mixtures, were quite variable and even the fresh mixtures differed considerably from time to time. Whether this is due to the effect of the sulphurous acid on the different indicators or to some other cause has not yet been determined, and only the limits of variation of the mixtures giving a certain pH are shown in any of the work where the pH was used. The oxygen content of the water in which the fishes were killed was taken from time to time by the Winkler method. Carbon dioxide was determined occasionally by titration with sodium carbonate and phenolphthalein indicator, but this method is

apparently only a rough one as indicated by the more delicate methods of hydrogen ion determination.

3. Presentation of the results

A. *Sulphuric acid*

When sulphuric acid is introduced into water containing carbonate, no free mineral acid remains in the water until all the carbonates have been transformed into sulphates with a liberation of carbon dioxide, and this presumably occurs when the pH is about 5.0. Accordingly in most of the tests made the acidity produced by the addition of sulphuric acid was due to *carbon dioxide liberated.* On account of this fact and the rapid escape of the carbon dioxide, it is important that experiments be performed in running water. Also it is obvious that since average sea-water is about .002 normal carbonate a large amount of acid can be introduced before any marked change in hydrogen ion concentration can take place. Thus it will be seen from table 1 that the introduction of 30 parts per million only lowered the pH to 7.25. It will be noted further that up to nearly 32 parts of acid added per million no injurious results were noted on small herring, and that probably with 39 parts per million the injury noted was due to mechanical injury of the fish which died. Fishes often die soon after being seined and occasional deaths in such fresh material are by no means due to the poison. It will be noted that death among the young herring occurred only with the addition of 39 parts of acid per million, which brought the pH down to 6.85 or just on the acid side of true neutrality. This appeared to be fatal to the young herring after 8 hours exposure and is little less than the concentration which Whitley (1905) found would injure the eggs of the plaice immediately after fertilization; for further work on the effects of acid see cited works of Loeb, Medes and Moore. The herring is a pelagic fish living near the surface in the open waters, frequently at some distance from shore, and coming to shore to breed. Its remarkable sensitivity to differences in hydrogen ion concentration as shown by the action in the gradient tank have already been discussed by the writer and Powers (1915). In a few gradient experiments, in which a very slight difference in pH was produced by the addition of a very small amount of sulphuric acid to the water running into one end, the herring showed an ability to recognize the differences between pH 8.1 and pH 8.2 at the two ends of the tank. The graph of one of these reactions is shown in chart 1, graph 1, from which it would appear that the herring is able to recognize differences in hydrogen ion concentration correspondng to differences in

pH of .025. Whether or not the addition of such acids in the sea-water would prevent runs of herring to a greater degree than the introduction of the sewage cannot be definitely determined, but 35 parts of sulphuric acid per million diluted 100 times with sea-water would probably give only a very slight reduction in pH; and with the tendency of the fresh water or more dilute sea-water to remain at the top where CO_2 can escape, it seems more than probable that the effects of direct addition to the acid would very quickly disappear through the escape of the carbon dioxide. In the case of raw sewage the heavier materials settling to the bottom over large areas continue to decompose, producing many toxic substances as well as increasing the acidity. This would tend to prolong almost indefinitely the occurrence of acid as compared with the direct introduction.

A considerable number of experiments were performed on the viviparous perch, a fish of some importance for food, which lives in the shore water and often occurs in considerable numbers in tide pools. This species is much more resistant than the herring. Table 1 shows the resistance of the perch to the conditions produced by the addition of sulphuric acid. The addition of 39 parts per million which gave a pH of 6.85 did not kill the fishes. They appeared in several experiments to become slightly intoxicated and then to acclimatize. With the addition of 65 parts per million which lowered the pH 6.3, the fishes died only after 5 hours exposure. The perch thus seem to be much more resistant than the herring, being about five times as resistant to the effects of sulphuric acid, as shown by the comparisons of the species in table 3. They select a more acid water than do the herring (chart 1, graph 2).

Flat fishes are usually very resistant to all sorts of conditions. This characteristic is probably one common to all benthos. It was only with an addition of 291 parts of sulphuric acid per million that the fishes were killed at all. This brought the pH down to 4.35. 110 parts per million, which killed the perch in 40 minutes, had little or no effect on the flat fishes during a period of 24 hours except to increase their respiratory activity. Whitley found that the addition of about 40 parts of acid per million, which gave a one-thousandth molecular solution of hydrochloric, caused considerable mortality in one of the European flat fishes, the plaice. This mortality occurred only in eggs subjected to acid immediately after laying, the older stages increased in resistance very rapidly, so that he referred to them as "remarkably resistant." Injury can hardly come to these bottom fishes with the addition of acid as a result of the Miles Process of sewage treatment. Accumulation of decomposing detritus resulting from direct introduction of raw sewage would seem to be much more likely to have a detrimental effect.

B. *Sulphurous acid*

The use of SO_2 as bubbled through the sewage has been found to be the most economical acid method. It also has a very marked germicidal effect, the number of bacteria being reduced from many thousand to a few hundred per cc. This acid is accordingly most desirable for use in sewage treatment. It is, however, ten times or more as toxic (Shelford, 1917) to fishes as sulphuric acid, but has the advantage of escaping from the water very rapidly, and since it is present in the effluent before it is mixed with the sea-water, the bubbling of compressed air through the effluent or a simple stirring or other exposure would very quickly remove any dangerous effect which it might have on organisms. It differs in this respect from H_2SO_4, which liberates poisonous carbon dioxide by a chemical reaction which takes place only *after* the effluent and the sea-water are mixed. Thus on account of the ease with which it may be removed and its germicidal effect it is much more desirable for use than H_2SO_4. Turning to table 3 we note that from 2.3 to 2.9 parts per million are required to kill a 2.25 gram herring in an hour, and that 4 parts per million are very quickly fatal. It was found with the gradient tank that herring recognize a difference of one part per million in the concentration range from 0 parts per million at one end to 3 parts per million at the other (chart 1, graph 3). They turn back from slightly higher concentrations but are positive to very strong solutions (chart 1, graphs 4 and 5). In the case of the perch, 3.9 to 4.3 parts per million killed the fish in $3\frac{1}{2}$ hours while 9 parts per million were very rapidly fatal. Only one flat fish was killed in the sulphurous acid water, and this after 40 minutes exposure to water containing about 9 parts per million. In sulphurous acid the perch is only a little over twice as resistant as the herring, and the flat fish about five times as resistant as the perch. The question of treatment of the effluent by allowing it to flow over limestone received a little attention. It was found, however, that in running water calcium hydrogen sulphite is less toxic to herring than sulphurous acid, but in standing water the salt is more toxic than the acid on account of the rapid escape of the SO_2. Thus in standing water requiring .6 cc. of N/100 iodine per 100 cc. in the cases of the three compounds — sulphurous acid, calcium hydrogen sulphite and calcium sulphite — death resulted in 85 minutes in the acid, and in about 50 minutes in each of the other two. There would seem to be no advantage from the standpoint of fishes, in the treatment of the effluent with limestone.

C. *Acids from munition works and their effect upon fishes*

We have already noted that the killi-fishes were repelled from the New Jersey tide waters by the acids from muition works (Shelford, 1918b).

The treatment of these acids with limestone has been proposed. The acid effluent consists of nitric and sulphuric acid. The calcium sulphate produced by running the acids over limestone would be precipitated on account of its practical insolubility. The question of the effect of calcium nitrate on marine animals remained to be investigated. A few experiments, in which a small amount of calcium nitrate was added to sea-water running into one end of the gradient tank, showed that the calcium nitrate raised the pH of the water and both the viviparous perch and herring were positive to it as shown by graphs 6 and 7 in chart 1.

4. General discussion

These investigations while incomplete and faulty in that they could not deal with the most sensitive stage in any of the fishes which were investigated, indicate clearly that the use of SO_2 is preferable from the standpoint of organisms to the use of sulphuric acid; that its greater toxicity is offset by the rapidity with which it leaves the effluent before it is mixed with sea-water or stream water in case the introduction is not directly into the sea. Unfortunately one can only estimate the possibly greater sensitiveness of the younger stages of herring. Since a dilution of an effluent which is 35 parts per million to one tenth, without allowances for the inevitable losses into the air before such a mixture can take place, gives about the minimum fatal concentration for a 2¼-gram fish, it would be surprising if a dilution to one onehundredth could have detrimental effect on herring at any time, for this would fall below reasonable limits of toxicity and probably also below the limits which the sense organs of the fish could recognize. The concentration which appears to be about the minimum fatal dose for the 2.25 gram herring is also less than the weakest concentration which produced abnormal development in the egg of the large red sea-urchin in the work of Miss Hall (1918). A slight treatment of the effluent by some aërating process (Bartow, 1917) would render feasible the use of the Miles Acid Process in the treatment of sewage, and would leave intact all the benefits from the standpoint of public health. It would render possible the recovery of the valuable substances contained in sewage, as friends of the process have claimed for it, and at the same time make the prospects for an abundance of marine fishes near large cities and towns infinitely better than under conditions in which raw sewage is introduced directly. It is to be hoped that those who saw only the profits to be gained from the sale of recovered products may be persuaded to advocate the introduction of the process on account of the abatement of nuisance, benefits to public health, and probable benefits to fisheries.

ACKNOWLEDGMENTS

The author wishes to thank Professor Trevor Kincaid for assistance in collecting and for identifying the fishes. The entire expense of the work was borne by the Puget Sound Biological Station.

TABLES

TABLE 1 *Showing the general results of the addition of sulphuric acid to sea-water and the effect on fishes*

cc. N/10 H_2SO_4	Pt. per million	Normality	cc. CO_2 per liter	pH.	SPECIES AND RESULTS		
					HERRING		
					Died after	O.K. after	
-------	-------	----------	-------	7.6	----------	Indefinite	
-------	-------	----------	-------	7.45	----------	Indefinite	
6.2	30.3	.00062	7.0	7.25	----------	Indefinite	
7.1	31.7	.00071	8.0	7.05	----------	8 hours	
8.0	39.2	.00080	9.0	6.85	480 minutes		
15.5	65.9	.00155	17.3	6.3	60 minutes		
					VIVIPAROUS PERCH		FLAT FISH
					Died after	O.K. after	Died after
-------	-------	----------	-------	7.6	----------	Indefinite	No effect
-------	-------	----------	-------	7.45	----------	Indefinite	No effect
6.2	30.3	.00062	7.0	7.25	----------	Indefinite	No effect
7.1	31.7	.00071	8.0	7.05	----------	Indefinite	No effect
8.0	39.2	.00080	9.0	6.85	----------	Acclimatized	----------
15.5	65.9	.00155	17.3	6.3	300 minutes	----------	No effect 1 day
22.6	110.7	.00226	25.4	5.5	40 minutes	----------	No effect 1 day
59.5	291.5	.00595	66.6	4.35	35 minutes	----------	D'd after 127 min.

TABLE 2. *The effect of sulphurous acid on fishes*

Pt. per million of H_2SO_3	Approximate Normality	pH.	SPECIES AND RESULTS		
			HERRING		
			Time to death	Remarks	
1.447 — 1.654	.000040	----------	----------	No effect	
2.067 — 2.274	.000050	8.0	----------	No effect	
2.481 — 2.315	.000064	7.6	----------	No effect	
2.315 — 2.894	.000069	7.6	(1) 60 minutes	Several others survived several hours	
3.514 — 3.729	.000083	7.25	60 minutes		
3.928 — 4.341	.000091	----------	13 minutes		
			VIVIPAROUS PERCH		FLAT FISH
2.315 — 2.894	.000069	7.6	----------	No effect	No effect
3.514 — 3.721	.000083	7.25	----------	No effect	No effect
3.928 — 4.341	.000091	----------	210 minutes	----------	No effect
5.168 — 5.375	.000125	----------	210 minutes	----------	No effect
7.539 — 7.856	.000185	----------	60 minutes	----------	No effect
8.889 — 9.097	.000222	----------	8 minutes	----------	Died in 40 min.

TABLE 3. *Relative resistance of different species of fish*

SPECIES	Resistance in H_2SO_4; running	Wt. Gm.	Resistance in H_2SO_4; standing	Wt. Gm.	Resistance in H_2SO_3; running	Wt. Gm.
Surf Smelt ----------	----------	----------	8	4.0	----------	----------
Herring ----------	10	2.25	10	2.25	10	2.25
Viviparous perch -----	50	22.0	25	15.0–20.0	21	22.0
Flat fish ----------	150	5.0–10.0	----------	----------	1100	6.0

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CHART 1

Graph 1 shows the movement of herring in the gradient tank shown in figures 2 and 3 of plate 19. The back and forth movement of the animal is drawn to a vertical time scale with minutes divided into ten-second periods. The fish started in the pure sea-water end of the tank, reached the other end in about 15 seconds, and turned back, reaching the point where it started at the end of 35 seconds. The dark portion of the heading indicates a substance added to the sea-water and gives a rough idea of the concentration which decreases from left to right. In this graph, enough acid was added to the sea-water to lower the pH from 8.2 to 8.1; the herring avoided the more acid water. The vertical lines indicate thirds of the tank.

Graph 2 shows the reaction of a viviparous perch in a tank the same as shown in graph 1. Enough sulphuric acid was added to change the pH from 8.2 to 8.1 and the perch selected the more acid water.

Graph 3 shows the avoidance of 0.8 part per million of sulphurous acid by a herring.

Graph 4 shows a positive reaction of a perch to 60 parts per million of sulphurous acid, which resulted in intoxication in 3.5 minutes. The crosses indicate intoxication after which death followed.

Graph 5 shows a positive reaction of herring to the same concentration of sulphurous acid. This suggests a possible danger from sulphurous acid, but the positive reaction takes place only in concentrations usually higher than the undiluted Miles Process sludge; ×'s as in graph 4.

Graph 6 shows the reaction of a viviparous perch to calcium nitrate. It is possibly very slightly negative. The alkalinity of the water is increased by it, as shown by the figures at the end which indicate the pH.

Graph 7 shows the positive reaction of a herring to N/2500 normal calcium nitrate; figures as in graph 6.

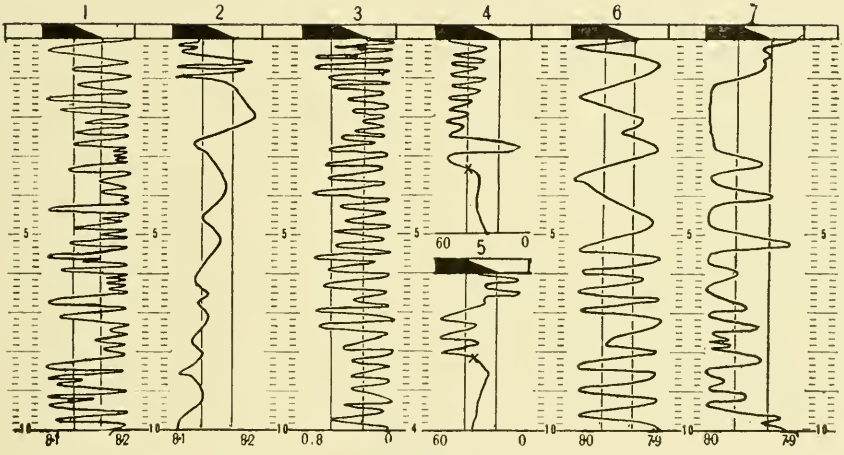

CHART 1

PLATE 19

Fig. 1 show the arrangement of glassware by which running-water mixtures of sea-water, acids, etc., were obtained. *A* is the dropping chamber, made up of the bulb of a 50 cc. pipette with a piece of tubing drawn to a dropping point, in this case the lower half of a broken 1 cc. pipette, projecting into it from above. The dropping tube was connected with a supply bottle of acid or other chemical and the number of drops regulated with a screw pinch-cock. The dropping chamber should be two or three feet above the mixing bottle (*B*); it is shown closer here merely for convenience in drawing. The mixing bottle (*B*) held only about 200 cc., so as to make changes of flow easy. The water entered through the left tube, which extends to the bottom very close to the one from the dropping chamber. The mixture left the bottle through the right tube ending at the lower level of the cork. *C* shows the 100 cc. cylinder into which the flow could be turned for taking samples by means of pinch-cocks. *D* is the experimental bottle, holding two to three liters; it is the one into which the fish were introduced.

Fig. 2 shows a cheap form of gradient tank which was found very effective. Water was introduced into the two ends through tees as shown below the inlet pipes (*i*). It flowed out at the center from both top and bottom, through a hole on each side. Instead of some of the elaborate arrangement of pipes described elsewhere, the tubes leading from the openings projected only a little outside of the tank; rubber tubes were attached to these and brought from the bottom openings to one side and from the top openings to one end where they were joined with glass Y's (*o* and *o'*). The outflow was adjusted by means of pinch-cocks so that the same amount came from each side, and the same total from the top and from the bottom. The front was of glass, in two panes. The tank was approximately 120 cm. long, 13 cm. wide and 15 cm. deep.

Fig. 3 shows a longitudinal section of the gradient tank. The *M* end is the modified water end shown by black lines; the *P* end is the polluted water end. There is a suggestion of a gradient between the two kinds of water in the center third. The thirds are indicated by the short vertical lines below.

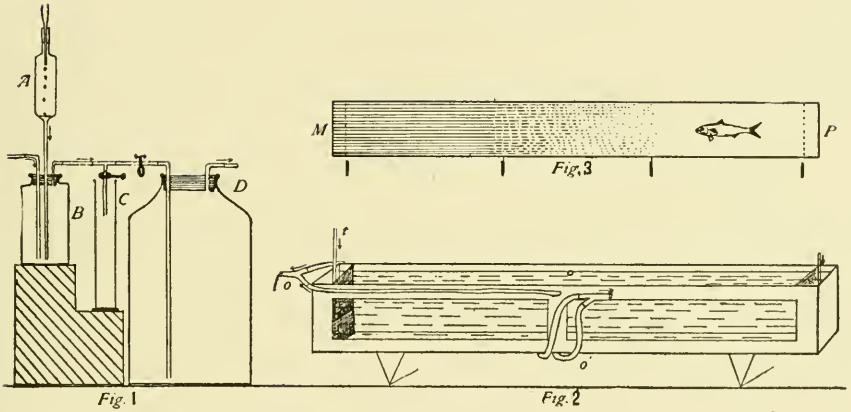

Fig. 1 Fig. 3 Fig. 2

PLATE 19

Some Experiments on the Resistance of Sea-urchin Eggs to Sulphurous Acid

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Recently a method for the treatment of the sewage of cities having over 50,000 inhabitants has been suggested whereby, thru treating with sulphuric or sulphurous acid (Miles Acid Process), several valuable substances will be recovered, and the effluent which goes into the rivers or bays will be essentially free from dangerous bacteria. This will prevent typhoid infection of oyster beds; but there arises a question as to whether the acid which goes into the water will injure oysters and other valuable animals. Considerable work along this line has been done with adult forms by Dr. V. E. Shelford. This paper presents a rather brief study of the effects of sulphurous acid on eggs and larval stages. The work was done in the summer of 1918 at the Puget Sound Biological Station at Friday Harbor, Washington.

The material used in most of these experiments is the large purple sea-urchin, *Strongylocentrotus franciscanus* Ag. This occurs abundantly along clean rocky shores at depths from 6 to 18 meters. This is a particularly good species for embryological work, as the eggs are large and fairly clear and the different cleavage stages can be easily followed. Another advantage is the very large number of eggs furnished by one individual. These can be obtained unfertilized, so that the earliest stages are always to be had for experimental work. The adults were exposed to the same condition as the eggs for comparison of their resistances. Experiments were also tried on medusae (*Phialidium gregarium* Haeckel and *Thaumantias cellularia* Haeckel), as index organisms.

A little preliminary work was done on gastropod mollusk eggs which occur in great abundance on Zostera (eel-grass) and Ulva. These, however, are encased in jelly, which with the egg membrane, prevents the entrance of the acid when solutions weaker than N/300 of acid were used. In the Miles Acid Process solutions of this strength would never be reached. A naked egg was therefore chosen for the work.

On account of the instability of sulphurous acid, solutions of this had to be renewed frequently. Furthermore the exact reactions of sulphurous acid in sea-water are not known. Whether it merely dissolves and by its own dissociation gives a certain hydrogen ion concentration or

whether it reacts with the sea-salts liberating carbon dioxide is yet to be determined. In these experiments the solutions were all used fresh, as it was found that the same hydrogen ion concentration gave different amounts of iodine titration when freshly made up and after standing for several days.

The hydrogen ion concentration was determined by colorimeters made according to the formulas of Clark and Lubs (1917), and is expressed in terms of the pH. The buffer solutions for these were made up by Dr. E. B. Powers of Colorado College. The average sea-water in Puget Sound varies in pH from 7.8 to 8.4. The approximate normality and corresponding pH of the sulphurous acid used is given in table 1. A correction of .15 cc. had to be made to allow for the absorption of iodine by the sea-water.

Table 1. *Showing the approximate normality of H_2SO_3 as found by titration with iodine; and the corresponding pH*

pH	cc. N/100 iodine per 100 cc.	Approx. normality
6.5	5.5 to 6.0	N/1800
6.65	4.0 to 4.5	N/2300
6.85	3.2 to 3.7	N/3000
7.05	1.9 to 2.1	N/5400
7.25	1.3 to 1.6	N/7500
7.45	.8 to 1.0	N/12000
7.6	.7 to .8	N/15000
7.8	.68 to .7	N/17000
8.0	.6 to .66	N/20000

Three types of experiments were performed: (1), Testing the sensitivity of unfertilized eggs and sperms (table 2); (2), Testing the sensitivity of fertilized eggs to acid at different stages of their development (table 3); (3), Testing with 4 to 6 hours exposure to the acid at different stages and then developing further in sea-water (table 4).

TABLE 2. *Effects of H_2SO_3 on unfertilized eggs*

Series	Approx. pH	Approx. normality	Stage of development	Results of exposure
E-17-a	6.9	N/3000	Unfertilized eggs exposed to acid 1 hr. 30 min.	Eggs fertilized in acid 90% cleavage; normal blastulae in 36 hrs. In sea-water developed evaginated gastrulae in 84 hrs.
			" "	Eggs fertilized in sea-water 85% cleavage; apparently normal gastrulae in 96 hrs.
E-17-c	6.9	N/3000	Unfertilized eggs exposed to acid 2 hrs.	Eggs fertilized in acid formed few early blastulae in 36 hrs. In sea-water, became abnormal in 72 hrs.
			" "	Eggs fertilized in sea-water formed apparently normal blastulae in 36 hrs. In sea-water, formed evaginated gastrulae in 96 hrs.
E-9-b	7.2	N/7500	Unfertilized eggs exposed to acid 40 min.	Fertilized in acid and grown for 12 hrs. In sea-water, normal gastrulae in 55 hrs.
			" "	Fertilized in sea-water normal gastrulae in 55 hrs.
E-9-b	7.2	N/7500	Unfertilized eggs exposed to acid 6 hrs.	Fertilized and grown in acid 16 hrs. Few early blastulae; dead in 2 days.
			" "	Fertilized in sea-water, same as acid.

To test the sensitivity of unfertilized eggs and sperms, the eggs were placed in acid, then taken out and some fertilized in acid and others in sea-water, at definite intervals (table 2). The sperms were motile in the acid but seemed to be inhibited so that cleavage was delayed. However, fertilization took place in all cases, *i e.*, with or without acid, cleavage going to the gastrula stage in a pH of 7.6—8.0 (N/15000—

N/20000), to the blastula and gastrula stages in a pH of 7.25 (N/7500), and to the evaginated gastrula stage in a pH of 6.7—7.05 (N/3000—N/5400).

From table 2 it may be seen that fertilization and cleavage will take place after 6 hours of treatment with H_2SO_3 (pH of 7.2—8.0), altho abnormalities appear after a period of 1½ hours of treatment. It may be that sulphur dioxide has the power of decreasing oxidation and therefore of prolonging the life of the unfertilized egg. Loeb (1917) found that potassium cyanide has this effect.

Table 3. *Effects of H_2SO_3 on fertilized eggs*

Series	Approx. pH	Approx. normality	Stage of development	Results of exposure	Time observed
E-15-a	6.65	N/2300	Fert. 25 min.	Developed to 4 cells, 2% cleavage, death..	20 hrs.
b			Fert. 45 min.	Developed to 16 cells, 3% cleavage, death..	20 hrs.
f			1 cell	Developed to blastula, 80% cleavage	2 da.
j			4 cells	Developed abnormal blastula, 89% cleav..	3 da.
l			32 cells	Developed abnormal gastrula	3½ da.
n			Blastula	Developed abnormal gastrula	3½ da.
E-16-a	6.7 to	N/5400	Fert. 30 min.	Developed to 8 cells, 1% cleavage	23 hrs.
c	7.05		Fert. 75 min.	Developed evaginated gastrulae, 80% cleav.	2½ da.
f			1 cell	Developed evaginated gastrulae	4 da.
l			8 cells	Developed evaginated gastrulae	4 da.
o			Blastulae	Developed evaginated gastrulae	4 da.
E-10	7.25	N/7500	Fert. 2 hrs.	Developed normal blastulae	1 da.
E-3			Blastulae	Developed normal gastrulae	18 hrs.

In the second type of experiment the eggs were fertilized in seawater, then placed in H_2SO_3 at different stages of development (from 25 minutes after fertilization to the blastula stage) and left to finish their growth under these conditions (table 3). Using acid with a pH

of 6.65, all eggs subjected to the acid before cleavage began, were badly inhibited, the 16-celled stage being the limit of growth. Those put in while from 1—8-celled became abnormal at the blastula stage; those from 32-celled to the blastula formed abnormal gastrulae. With a pH of 6.7—7.05, those put in 30 minutes after fertilization developed only to the 8-celled stage; all others formed blastulae or abnormal gastrulae. Using a pH of 7.25, eggs fertilized for two hours but with no cleavage, formed normal blastulae; blastulae and gastrulae developed normally. Similar results were obtained with a pH of 7.6—8.0.

In the third type of experiment fertilized eggs at different stages of development were put into H_2SO_3 for 4—6 hours, then returned to sea-water for further growth (table 4). This method seemed to cause fewer abnormalities than either of the other experiments. In a pH of 6.5, 2-celled eggs in acid for 4 hours developed normally, 8-celled to blastula stages in acid for 6 hours developed normal blastulae and gastrulae. In a pH of 6.65, blastulae in the acid for 6 hours formed normal gastrulae. Gastrulae put into acid for 6 hours continued to develop normally after return to sea-water.

TABLE 4. *Effects of exposure to H_2SO_3 for 4—6 hours*

Series	Approx. pH	Approx. normality	Stage of development	Time in acid	Results of exposure
E-1	6.5	N/1800	2 cells	4 hours	Formed normal blast.
			8 cells	6 hours	Formed normal blast.
			blastulae	6 hours	Formed normal gast.
E-18-a	6.65	N/2300	1 cell	6 hours	Formed normal gast.
b			2 cells	6 hours	Formed normal gast.
E-16-p			blastulae	6 hours	Formed normal gast.
E-19-d			gastrulae	6 hours	Normal gastrulae

When testing the adult sea-urchins, one large and one small individual were used in each concentration. With a pH of 6.1 both animals lived for 1 day and 21 hours. The acid water was kept running the first day but was unchanged during the second. It was clear and free from odor at the end. In the second jar, with a pH of 6.85, the small one died in 20 hours and the large one in 1 day and 20 hours. The water was murky and foul-smelling, however. This would seem to indicate a germicidal effect in the stronger solution. In the experiments with medusae four concentrations of acid were used, with pH of 6.75, 7.45, 7.6, and 7.8. The water was changed frequently to keep the pH constant. *Thaumantias cellularia* was more active in all concentrations at first, but died in 23

hours in all. *Phialidium gregarium* showed no pulsations in the 6.75, the only sign of life being a closing of the bell when stimulated. These in a pH of 6.75, died in 24 hours. Those in the other concentrations lived 3 hours longer but showed only occasional or very slow pulsations during the last 10 hours.

SUMMARY AND DISCUSSION

Unfertilized eggs exposed to H_2SO_3 of N/7500 or weaker for a period not longer than 1½ hours develop normally when fertilized either in acid or in sea-water.

Eggs in different stages of development exposed to H_2SO_3 of N/7500 or weaker, develop normally.

Eggs exposed at different stages of development for 4—6 hours, or a tide period, to strengths from N/1800 to N/2300, were not injured but developed normally.

Altho death does not occur immediately in solutions stronger than N/7500, still abnormalities appear sooner or later which prevent the formation of normal adults. This condition would be as fatal to the life of the species as death. In the Miles Acid Process strengths over N/10000 would probably never be reached, and even greater dilutions would be probable since sulphur dioxide passes off rapidly. It seems then that the sea-urchin would be able to live in the waters which receive sewage treated in this manner. Since the sea-urchin normally lives on clean rocky bottom free from contamination it is probably more sensitive than the clam and oyster which live in similar depths, but where the pH is more variable and where there is liable to be more contamination. The sea-urchin may be considered as an index organism for these forms. We may predict therefore that the shellfish would not be destroyed by this process when sulphurous acid or sulphur dioxide is used.

I wish to thank Dr. V. E. Shelford, at whose suggestion this work was undertaken, for his interest and helpful suggestions. I am indebted to Dr. J. F. Bovard and Dr. C. M. Child for their assistance with the embryological work.

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Fungi found on Codium mucronatum

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There are comparatively few fungi reported from saline habitats. Those that are found under such conditions are microscopic species and, altho they are mostly parasitic on marine algae, some few saprophytic forms have been described in very recent literature. Of the total number of marine fungi that have been reported there are a very few species belonging to the Phycomycetes, about 24 to the Pyrenomycetes and 14 to the Fungi Imperfecti. In case of the marine Phycomycetes almost, if not all, belong to the Chytridiaceae. These are such minute structures that they are often not noticeable, and the real nature of the body described as a fungus is open to question. Since the life history of some of these are so incompletely known it is not surprising that we know so little of the salt-water species. Nevertheless, as early as 1865 Cohn[1] described three marine species of Chytridineae and pointed out[2] the difficulty in working with such minute structures and the ease with which they had often been mistaken for the fruit bodies in certain marine algae. Magnus[3] soon added to these *Chytridium tumefaciens* and *C. sphacellarum* and again reported *C. Plumulae* Cohn. Kibbe[4], who reports *Chytridium alarium* Kibbe on *Alaria fistulosa,* has cited the literature on the marine Phycomycetes and Pyrenomycetes so adequately that we will not repeat it here.

As to the Fungi Imperfecti occurring in saline habitats, Sutherland[5] has discussed the previous literature and described eight new species of Imperfecti representing widely different subdivisions which leads one to the belief that there might be a fairly extensive fungous flora in salt water.

During the summer of 1914 while studying the morphology of *Codium mucronatum* several specimens of a Chytridium were observed on the utricles. A search was made for the zoospores but they were not found

[1] Cohn, F. Chytridii species novae marinae. Hedwigia, Vol. 4, pp. 169-170. 1865.

[2] Cohn, F. Beitrage zur Physiologie der Phycochromaceen und Florideen. Archiv. f. Mikrosk. Anat., Vol. 3, pp. 1-60, *pl.* 1-2.

[3] Magnus, P. Chytridium. Sitzungs-Ber. d. Ges. nat. Freun z. Berlin. Vol. for 1872, pp. 87-90. 1872.

[4] Kibbe, A. L. Chytridium alarium on Alaria fistulosa. Puget Sound Marine Sta. Pub., Vol. 1, pp. 221-226, *pl.* 39-40. 1916.

[5] Sutherland, G. K. Marine fungi imperfecti. New Phytologist, Vol. 15, pp. 35-48. *Text figs.* 1-5. 1916.

until the summer of 1917. At this time there were also found the Rhizophidium and Stemphylium described below.

Chytridium codicola, sp. nov. (Pl. 20, Figs. 5-7). Zoosporangia erecta, sessilia, globosa vel ovoidea, 20—34 μ diametro metiens, hyalina; cytioplasma primo multis cum guttulis, deinde granulosum zoosporis nondum perfectis; operculum clausum invisible; apertum rotundum, 6—8 μ diametro, prope apicem sporangi; mycelium saccatum, simplex, vesiculosum, guttulatum, hyalinum; sporangia perdurantia endophytica non visa; zoosporae globosae, uniciliatae, hyalinae, uniguttulatae, 3—4 μ.

Habitat in utriculis vivis Codii mucronati. Washington. Aestate.

This species was collected on *Codium mucronatum* growing below the littoral zone and attached to rocks on the southern shore of Turn Island. This particular collecting ground is a transition between the Ulva-association and the Laminariaceae-association with Alaria, Laminaria and Ulva as the dominant genera and *Codium mucronatum* as a secondary species. The individual plants of *Chytridium codicola* were attached to the walls of the living utricles usually a short space from the mucrons. The zoosporangium is erect, sessile, spherical to ovoid, 20—34 μ in diameter, hyaline. At first the cytoplasm is many-guttulate but becomes granular before spore-formation. The wall is smooth and of one thin layer. The operculum when closed follows the contour of the zoosporangial wall so evenly that it cannot be detected until open. At maturity of the zoospores the operculum opens and remains attached to the margin of the aperture or disappears entirely. The circular aperture is about 6—8 μ in diameter and is located at or near the apex of the sporangium. The penetration tube of the zoospore develops into a saccate mycelium which is quite simple though often swollen or vesiculate, guttulate and hyaline. No endophytic resting sporangia were observed. The zoospores are globose, hyaline, uniguttulate, uniciliate and 3—4 μ in diameter.

Rhizophidium codicola, sp. nov. (Pl. 20, Figs. 1-4). Zoosporangia erecta, sessilia, globosa vel piriformia, 16—24 μ diametro metiens, hyalina; cytioplasma reticulatum, deinde granulosum, zoosporis nondum perfectis; mycelium est cellula irregularis, hyalina, multis cum filis ramosis, minutissimis, vix cytioplasmate hospitis distinguendis; zoosporae globosae, hyalinae, uniciliatae, 2.5—3 μ, ex sporangi apertione irregulare egressae.

Habitat in utriculis vivis Codii mucronati. Washington. Aestate.

This minute organism was found with *Chytridium codicola* on the utricles of *Codium mucronatum* collected on the southern shore of Turn Island by Miss Annie May Hurd during the summer of 1917. It was found in more abundance than the Chytridium. The zoosporangia are erect, sessile, sperical to almost pyriform, 16—24μ in diameter, hyaline; the cytoplasm is reticulate and then granular before zoospore formation. The mycelium is an irregular, hyaline cell with many radiating, branched,

delicate protoplasmic threads. These hyphae are very minute and often can barely be detected and distinguished from the cytoplasm of the host. The zoospores are globose, 2.5—3 μ in diameter, hyaline, uniciliate and contain one prominent oil globule. The zoospores are released from the zoosporangium through an irregular aperture.

Stemphylium Codii, sp. nov. (Pl. 20, Fig. 8). Epithelio-sori, ubi adsunt, irregulares, non limitati; hyphae subramosae, septatae, hyalinae, dilute maturitate fulvescentes, 5—7 μ; conidia subglobosa vel ellipsoidea, saepe irregularia, sessilia aut breve-pedicellata, apice rotunda, base late rotundata, 3—pluri-septata, septis constrictis, 37—46$\times$24—30 μ.

Habitat in thallis mortuis Codii mucronati. Washington. Aestate.

Clusters of utricles and branches of *Codium mucronatum* attacked by this fungus were pallid green, and where the fungus was abundant there was considerable distinegration. The sori of *Stemphylium Codii* are usually endothelial and very scattered and ramose, when superficial they are irregular and not definitely limited. The mycelium is somewhat branched and made up of septate, very slightly lomented, hyaline hyphae which are 5—7 μ in diameter. At maturity the hyphae change to dilute fulvescent in color. The conidia are borne on the prostrate mycelium, sessile or short pedicelled, but sometimes intercalary. They are subglobose to ellipsoid and often irregular in form, 3—many-septate; septate constricted, dark brown, 37—46$\times$24—30 μ. When the conidia are pedicellate the tips are rounded and the bases are broad and rounded.

PLATE 20

Rhizophidium codicola. ×800

Figs. 1, 3, 4. The organisms in a vegetative condition. Figs. 3 and 4 show early stages in the formation of the zoospores.

Fig. 2. An empty zoosporangium with the irregular mouth, and the zoospores escaped.

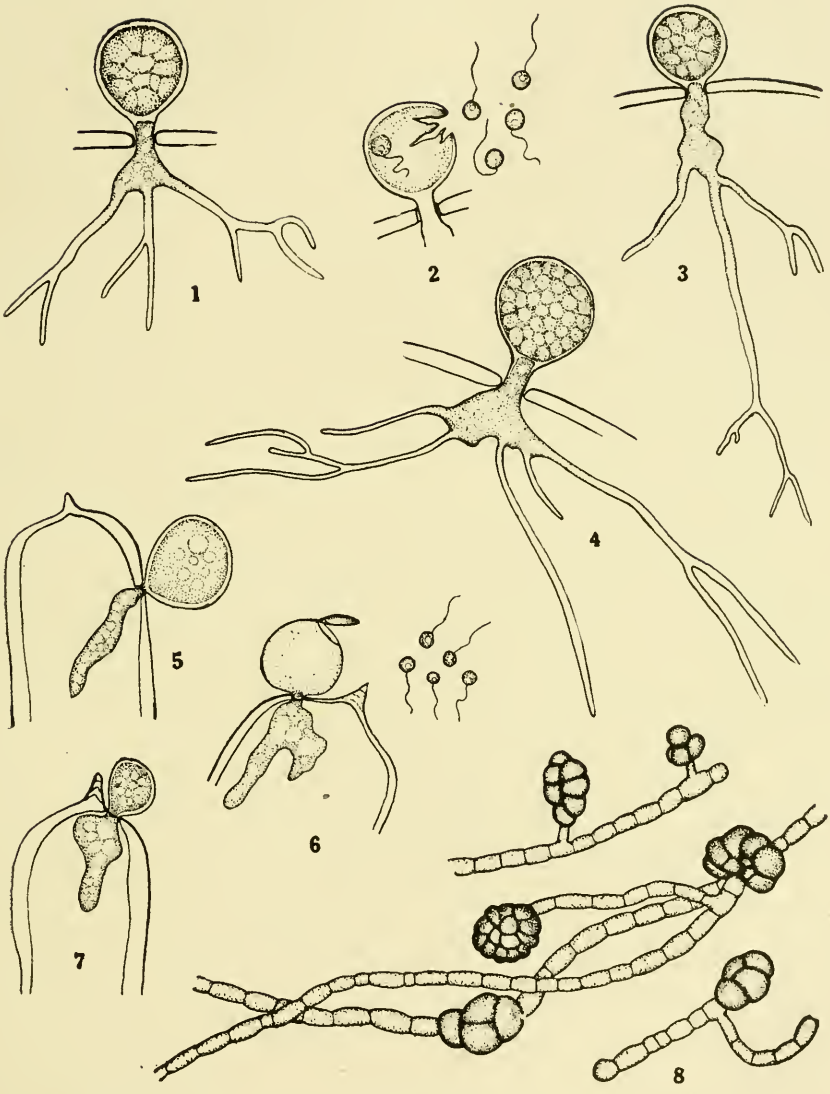
Chytridium codicola. ×350

Figs. 5, 7. The saccate mycelium and vegetative condition of the zoosporangium.

Fig. 6. An empty zoosporangium with the operculum open. The unicilate zoospores are shown.

Stemphylium Codii. ×350

Fig. 8. The prostrate mycelium with the muriform conidia. Both the intercalary and the pedicellate conidia are represented.



S. M. Zeller, Del. PLATE 20

Partial List of the Animals Yielding Embryological Material at the Puget Sound Biological Station

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The following record is compiled from the casual observations made in the conduct of a class in invertebrate embryology at the Puget Sound Biological Station during the summer sessions of 1917 and 1918. Complete data on the seasons of reproduction and on the breeding habits of the common marine forms in the vicinity of Friday Harbor would be most desirable and useful, but cannot yet be realized.

This record pretends merely to list the animals that undergo a part or all of their phases of reproduction during the time from late June to early in August; and although it is not a complete list it is hoped that it may serve as a partial guide to those interested in embryological material for classes and for research.

The record is arranged by phyla, giving the animals known to be either laying eggs, or having just passed this stage, during the time the Station was in session. It is intended to state whether the forms are abundant, common, or occasional; and also whether or not they may be found during the entire session. While the list is for the most part invertebrate, one or two vertebrate forms are included on account of the excellence of certain material.

That successive seasons vary considerably in the nature and abundance of available embryological material is certain; and a record of even a few forms over a number of years would help establish a norm from which a certain expectancy might be anticipated. From the experience of each season as the session closes (1917 and 1918), it becomes more certain that the list of reproducing forms may be very considerably extended. Prof. C. M. Child, Dr. H. B. Torrey, Dr. H. S. Brode, Dr. J. W. MacArthur, Dr. Nathan Fasten, and Mr. M. C. Riddle have called attention to numerous facts which are included; and of their generous interest acknowledgment is gratefully made. Specially are we indebted to Prof. Trevor Kincaid, who gave many suggestions out of his long experience in collecting in Puget Sound waters.

While the Biological Station happens to be in session when it is most favorable for investigators to get away from college duties, it does not coincide with the time when most of the animal life is at the height of its

reproductive phase. Some forms, like the echinoderms, have passed their egg-laying period before the Station opens, so that usually only a few belated forms may be observed. Others, as the coelenterata, are abundant, and present wonderful opportunities for work with the eggs of medusae and ctenophora. The same is true of the ascidians. In still other groups the season is too early. However, the University of Washington plans sometime soon to keep the station open throughout the whole summer; this will greatly extend the possibilities for embryological work, so that many forms not in this list may be discovered.

Porifera

Grantia (?). Common on the piles and floats near the laboratory and in the hauls with the trawl, June to August. Individuals kept in the laboratory failed to discharge eggs or larvae, but eggs were easily obtained from teased material.

Esperella adhaerens Lambs. Abundant on Pecten shells, from June to August. Eggs obtained 1917, but none showed signs of development.

Myxilla parasitica Lambs. Abundant on Pecten shells brought up by trawl during month of July. Eggs were obtained, but none were found in process of development (1917).

Coelenterata

The eggs of the medusae given below may be obtained and fertilized, when the adults are mature, merely by leaving a number of individuals in a dish of water for one to several hours. Many lay eggs at night. The eggs will be found on the bottom of the dish, or floating if the water is sufficiently agitated by the movements of the medusae.

Phialidium gregarium Haeckel. Abundant in Friday Harbor and easily obtained from docks in front of the laboratory, June to August. Eggs may be obtained in quantity and are large and transparent. The hydroid was raised in 1917 by Mrs. T. C. Frye, and proved to be *Clytia inconspicua* (Forbes) Fraser.

Sarsia rosaria Haeckel. Common in Friday Harbor and can be obtained from docks in front of the laboratory, June to August. During the summer of 1918 they were less common than in 1917. They vary greatly in numbers from day to day. The eggs are large and clear, and cleavage is easily followed. The hydroid was raised in 1917 by M. C. Riddle, and proved to be a Syncoryne.

Stomatoca atra Agassiz. Common in Friday Harbor, and may be easily obtained from the docks in front of the laboratory, June to August. It was fairly abundant during the summer of 1917, and less so in 1918. The number of individuals is variable from day to day. The eggs are rather small and with yolk, so they are not transparent. Individuals lay

a great many eggs, and more often at night than during the day. The hydroid of this medusa is a tubularian, probably some species of Perigonimus.

Polyorchis minuta Murbach & Shearer. Common in Friday Harbor, and easily obtained from the laboratory floats, June to August. It is never seen in large numbers and is variable from day to day. The eggs are large and clear. It was rather scarce during 1918. The hydroid of this is not known. An attempt should be made to raise these from the eggs.

Aequorea forskalea Peron and Lesueur. Abundant in Friday Harbor during June, July and August. It is easily obtained almost any day from the laboratory docks. The eggs are large and clear, and the cleavage stages are easily followed. The hydroid is a minute Campanulina.

Thaumantias cellularia Haeckel. Abundant in Friday Harbor at almost any time from June to August. They sometimes appear in great numbers, and can be obtained from the Station floats. The eggs are the largest of any among the common medusae found in this vicinity, and are transparent. The hydroid is a Thaumantias. Development of the eggs in the laboratory is difficult since the cleavage stages are very sensitive to any change of conditions.

Diphyes sp. Occasional specimens were picked up floating by the Station docks. Many were seen during the summer of 1917, a few in 1918. Eggs were obtained in 1917, and Mr. M. C. Riddle observed part of their development.

Haliclystis sp. Common on the eelgrass at False Bay on the west shore of San Juan Island; occasionally found on eelgrass in Argyle Bay, also near Twin Island. They can be obtained at extreme low tides. Developing eggs were secured in 1917 in early August.

Aurelia sp. Adult specimens are scarce. Two ephyra were casually picked up at the Station floats in 1917 and one in 1918.

Epiactis prolifera Verrill. Abundant on the eelgrass at low tide in Friday Harbor. This is the only actinian observed to be reproducing. The eggs are large, but opaque with reddish brown yolk. It seems likely that development occurs to an advanced stage within the parent body, and that the young migrate from their position of origin to their conspicuous location outside, attached to the body of the parent in a manner imitative of budding. As all sizes of these young are obtainable all summer the reproductive season is probably quite prolonged.

Caryophyllia sp. A simple coral common on rocks exposed at low tide on the exposed channel of Turn Island; also on the Sucia Islands. In 1917 swimming larvae thought to be those of Caryophyllia were noticed in a dish in which some specimens had been brought into the laboratory.

Mnemiopsis sp. Common forms easily obtained from Station floats,

June to August. The eggs are beautifully clear; the bilateral cleavage may be perfectly followed in the living eggs. This is one of the best forms for the study of cleavage. The whole development may be followed in 3 or 4 days, and the young may be obtained by towing.

Pleurobrachia sp. Common thruout the season in Friday Harbor, and obtainable from the Station floats. These were not as abundant during 1918 as in 1917. The eggs are large and clear, and are easily raised in the laboratory.

Beroe sp. Not as common as Mnemiopsis or Pleurobrachia. These come rather late in the season, July to August. The eggs are smaller than the forms mentioned above. The animal can easily be raised in the laboratory.

Echinodermata

Orthasterias forreri forcipulata Verrill. Occasionally brought up in the trawl. Ripe eggs and sperms were obtained during the season 1917, and gave normal bipinnaria larvae in the course of four or five days.

Solaster dawsoni Verrill. Commonly secured by means of the trawl thruout the season. Eggs and motile sperms may be obtained. Specimens taken July 15, 1918, showed immature ova.

Evasterias troschelii (Stimpson) Verrill. Common in hauls of the trawl and occasionally found along the shore. They carry eggs and motile sperms, June to August.

Ophiura brevispina Say. Abundant in certain localities in hauls of the trawl. Eggs are present, but the sperms are only in part motile. They may have a fall reproductive season.

Amphioplus abditus Verrill. Occasional under rocks at low tide from Minnesota Reef to Argyle Bay. The eggs were ripe but the sperms were only in part motile. They may have a fall reproductive period.

Gorgonocephalus eucnemis Müller & Troschel. Occasionally secured with the trawl. The gonads are turgid with heavy yolk-filled eggs. The sperms are in part motile. They may have a fall reproductive period.

Strongylocentrotus drobachiensis O. F. Müller. A very abundant form obtained along shore and in shallow trawling. Occasionally individuals will be found to contain ripe eggs or sperms late in June or early in July, but for the most part the gonads are spent, and regressive changes are going on.

Strongylocentrotus franciscanus A. Agassiz. Abundant along shallow water from Pt. Caution to Turn Island. The season is evidently earlier than June, when the Station opens. Occasionally during June, July, and August individuals will be found containing ripe eggs or sperms. During July 1917 this urchin yielded material in abundance, but this was not so in 1918, when it was exceptional to find specimens with ripe reproductive

products. A number of mature females were obtained near Turn Island thruout the month of July, 1918.

Strongylocentrotus purpuratus Stimpson. Rare specimens now found on reefs near Friday Harbor, formerly quite common. Occasionally individuals will be found with ripe eggs or sperms, but for the most part they are spent before June.

Echinarachnius excentricus Eschscholtz. Common forms found in sand at False Bay. By July 7, 1917, and July 7, 1918, these forms were quite spent.

Cucumaria japonica Semper. Abundant along shore among the rocks, and at low tide. Eggs taken in the early part of the season, altho apparently ripe, failed to develop when fertilized. The probability is that their season was just coming on. The eggs are small and opaqne.

Cucumaria chronhjelmi Theil. Abundant along shore and in hauls by the trawl, specially in the mud of East Sound. The eggs are small, greenish and opaque. No development was obtained during the June to August session altho eggs and sperms apparently ripe were found. The season probably came later.

Leptosynapta inhaerens (O. F. Müller) Verrill. Abundant at False Bay. The phases of reproduction passed early in the summer. No ripe eggs could be obtained by July 7, 1918.

Stichopus californicus (Stimpson) Edwards. Abundant forms in shallow water near the Station, and by trawling. The eggs are large, translucent and colored a delicate pinkish cream. No developing larvae have been obtained, but the indications are that the season for this holothurian is later rather than earlier. By July 15, 1918, individuals were found yielding well formed eggs which discharged freely from the torn ovaries. Owing to the size and clearness of the eggs, the development of this must be a wonderful sight. Fertilization was obtained with these eggs. Cleavage did not begin for five hours after the addition of sperms, and but few developed further than the 4-celled stage. Either the eggs or the sperm may not have been mature. Careful observation should be made on these forms as the indications are that good embryological material may be found in this holothurian.

The following starfish had passed their season (1917): *Pisaster ochraceus* (Brand) A. Agassiz, *Luidia foliolata* Grube, *Leptasterias hexactis* (Stimpson) Verrill, *Pycnopodia helianthroides* (Brand) Stimpson, *Dermasterias imbricata* (Grube) Perrier, and *Pteraster tesselatus* Ives. However, judging from the voluntary extrusion of sperms from the males of Piasaster and Luidia, the season may have been on shortly before the Station opened. In former years Leptasterias has been known to begin

development the latter part of February, with completion in April or early May.[1]

Mollusca

Saxidomus giganteus Deshayes. Common on beaches at low tide. Ripe eggs and motile sperms were seen by Professor Trevor Kincaid during the first week of July, 1918. Pecten larvae were found in tow on July 1, 1918.

Mya arenaria Linnaeus. Common mud clam fund in sand and mud of nearby beaches. The eggs and motile sperms may be obtained from torn gonads.

Mytilus edulis Linnaeus. Common mussel found on rocks and piles. The eggs and active sperms are easily obtained from torn gonads.

No marked activity of the gonads was noted in Marcia, Paphia, Entodesma, Macoma, Monia, or Cardium; so these were considered out of season (1917).

Lacuna porrecta Carpenter. A very abundant mollusk with wide distribution, living on kelps and eelgrass. The eggs are doughnut-shaped and may be obtained in all stages of development thruout the season.

Haminea vesicula Gould. Abundant at low tide among the Ulva and eelgrass. The height of the reproductive season is July and August. Common on the beaches at Brown Island and Newhall's Beach. The egg masses are 12-38 mm. in height, and are whitish balloon-shaped bodies, with numerous eggs enclosed in a gelatinous substance. These may be anchored in mud or on eelgrass. The masses are best collected between five and nine o'clock in the morning, to obtain the earliest cleavage stages; for the mollusks seldom are found laying after the early morning hours. The habits of this animal are well described by Leonard.[2]

Thais lamellosa Gmelin. A very abundant mollusk found on the rocks at low tide everywhere. The egg capsules are commonly called "Sea Oats." The height of their season is before the station opens, the last of June; but belated individuals have been found depositing their capsules of eggs as late as July 7 (1918). For the most part, by June the capsules will be found empty or the embryo in very advanced stages.

Argobuccinum oregonense Redfield. A very common mollusk found on reefs. The eggs in all stages of development may be obtained from June to August. It is one of the best sources of embryological material because of the abundance, accessibility, and ease with which the animals may be raised in the laboratory. Care must be exercised in removing capsules from the rocks, so as not to rupture them, for unfortunately develop-

[1] Osterud, H. L. Preliminary observations on the development of Leptasterias hexactis. Pub. Puget Sound Biol. Sta. Vol. 2, pp. 1-15. 1918.

[2] Leonard, Ruth. Early development of Haminea. Pub. Puget Sound Biol. Sta. Vol. 2, pp. 45-63. 1918.

ment does not proceed normally in sea-water. Specimens brought to the laboratory have laid eggs on the side of live-boxes out of doors, but none have given normal results in the laboratory aquaria. The early stages are easily followed, but later stages necessitate stained mounts. The veliger stage is passed in the shell.

Acmaea pelta Eschscholtz, and *Acmaea patina* Eschscholtz. These two limpets are very abundant on the rocks between tide levels. The ovaries are crowded with large angular eggs with large germinal vesicle and distinct chorion; so are evidently approaching maturity (July 1917).

Crepidula adunca Sowerby. Occasional animals associated always with other shells. They are found in shallow water in trawling and along rocky shores. The eggs are very large and filled with a yellow yolk which makes the eggs quite opaque. Six to eight eggs are laid in a single balloon-shaped capsule, and several capsules attached in a small bunch. The eggs are laid early in July (1918).

Cryptochiton stelleri Middendorf. Common on reefs at extreme low tides, and in trawling in shallow water. They had passed their season before the last of June in 1917, and by July 15 in 1918.

Katherina tunicata Sowerby. Abundant forms easily collected along shores at low tides. They were probably just approaching a late reproductive season. Ripe eggs and motile sperms were obtained during late summer in 1917, and also by July in 1918. The eggs are small, dark-colored and opaque.

Tonicella lineata Wood. Common forms along shores at low tide. They yielded ripe eggs and motile sperms about the first of August in 1917.

Mopalia muscosa Gould. Common form along shore at low tide. They yielded ripe eggs and motile sperms near Aug. 1 in 1917, and also during July in 1918. The eggs are small, opaque and very dark.

Phyllophysia sp. A rare green-colored nudibranch found on the eelgrass of Fisherman's Cove. Freshly laid egg masses were brought up with the trawl on July 8, 1918.

Aeolis sp. A very abundant nudibranch which lives on the eelgrass and kelps. The animal is a small aeolid with grayish orange-tipped branchiae. The egg masses were being deposited June 24 to July 16 in 1918. The height of the season is passed by this time, but occasional specimens may be discovered laying eggs even later. The egg capsules are deposited in whitish or pinkish gelatinous strings constricted somewhat like sausages. This string is wound in a zig-zag spiral from a central starting point, producing a rosette which may be as much as 38 mm. across. The animals may be brought into the laboratory, where they will continue their egg laying.

Anisodoris nobilis MacFarland. A common yellow nudibranch found

on reefs at low tide and on piles under wharves. This is commonly called the sea lemon. The egg masses are common during the early part of the summer session, June 24 to July 16. The eggs are laid in a yellow ribbon 16-22 mm. wide, attached at one edge and wound in a spiral which is closer on the attached than on the free edge. This animal may be brought into the laboratory, where it will continue to lay eggs.

Melibe lenina Gould. Occasional. A large nudibranch found on the eelgrass on the channel side of Brown Island. The egg masses are similar in form to those of other doridae, except that they are creamy-white in color. It is found early in season rather than later.

Annelida

Nereis virens Sars. Occasional thruout June to August. While the adult specimens are abundant on the beaches near the Station, but comparatively few animals are taken that yield ripe eggs and sperms.

Nereis agassizi Ehlers. A single specimen was picked up near Brown Island on Aug. 4, 1917; and shed its eggs in the laboratory during the night. It is possible these may be obtained earlier. Occasionally they have been seen in the act of swarming, but no definite period can be set. The larvae are taken in considerable number in the tow early in the season.

Serpula columbiana Johnson. Abundant. The worm may be most easily collected at Turn Rock or Minnesota Reef, where its tubes will be found attached to the numerous smaller rocks, which may be brought into the laboratory with the worm uninjured. They yield quantities of eggs. The extrusion of the polar bodies is very conspicuous and development progresses rapidly.

Amphitrite robusta Johnson. Abundant forms living in sand-tubes under rocks at low tide. They yield eggs and partially motile sperms toward the end of the season, and are then probably approaching their season.

Amphitrite spiralis Johnson. Abundant forms living in sand tubes under rocks at low tide. The season for egg-laying is probably during August, since partially ripe eggs and sperms can be obtained during the last days of July and early in August.

Polynoe squamata A. & M. Edwards. Common forms. They were approaching their season on Aug. 4, in 1917, and somewhat earlier, July 6, in 1918, judging from well developed eggs and motile sperms found in several individuals.

Arenicola claparedei Levinsen. Abundant in sand at False Bay. Recently laid egg masses were numerous by July 7, 1918.

Glycera, Clymenella and Arenicola appeared to be spent when examined during the season of 1917; but Arenicola was in the midst of egg-laying on July 7, 1918.

Molluscoidea

Terebratella transversa Sowerby. Common in shore collecting and trawling. It yields numerous translucent eggs; but no motile sperms have been found.

Platyhelminthes

Leptoplana sp. Fairly common under stones on Turn Rock, Minnesota Reef and adjacent shores at low tide. The animals will deposit eggs in dishes or on submerged slides. Egg patches contain a single layer of eggs, one to each capsule, embedded in a yellow matrix. The heavy yolk hinders the study of later stages. The eggs are very hardy and withstand stagnation conditions for some time. Their development is slow.

Cerebratulus montgomeryi Coe. No ripe eggs were obtained, but many pilidium larvae were found in tow during the latter part of the season.

Arthropoda

Epialtus productus Randall. Abundant on eelgrass, on kelps, and on piles under the docks; common to a depth of at least 40 fathoms. Individuals carrying eggs were found frequently thruout the summer session.

Telmessus cheiragonus Rathbun. Occasionally found at Brown Island, Arglye Lagoon, Turn Island, Madrona Point to Minnesota Reef. Females carrying eggs were observed during the summer of 1917 and early in July of 1918.

Hyas lyratus Dana. Abundant forms with wide distribution. Common in trawling. Females carrying eggs, also early stages of development, were observed during the summers of 1917 and 1918.

Hemigrapus nudus (Dana) Rathbun. Abundant crabs along shore. It is the common purple shore-crab. Females carrying eggs are commonly found early in the session.

Upogebia pugetensis Dana. Common in sand-beaches along with Nereis. Specimens with eggs found occasionally; the eggs hatch out as zoea. Their period extends at least from June to August.

Upogebia sp. This was found carrying eggs during the summer session.

Caprella sp. A very abundant amphipod found on the eelgrass generally, and in large numbers among the hydroid colonies on the eelgrass at False Bay. The eggs are carried in brood sacs from which the young are liberated as zoea. Larvae were well advanced by July 7, 1918.

Balanus aquilla Pilsbry. Common. A very large barnacle brought up in the trawl. It yields embryos in various stages. The larvae are liberated as nauplii. The eggs in early stages of development were found on July 15, 1918.

Balanus cariosus (Pallas) Darwin. Common shore barnacle. Developing embryos were found in the mouth cavity and liberated as nauplii.

Balanus evermanni Pilsbry. Abundant form along shore. Developing embryos were found which were liberated as nauplii.

Peltogaster sp. Found rarely as parasite on abdomen of hermit crabs. Individuals full of developing nauplii were found in the summer of 1917. The animals were also found in large numbers in the atrial cavity of the tunicate, *Styela gibbsii* (Stimp.) Herd. The eggs had not yet begun development.

Bopyrid sp. Occasional. It is a parasitic isopod found in the gill-chamber of shrimps. Males and females were found together, the female full of eggs which were unfertilized.

Cancer gracilis Dana. Common crab found in shallow water and in trawling. One specimen was found with eggs in trawling off O'Neil Island. The eggs masses were very large and the eggs in the early stages of development (July 16, 1918).

Cancer oregonensis (Dana) Rathbun. Found in shallow water on rocky points near the Station. The females carry eggs during the summer.

Petrolisthes eriomerus Stimpson. Found on rocky shores near the Station. The females carry eggs during the summer.

Lophopanopeus bellus (Stimpson) Rathbun. Found on rocky shores near the Station. The females carry eggs during the summer.

Oregonia gracilis Dana. Found at nearly all depths in the vicinity of the Station. They are especially abundant on the piles of the Friday near the Station. The females carry eggs during the summer.

Scyra acutifrons Dana. Found on the northeast side of Brown Island at 20-50 fathoms. The females carry eggs during the summer.

Chordata

Corella willmeriana Herdman. Abundant on wharves and flats in the harbor and quite commonly brought up in trawling. The eggs are large and clear and may be found in various stages of development in the atrial cavity. This furnishes splendid class material thruout the season.

Cynthia haustor Stimpson. A common tunicate on reefs at low tide and in deeper waters. Eggs in various stages of development may be found in the atrial cavity.

Styela stimsoni Ritter. Common under rocks on reefs. The eggs were large and clear but still remained in the ovary at the end of the session in August 1917.

Styela gibbsii (Stimpson) Herdman. Common under rocks on reefs. The eggs were large and clear but still remained in the ovary at the end of the session (August 1917).

Chelysoma productus Stimpson. Common in trawling, and known as the horse-shoe ascidian. Large clear eggs were found in the ovaries at the end of the season (August 1917).

Vertebrata

Hydrolagus colliei (Lay & Bennett) Gill. Common in trawling, but rarely are the eggs of these found.

Raja binoculata Girard. Common skate found in waters of the Sound. The egg cases brought up in trawling during July very often contain a good series from the eggs with the blastodermic disk formed to young with the yolk-sac nearly absorbed. When the trawl brings up these egg-cases it nearly always brings up at the same time six and perhaps more. There may be one to five eggs in a single case, and almost invariably the eggs from the different egg cases may be arranged in a series, thus suggesting that the skate lays her eggs all in one place or nest.

A species of cottoid fish lays large masses of eggs which are clear and stuck together with a gelatinous substance. They may be seen at low tide fastened to the side of rocks. These are found only early in the season, June to early July.

Some Experiments with Fucus to Determine the Factors Controlling its Vertical Distribution

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Even a casual observation convinces one that Fucus is rarely found below the low-tide line, certainly rarely if ever reaches the high-tide line, and occupies rather a small portion of the area between its upper and lower limits which one would expect it to cover. During the summer of 1918, at the Puget Sound Biological Station, the writer attempted to determine some of the factors governing the local distribution of *Fucus evanescens* Agardh. This is by far the most common species of the region, in fact, except in rare cases, the only one in the immediate locality.

Studies were made on San Juan Island, Brown Island, some of the Sucia Islands and Turn Rock. My observations showed that the lower and upper limits of the Fucus zone were very variable on exposures in different directions. The lower limit is higher and the upper limit lower on north, northwest and northeast exposures. Thus the zone is comparatively narrow, and indeed is usually wanting on high and steep north shores. The upper limit of the Fucus zone on the south, southeast, east and southwest exposures is often almost a straight line parallel to the high-tide line. The upper limit of barnacles was taken as the high-tide line. The Fucus zone on these exposures often almost reaches this upper limit. The lower limit of the zone on south exposures is usually about the zero tide-line. Some Fucus is found below this, but only a little, and only near that line.

Turn Rock is an oblong reef composed entirely of rock and is about 40 by 100 meters at a −1.1 tide. A ridge about 6 meters above a −1.1 tide extends from the southeast to the northwest. According to the water marks on the concrete base of the lighthouse, the high-tide line was one meter above its highest point. The lower limit of the Fucus zone was ⅔ meters above this −1.1 tide. There were only a few scattered plants at the low-tide line, and below this practically none. The northwest half of the rock had much less Fucus growing upon it than the southeast half. The north third of the rock was practically destitute of this plant. The Fucus that did grow on the northwest half was just over the top of the ridge and on the south and southeast sides of the higher projections.

These observations led the writer to measure the vertical space occupied by the Fucus zone on various shores. Measurements were made for some distance north and south of the Station laboratory and also on the southeast and northeast exposures about Point Caution. The shores

of all these regions are of solid rock with occasional stretches of gravel or boulders. The average vertical width of shore occupied by the Fucus zone on east slopes was over 1.5 meters. This average includes the coves, and the shores covered by wharves and by the Station laboratory, all of which showed little or no Fucus. The average vertical width of shore occupied by the Fucus zone on the south was over 2 meters, and on the north less than ⅓ meter. The higher the shore on the north, northwest and northeast exposures, the narrower was the Fucus zone.

The following are some of the more pertinent facts observed in a general study of various kinds of shores:

1. Shores under overhanging trees have little or no Fucus.
2. Average north shores have a very narrow Fucus zone.
3. North exposures with a high shore-line have no Fucus zone.
4. Exposures abounding in Fucus have none under wharves, Station Laboratory, etc.
5. The darker a cove the less Fucus on its shores.

The graphs drawn from the measurements of the Fucus zone on both sides of the Station and about Point Caution are shown in chart 1. It will be noticed (chart 1) that the Fucus zone practically disappears under the wharf and that there is practically none under the laboratory. Observe how narrow the zone is on north slopes. From this it appears that light is a factor.

Experiments were then set up with a view to determining the effect of different light intensities on Fucus, and if possible to find how much light was necessary for its growth.

In order that the plants might be grown under different light intensities four lath shade-crates were constructed. These were ½ m. deep and 1 m. square. Both top and sides were made of laths. The fraction of the total area which was left as space between the laths was the fraction of total daylight which could enter. The plants were left in their natural habitat in all cases, and the crates set over them. With mature plants the following series was arranged:

1. Plants in total daylight, by leaving them wholly uncovered.
2. Plants covered with a lath shade-crate, thru which only half the light could enter.
3. Plants covered with a lath shade-crate thru which only a fourth the light could enter.
4. Plants covered so that only an eighth the light could enter.
5. Plants under inverted tubs, and thus in total darkness.

Heavy cords were tied to each upper corner of the shade-crates, and to each of these a heavy rock was tied. The rock rested on the shore. This was security against the action of the tides. The tubs were held

in place by laying a heavy rock upon each. It was also necessary to cut a small slit in the top of each tub to allow the air to escape while the tide was rising. Two tubs were used, but only one crate of each kind was constructed.

These shade-crates were placed over mature plants; two weeks later young plants were also placed under them. In this manner the Fucus plants were grown under conditions as normal as possible, except for light. Since Fucus grows in various latitudes, the slight variation in temperature caused by the shade-crates was considered negligible. It is true that the denser the shade the greater the atmospheric moisture. It is also probable that bacteria are more abundant in darker habitats. On the other hand diatoms, having chlorophyll, are likely more abundant in light. Diatoms are among the most destructive parasites of seaweeds. While, therefore, other factors may have varied some, these were avoided so far as possible.

The crates were examined every week for nearly six weeks. No change could be noticed until about the end of the third week. At this time the plants growing in darkness had become a darker brown, and decay had begun in spots, specially about the edges. The plants under the crates which only 1/8 of the light penetrated were also darker in color, but no indication of decay could be seen. By the end of the fourth week these plants had also begun to decay. Under both crates, a considerable number of the Fucus plants softened just above the hold-fasts and broke loose. No difference could be observed in the plants under the crates which more than 1/8 of the light entered.

TABLE 1. *Mature Fucus in natural habitat exposed for 6 weeks to different light intensities*

Light	Decomposition	Color
Darkness	Marked	Blackish
1/8 light	Distinct	Darker
1/4 light	Doubtful	Doubtful
1/2 light	None	Unchanged
Full light	None	Unchanged

At the end of the six weeks (table 1) all of the plants under the crates that allowed less than 1/8 of the light to pass thru were dead or were no longer growing. The conditions for the growth of Fucus were as nearly the same as possible for all crates, except for the amount of light received. Unfortunately the observations could not be continued for a longer time; more conclusive results might have been secured concerning the plants receiving more than 1/8 of the total light. The results show, however, that 1/8 daylight is roughly the minimum limit for Fucus, unless juvenile plants are more sensitive.

Even a very casual observation shows that Fucus is not abundant on a gravelly beach. When it does occur in such a habitat it is nearly always found on boulders or firmly planted rocks which project above the surface. There are probably two factors which make the growth of much Fucus on gravel impossible:

1. The movement of stones by the action of the tides, thus causing the oospores to be rubbed off.

2. The smoothness of the stones, thus causing them to become thoroughly dry during low tide and interfering with germination.

It is possible that decomposing plant and animal deposits on flat beaches with little movement of gravel affect Fucus unfavorably. Three attempts were made to grow the oospores of Fucus on smooth beach stones and shells between the low- and high-tide lines. Fucus plants containing large, slimy fertile tips were secured in the evening from Fucus beds. These were wrapped rather tightly in a towel and left there over night. In the morning they were unwrapped and dried in a room for 30 minutes. The tips containing the conceptables were broken from the plant body and placed in a battery jar containing seawater. In about 10 minutes the oospores were discharged. In 30 minutes the oospores had settled to the bottom of the jar, and the plant-tips, together with all but a very small amount of the water at the bottom of the jar, were poured off.

Shells of average size, and white, smooth stones, were placed in a flat porcelain pan and covered with seawater. A pipette was used in planting the oospores on the shells and stones. They were then left quietly in the pan for 48 hours. The water was changed on the second day by means of a rubber siphon. After 48 hours the sporelings usually were 10-celled, and were securely fastened to the stones or shells by a layer of mucilaginous substance. Stones and shells were planted under various conditions. All were fastened to the substrata to prevent the sporelings from being rubbed off. A series of experiments was begun as follows:

1. A shell and a stone were placed on the beach.
2. A set of each was placed in a Fucus bed.
3. A set was placed under the crate that received ½ light.
4. A set was placed under the crate that received ¼ light.
5. A set was placed under a crate that received ⅛ light.
6. A set was placed under an inverted tub, under which was total darkness.

The first set of sporelings on the beach disappeared, rocks and all, one week after they were started. However, they were not growing. Two other sets were started at once. These did not grow and had disappeared from both stones and shells by the eighth day. Observation showed that the smooth stones and shells were dry for two or three hours

during low tide each day, while the rock upon which Fucus grew was moist in the angles and cracks. The sporelings were not able to withstand these severe periods of desiccation. This seems to be a strong factor in almost excluding Fucus from the beaches.

The stones and shells serving as substrata for the sporelings in the Fucus beds, as well as those underneath the crates which received 1/8 and 1/4 light, were moist for longer periods than on the beaches. However sufficient moisture was not retained on their smooth surfaces to produce growth in the sporelings and they were lost from the shells on the 15th day. Since the sporelings died among the Fucus beds at about the same time as under the crates, light cannot be considered a very important factor here. The sporelings attached to shells and stones under the crate receiving 1/8 light grew for about three weeks. They gradually lost much of their color and lost all symptoms of growth; but they were still attached to both the stones and the shells at the end of five weeks. They probably grew until the reserve food of the oospore had been exhausted, together with whatever food the plant was able to manufacture under the reduced amount of light. The food requirements of the young plants were probably too great for the conditions; thus growth ceased and death would undoubtedly have followed.

The sporelings attached to the shells and stones under the inverted tubs grew for about a week only. Apparently when the food stored in the oospore was used up, growth ceased, since photosynthesis could not go on in total darkness. At the end of the 8th day they had become much lighter in color, and no more growth could be detected. By the end of the third week some had been lost from their substrata; and by the end of the fourth week none could be found. Under the crate receiving 1/8 light, and under the inverted tub, evaporation was reduced to such an extent that they had sufficient moisture for growth; but the light was so reduced that probably the necessary food could not be manufactured since death resulted.

To study the effects of submergence, young plants that had about two lobes, and also mature plants, were suspended in the sea at different depths. Chips of rock to which the Fucus plants were growing naturally were secured. The locality was just off shore from a Fucus bed. The depth of the water at the spot was about 9 meters. Heavy cords each 7 meters long were used. Rocks containing the Fucus plants were fastened to the cords at intervals of 3 dm. for the first 3 m. from the surface. Below 3 m. the rocks containing the Fucus plants were fastened at intervals of 6 dm. Two cords containing young plants and two containing mature plants were suspended. These were examined once each week.

At the end of three weeks the plants below 12 dm. had a dark color; the plants above this showed little change at this time. At the end of four weeks the younger portions of the plants below 12 dm. were dropping off, leaving only the half decomposed bases. The plants above 12 dm. still had some of the younger portions adhering at the end of nearly six weeks; but no growth had taken place, and many of the younger parts were easily shaken from the older portions of the plants. On three cords, no young portions of the plants were adhering to the bases after five weeks. On the fourth cord young portions of the plants were found adhering to the older parts at a depth of 1.5 meters. All the cords were suspended within a radius of 10 feet, and conditions were seemingly uniform. Two plants at a distance of 1 m., three plants at a distance of about 4.5 meters, and five at a distance of about 6 meters or more below the surface of the water, had become decomposed at the hold-fast or just above it, and the plants were lost. Table 2 is a summary of these results.

Table 2. *Fucus with natural attachment suspended in the sea at different depths*

Depth below surface	Period of time	Condition of plants
3 dm.	6 weeks	No growth; little decomposition
Above 1 m. and below 3 dm.	6 weeks	No growth; more decomposition
Below 1 m.	4 weeks	No growth; much decomposition; 10 plants lost from decomposition just above hold-fast

These plants were submerged all of the time, since the cords were suspended from floating logs. Had all plants been affected as soon and in the same manner, the cause might be submersion. However, since the plants in the deeper water were affected first, and to a much greater degree than those nearer the surface of the water, the writer considers that light is at least a much greater factor than mere submergence. It couldn't be temperature, for the difference from surface down is only a few degrees, and this same species persists on rocks until well into the winter, I am told. It could hardly be CO_2, for the movement of the water, specially about rocky shores in tidal streams would distribute the CO^2 rather thoroughly. It might be that bacteria would be more effective in darker regions; however, that again refers it to light.

Considering that sporelings might be affected more than were older plants, the following series of experiments was not set up for observation:

1. Sporelings constantly submerged, but under different light intensities.

2. Sporelings exposed to the air a part of each day, growing under different light intensities.

3. Sporelings that had 3 weeks of good growing conditions, and then were placed under different light conditions.

The sporelings were grown in a south window on shells in a glass dish. Oospores were obtained in the manner previously described. Porcelain bowls 1 dm. in diameter were used as containers for seawater in which the oospores were planted on shells and on microscopic slides. The water on these sporelings was changed daily at 8 A. M. and at 2 P. M. One series was placed in a south window and the other on an east shore above a Fucus bed. In series number 1 of the above, in which the sporelings were submerged continually, the following conditions were tried:

a. A bowl uncovered.
b. A bowl admitting ½ light.
c. A bowl admitting ¼ light.
d. A bowl admitting ⅛ light .
e. A bowl covered so that no light entered.

TABLE 3. *Sporelings submerged in bowls all the time under different light intensities*

Light received	1st week	2nd week	3rd week	4th week
Total light	Short hold-fast	Short hold-fast and more growth of plant body	Much growth of plant body; dark brown in color	Much growth of plant body; hold-fast dark brown color
½ light	"	"	"	"
¼ light	Slightly longer hold-fast	Slight increase in size of both hold-fast and plant body	Slight increase in size of plant body	Some growth but color some lighter
⅛ light	Longer hold-fast	Some growth of plant body	No growth and color; much lighter	Little or no growth and pale color; cells breaking down
No light	"	Little growth of plant body and hold-fast unchanged	"	"

The sporelings of series number 1 (table 3) were grown for about 4½ weeks. At the end of the first week of their growth, those receiving no light were the largest; those receiving ⅛ light were next in size. Little if any difference existed among those receiving all the light, ½ light, and ¼ light. The difference in size between those receiving less light and those receiving more, consisted in greater length of the hold-fasts of the former. At the end of the second week the sporelings receiving only ⅛ light and those receiving no light were still longer, but the part of the sporeling that develops into the plant body of those that received

all the light, was larger than the part of the sporeling that develops into the plant body of those receiving only 1/8 of the light and of those receiving no light at all. At the end of the fourth week the order of size was reversed. The sporelings receiving all the light were a third larger than those receiving 1/2 light and twice as large as those receiving no light. There appeared to be no difference in size between the sporlings receiving 1/8 light and those in total darkness. Those in total darkness had a much lighter color than those receiving 1/8 light. Evidently the growth of constantly submerged sporelings varies with the light.

The sporelings receiving most light had a dark brown color. As the light intensity was reduced, the color in the sporelings became lighter. All conditions were as nearly as possible the same except light. The larger size and better color of the sporeling in the light was previously explained in the case of sporelings receiving insufficient light. In series number 2 of the above, in which sporelings were exposed to the air one hour each day as the water was changed, the following conditions were tried:

a. A bowl uncovered.
b. A bowl permitting 1/4 light.
c. A bowl permitting 1/8 light.
d. A bowl permitting no light to enter.

Table 4. *Sporelings exposed to the air for one hour each day, and having different light intensities*

Am'nt of light received	1st week	2nd week	3rd week	4th week
Total light	Short hold-fast; 50% of sporelings came loose from substrata	Growth of plant body	Hold-fast branching; some growth of plant body; color dark brown	Continued growth; color dark brown
1/4 light	Some growth; few sporelings came loose from slide	"	Some branching of hold-fast; some growth of plant body	Slow growth continued and color good
1/8 light	Long hold-fast; practically no sporelings came loose from slide	Little growth of plant body; color much lighter	No growth; color very pale	No growth; color pale; cells breaking down
No light	Long hold-fast; no sporelings came loose from slide	Little growth of plant body; color pale	"	"

In this series (table 4) none of the sporelings were as large as were the sporelings of the same age when submerged all the time. This is probably due to the very smooth surface of the slide not retaining much water. Likely the sporelings were exposed to more drought than they could well resist. It was estimated that 25% of the sporelings became detached from the slide. The sporelings receiving 1/8 light and no light

were at first larger than those receiving all the light or ¼ light. However they soon lost their dark brown color and ceased to grow, while the sporelings receiving light had a dark brown color and grew. This again points toward the necessity of light. It does not show that aeration is helpful or detrimental. The desiccation of the sporelings on the smooth slide was probably more severe than they would have received on the rocky shore.

TABLE 5. *Sporelings three weeks old grown on shells in normal light, then continued under different light conditions*

Amount of light	1st week	2nd week	3rd week
Total light	Color dark brown; continued growth	Color dark brown; continued growth	Twice as large as those receiving ½ light
½ light	Color dark brown; some growth	Some growth	Half as large as those receiving full light
No light	Color dark brown; no growth	Color becoming pale; no growth	Sporeling coming loose from shells; no growth; cells appearing dead

In series number 3 of the above in which the shells bore healthy sporelings three weeks old, 3 conditions were tried (table 5). The oospores were germinated in a south window in an uncovered glass jar. These sporelings were then subjected to different light intensities as follows:

a. A glass jar uncovered.

b. A porcelain bowl admitting ½ light from above.

c. A porcelain bowl admitting no light.

At the end of 2½ weeks the sporelings in the open glass jar were twice the size and much healthier in color than the sporelings receiving ½ light. The sporelings which received no light had died. This shows that light is necessary even after the sporelings have attained a considerable size. The sporelings in the open glass jar had a protuberance on one side which may have been the beginning of the dichotomous branching characteristic of Fucus.

Sporelings 48 hours old were attached to shells that were fastened to cords each 7 meters long. The shells were fastened like those with young and mature plants and were suspended in the sea. There were 4 cords suspended. The shells were examined under the microscope once each week. At the end of the fourth week a difference was noted in the color of the sporelings; the deeper the submergence the lighter the color. Some had disappeared from the shells, probably having died. At the end of the fifth week (table 6), all of the sporelings had disappeared from one cord except four on the shell 3 dm. from the surface. One of these

Table 6. *Sporelings 48 hours old, then grown for 5 weeks on shells suspended in the sea at different depths*

Depth	Condition of plants
3 dm.	21 healthy sporelings; 3 small plants completely covered with diatoms
6 dm. and 1 m.	2 sporelings; little color; no growth
5 m.	1 small sporeling completely covered with diatoms and probably dead
Between 5 and 7 m.	No sporelings

seemed to be alive, and a later examination showed clearly that it was alive and growing. The other three were completely covered with diatoms. Another cord contained seven healthy sporeling 3 dm. from the surface of the water. Two shells on the same cord, one 6 dm. and the other 1 m. below the surface of the water, still retained two sporelings each. However, they had but little color and were thought to be dead. The other shells on the cord had no sporelings remaining on them. A third cord showed 13 healthy sporelings 3 dm. below the surface of the water, and each showed a protuberance on one side. One shell 5 m. below the surface of the water contained what was believed to be a sporeling. It was surrounded by diatoms, was very light colored, and the cells appeared to be breaking down. The fourth cord was lost in the Sound before any data was gathered.

The changes from the natural habitat were continual submergence, light and diatoms. If it were the effect of being continually submerged that produced death among the sporelings, all would have died, since they were all submerged alike. If it were caused by diatoms, one would expect those above low tide to be about equally affected, as is the case with Cladophora.

No living sporelings were found over 3 dm. below the surface of the water. A few sporelings were still held to the shells. In these, however, the growth which had taken place occurred during the first 2 weeks, and they were now considered dead.

Light in the water diminishes as the depth increases. The living sporelings were found at 3 dm. below the surface of the water. Shells submerged between 3 dm. and 6 m. still had occasional dead sporelings clinging to them. Below this no sporelings were found. The number of sporelings also decreases with the distance below the surface of the water, or the number of sporelings became less as the light decreased. Thus light seems to be a controlling factor. The shells below 6 m. contained no sporelings and no diatoms. The shells that contained living sporelings

contained diatoms also. Diatoms may be harmful but they are not a determining factor.

SUMMARY

1. Mature Fucus plants are more resistant to lower light intensities than are sporelings.

2. Desiccation of young plants is believed to prevent the growth of Fucus on gravel.

3. Reduced light intensities cause the death of well-grown Fucus plants 1 m. below the surface of the water.

4. Well-grown Fucus plants receiving less than ¼ total light become darker in color, and decomposition takes place.

5. Reduced light causes the death of oospores and sporelings when planted more than 3 dm. below the surface of the water.

6. Light is a controlling factor in determining the lower limit of Fucus.

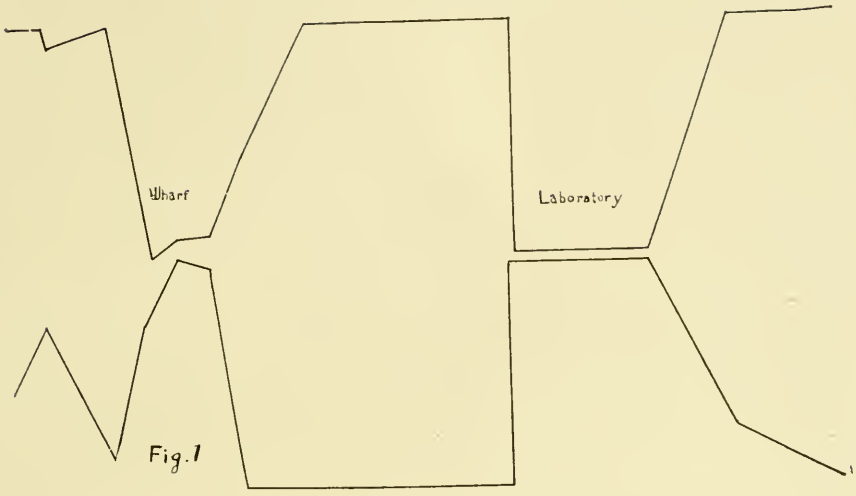
CHART 1

Horizontal distance on the chart is horizontal distance along the shore. Vertical distance on the chart is vertical distance on the shore.

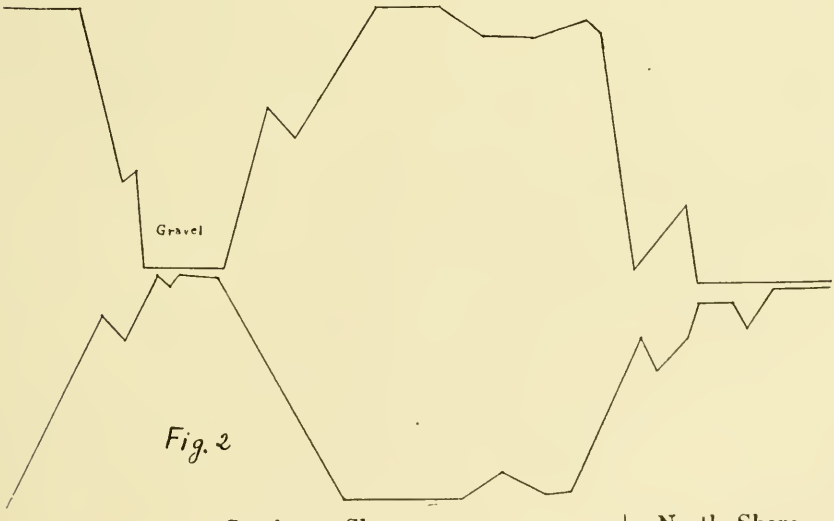
Fig. 1. Relative vertical width of the Fucus zone from north of the Station laboratory to south of Newhall's wharf. The lower graph marks the lower limit of Fucus, and its lowest point is the low-tide line. The upper graph marks the upper limit of Fucus, and its highest point is about ½ meter below the high-tide line. Distance between the two graphs therefore is the relative vertical width of the Fucus zone along this shore.

Fig. 2. Relative width of the Fucus zone in the Point Caution region from the rocky point townward from the streamlet to a point about 40 meters north of the cove in which the telephone cable enters the water. The lower graph marks the lower limit of Fucus, and its lowest point is the low-tide line. The upper graph marks the upper limit of Fucus. and its highest point is about ½ meter below the high-tide line. Distance between the two graphs therefore is the relative vertical width of the Fucus zone along this shore.

CHART 1



East Shore



Southeast Shore | North Shore

Morphology and Attached Stages of First Copepodid Larva of Salmincola edwardsii (Olsson) Wilson

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CONTENTS

1. INTRODUCTION

Although considerable has been written about the parasitic copepods of the family Lernaeopodidae, yet there is a woful lack of accurate information regarding them. Many of the generalizations concerning the family are, by some, based upon results obtained from the study of a single species, or from analogy with species of other families of copepods. For a number of years (1912-1918) the writer has been studying the parasitic copepod *Salmincola edwardsii* (Olsson) Wilson, one of the Lernaeopodidae, which attacks the brook-trout *Salvelinus fontinalis* Mitchill. The purpose of the present contribution is: (1), to clear up certain points regarding the morphology of the first copepodid larva of the species; (2), to present more accurate information concerning the attachment of the parasite to the host. For this work the author had an abundance of material on hand, which was obtained in the various trout hatcheries of the state of Wisconsin. This material was prepared according to the methods already outlined in his previous publications of 1914 and 1916.

2. HISTORICAL

The synnonomy of *Salmincola edwardsii* (Olsson) Wilson is rather involved. The copepod was first studied by Mayor in 1824, who identified it as *Lernaeopoda salmonea,* thus confusing the form with another species of the same name which was first described by Gissler in 1751, and studied by Linnaeus in 1761 and by Blainville in 1822. In 1840 Milne-Edwards reclassified the species worked on by Mayor under the genus Basanistes, and called it *Basanistes salmonea.* In 1868 Olsson proposed the name of *Lernaeopoda edwardsii* for the species under consideration, and showed that it was a true member of the genus Lernaeopoda. In 1915 Wilson established the new genus of the Salmincola and placed *Lernaeopoda edwardsii* under it. Thus we have *Salmincola edwardsii* (Olsson) Wilson. The synonomy of *Salmincola edwardsii* (Olsson) Wilson may be summarized as follows:

Lernaeopoda salmonea of Mayor in 1842.
Basanistes salmonea of Milne-Edwards in 1840.
Lernaeopoda edwardsii Olsson, 1868.

3. MORPHOLOGY OF FIRST COPEPODID LARVA

A. *External anatomy*

The first copepodid larva (Fig 1) passes a free-swimming existence in the water and is constantly hunting for a host to which to attach itself. Since this larva has already been briefly described elsewhere (Fasten 1912, 1913), the description need not be repeated here. However, the appendages (Figs. 2 to 10) require more attention.

The appendages are paired structures occupying the ventral surface of the cephalon (Fig. 1, c) and the thoracic segments (Fig. 1 th). The cephalon bears the first antennae (Fig 1, a′; Fig. 4), second antennae (Fig. 5), mouth tube (Fig. 2), mandibles (Fig. 3), first (Fig. 6) and second (Fig. 7) pairs of manillae, and the maxillipeds (Fig. 8). The first and second thoracic segments bear, respectively, the first (Fig. 9) and second (Fig. 10) pairs of swimming feet.

The first antennae (Fig. 1, a′; Fig. 4; Fig. 23, a′) are slender, uniramous structures consisting of four joints. The second of these joints bears two long, straight setae, whereas the third joint bears a stout spine. The fourth joint is somewhat thicker than the others and possesses eight thin setae.

The second antennae (Fig. 5; Fig. 23, a″) are located a little below the primary antennae. They are biramous appendages, bearing outer exopods and inner endopods. Each exopod consists of a single joint that terminates in two long spines. The endopod is three-jointed, consisting

of a very broad basal portion, a somewhat more slender middle part, and a third joint that carries a pronounced curved claw.

The mouth tube (Fig. 2; Fig. 23, m.t) is placed between the antennae, and is well developed in the free-swimming larva. It consists of an upper (Fig. 22, u. l.) and a lower lip (Fig. 22, l. l.), which are distinct and separate structures joining near their bases. This distinctness of the lips can be observed to best advantage when a section through the mouth region (Fig. 15) is studied. In the living larva the mouth tube is constantly moving above and below the anterior margin of the cephalon.

Beneath the mouth tube are the mandibles (Fig. 3; Fig. 22, mnd) and the first maxillae (Fig. 6; Fig. 22, mx′). Both of these are minute structures, uniramous and one-jointed. The mandibles (Fig. 3) are tipped with two spines, of which the inner is about twice as long as the outer. The first maxillae (Fig. 6) are chela-like in character.

The second maxillae (Fig. 7; Figs. 22 and 23, mx″) are large, powerful and distinctly three-jointed. The last of these joints bears a prominently hooked claw of great strength.

The maxillipeds (Fig. 8; Fig. 23, mxp) lie directly below the second maxillae and like these are uniramous, large, and very powerful. The maxillipeds possess three joints and terminate in a strong slightly curved claw.

The swimming feet are biramous structures (Figs. 9 and 10) possessing broad, laminated exopods and endopods. In a previous publication (Fasten, 1913, p. 38) the author made the following statement regarding the swimming feet: "The thorax has two segments and these bear the two biramous swimming feet, which end in broad laminated bases—the respective exopods and endopods. Each exopod termintes in four long feathery setae, whereas the endopods contain seven of these structures." Since making the foregoing assertions, the writer has carefully re-examined his material, and while the above facts hold true for the first pair of swimming feet (Fig. 9) they are only partially true for the second pair (Fig. 10), whose exopods have four feathery setae and whose endopods have six.

B. *Internal anatomy*

The internal anatomy has been worked out from the study of a great number of whole mounts of the organism, and particularly from the study of large numbers of sections of the first copepodid larvae made in transverse, sagittal, frontal and angular planes.

a. The eye of *Salmincola edwardsii* (Olsson) Wilson is fully developed in the free-swimming larval stage. Wilson (1911 and 1915), after studying the eye of *Achtheres ambloplitis* Kellicott, one of the Ler-

naeopodidae, comes to the conclusion that the eye in this whole family is extremely rudimentary in character. My own studies on the eye of *Salmincola edwardsii* do not bear out Wilson's assertions. Here the eye occupies a median position slightly below the center of the cephalon (Fig. 1, e). It is a tripartite organ (Fig. 18, e) whose structure is practically the same as that of the eye of the free-living marine copepod *Eucalanus elongatus* Dana, as described by Esterly (1908). For a complete description of the eye of *Salmincola edwardsii* see Fasten, 1916.

b. The attachment filament (Fig. 1, a. f.) plays a very important role in the attachment of the copepod to its host. It is located medianally at the anterior end of the cephalon, beneath the thin chitinous body wall. Its position and make-up can be seen to good advantage when a side view of the animal is studied (Fig. 22). It is then seen to consist of three parts: (1), a broad, ball-like bulla (Fig. 22, bu) situated immediately under the thin anterior margin of the cephalon; (2), a tube structure (Fig. 22, t), which makes its way backward from the posterior part of the bulla as far as the eye, and then coils upward in a single loop until it reaches the level of the commencement of the tube, where it enlarges into (3), a broad foot (Fig. 22, ft) which is attached to the frontal margin of the cephalon. The attachment filament secretes a transparent, viscid, glue-like substance that refracts light strongly. When this secretion comes in contact with fixatives containing corrosive-sublimate it coagulates and becomes darkly granular in appearance. In sections treated with Heidenhain's haematoxylin (Figs. 15, bu; Figs. 16 and 17, a. f; Fig. 25, bu and ft) it stains intensely black.

c. The glands which can be distinguished in the free-swimming larval form are as follows: the single frontal gland, the first and second pairs of antennary glands, the pair of shell glands and the pair of maxillipedal glands. All of these glands occupy the cephalon and are well developed.

The frontal gland (Figs. 16, 17 and 25, f. g), surrounds the entire tube of the attachment filament. It is quite a prominent structure, but most of it lies toward the ventral surface of the organism, between the oesophagus and the attachment filament. This is plainly seen in Fig. 17, f. g.

The glands of the first (Fig. 25, a′. g)and second (Fig. 11, a″. g) antennae are located, respectively, on either side of the ventral portion of the copepod below the first and second antennae. The primary antennary glands are somewhat smaller than those leading to the second antennae.

The shell glands (Figs. 11 and 17, s. g) and the maxillipedal glands (Figs. 11 and 18, mxp. g) are the most conspicuous glands found in the head; the former being about twice the size of the latter. Each shell gland originates dorsally a little above the lower portion of the second

antennary gland, and then traverses downward through the cavity lying back of the second maxillae (Fig. 17 s. g). The gland itself resembles a kidney in shape, and near the second maxilla it gives off a slender duct, which opens to the exterior after traversing the maxilla. The maxillipedal glands are somewhat bean-shaped and are situated behind the maxillipeds. They give off tubular ducts which make their way into the maxillipeds and there open to the outside.

Aside from these distinct glands there are numerous glandular cells located near the pharynx (Fig. 16, ph. g), which probably secrete enzymes useful in digestion. These cells are pear-shaped in character and possess prominent nuclei.

d. The muscles of the first copepodid larva are numerous, striated and well developed. Those which can be easily distinguished are: (1), the dorsal muscles (Figs. 1, and 18 to 20, m), running along the dorsal surface of the cephalon and first thoracic segment, and functioning in the operation of the abdomen; (2), the lateral muscles (Figs. 1, 11, 19 and 20, l), extending along the lateral margins of the first thoracic segment, and functioning in the movement of the second thoracic segment; (3), the muscles which operate the first pair of swimming feet (Figs. 1 and 11, m′), and are located in the lower lateral extremities of the first segment of the thorax; (4), the muscles which operate the second pair of swimming feet (Figs. 1 and 11, m″), and are located along the sides of the second thoracic segment; and (5), the dorsal abdominal muscles (Fig. 1, m‴), which aid in the movement of the abdomen. There are also a great many smaller muscles within the segments and appendages, but these could not be accurately traced. Connected with the foot of the attachment filament are numerous thin muscle strands (Figs. 24 and 25, ft. m), which operate in the discharge of the attachment filament from the body of the copepod. These muscles surround the bulla (Fig. 25, ft. m), originating below the extreme anterior surface of the cephalon and then passing downwards to become inserted in the foot of the attachment filament. One might easily overlook these muscles, for there are so many other structures crowded into the region where they are located. It was only after prolonged and careful study of a great number of larvae that these interesting muscles were discovered.

e. The digestive system is very difficult to trace by an examination of the entire organism. When thus studied the copepod is found to be so clearly transparent that the digestive system appears to be only partially developed, consisting of a large central cavity filled with yolk globules (Fig. 1, y), while the anterior and posterior portions of the gut seem to be lacking. The true nature of the digestive system, and for that matter of the entire internal anatomy of the first copepodid organism can only

be determined by careful reconstructions of sections of the larva, and this was the method of study pursued by the author.

The digestive system of *Salmincola edwardsii* is completely developed in the first copepodid larva and consists of a mouth tube, pharynx, oesophagus, stomach, intestine and anus. The lips of the mouth tube (Figs. 15 and 22, u. l and l.l) are separate at first, and then unite at the base to form an elliptical pharynx (Fig. 16, ph), which runs a short distance to about the place where the second maxillae originate, and there enters the oesophagus. This last named structure (Figs. 11, 17 and 18, oe) is a thin, circular tube, which extends through the rest of the cephalon and enters the spacious stomach (Figs. 11, 19 and 20, st). In Fig. 11, the connection between the oesophagus and stomach is particularly well shown. The stomach is large, cone-like, and most of it lies within the center of the first thoracic segment. In the living state it is filled with spherical yolk globules (Fig. 1, y) which stain intensely black with Heidenhain's haematoxylin. These are the black masses seen in the stomach (st) in Figs. 11, 19 and 20. Toward the posterior region of the first thoracic segment the stomach tapers into a slender intestine (Figs. 11 to 13, and 21, i). The intestine is fully developed. It extends thru the middle of the second segment of the thorax and thru the abdomen, terminating in the center of the extreme posterior margin of the third abdominal segment as the anus. This is well shown in Figs. 13 and 14 (an).

These observations bear out what Claus (1862) has observed concerning the digestive system of *Achtheres percarum* Nordmann, but are not in accord with what Wilson (1911) has found in the larva of *Achtheres ambloplitis* Kellicott. Wilson says, "Claus has described this first copepodid stage in *Achtheres percarum* as possessing a completed digestive system, capable of functioning. But he stands alone in such a statement. Nordmann, Kollar, Vejdovský and Kellicott all present the larval digestive apparatus at this stage as only partially developed, and thus agree with what was found in the present species." However, it must be pointed out that all the investigators mentioned by Wilson as well as himself, determined the internal anatomy of the first copepodid larvae largely through an examination of the entire animal. As stated above, this method of studying such minute, transparent larvae is rather delusive and inadequate.

The histological structure of the digestive system is very similar to that of other crustacea. Interiorly it is lined by a layer of simple columnar epithelium, whose cells contain prominent nusclei. In the stomach these epithelial cells attain a very large size (Figs. 11, 19 and 20, st). Surrounding the epithelial layer is a thin outer layer of fibrous connective tissue. Outside of this are the thin mesenteries which suspend the diges-

tive tract from the body wall of the organism. This arrangement can be seen in Figs. 19 and 20.

f. The nervous system is prominently developed in the first copepodid larva. The brain (Figs. 11 and 18, b) is a massive structure, consisting of the supra- and infra-oesophageal ganglia united into a single mass which completely surrounds the posterior portion of the oesophagus. Fig. 18 is a transverse section of the organism in the region where the brain (Fig. 18, b) is best developed, right below the eye (Fig. 18, e). Anteriorly, the brain gives off slender nerves (Fig. 11, b) to the antennae and the other mouth parts, while dorsally it gives off the optic nerve of the eye. Posteriorly a prominent nerve cord, which appears to be single (Fig. 19, n), makes its way from the infra-oesophageal ganglion along the ventral surface of the stomach. Whether this ventral nerve cord later divides and gives off nerves to the swimming feet and abdomen could not be determined.

g. The reproductive organs have not yet developed in the first copepodid larvae, and therefore it is impossible to distinguish males from females. When sections of these larvae are examined, however, one can always see a large, undifferentiated mass of germinal mesoderm along the ventral and lateral margins of the thorax (Figs. 11, 19 and 20, g.m). This mass later produces the various portions of the reproductive organs.

4. ATTACHED STAGES OF COPEPOD

The first copepodid larva is constantly swimming about in search of a host to which to attach itself. The organism is specific in its choice, only attaching itself to the brook trout, *Salvelinus fontinalis* Mitchill. When it comes in contact with the right host, attachment occurs. In a previous publication by the writer (Fasten, 1913) this was described as follows: "By the aid of the microscope I observed the process of attachment four times. As soon as the copepod comes in contact with the filament of the gill, its mouth parts are inserted into the flesh, and by means of the powerful claw-like second maxillae it begins to rasp the filament until it forms a cavity within it. As soon as this occurs, the anterior portion of the copepod's head, the frontal margin, is brought in contact with the cavity and the enclosed attachment filament is injected into the hole. The spherical mushroom body adheres to the flesh and the regenerating tissue of the gill soon encloses it tightly, thereby fastening the organism firmly. The mouth parts are then withdrawn from the flesh of the gill filament. In this condition the parasite remains attached for a short time. Then the second maxillae detach the posterior region of the attachment filament from the head margin and they themselves become permanently attached to this

end of the filament. Degeneration soon sets in and the organism changes considerably. The female copepod remains thus attached throughout life, while the male remains attached in this way until shortly before it is mature for copulation." Since the above statements, the author has made a very careful study of his numerous preparations and has discovered news facts, which clear up many of the obscure points regarding the attachment, as well as the later attached stages of the organism.

One of the things which could not be pointed out was the apparatus that was responsible for the discharge of the attachment filament from the cephalon of the copepod into the tissues of the host. The discovery of the thin muscles (Figs. 24 and 25, ft. m) connected with the foot of the attachment filament clarifies this obscurity. The contraction of these muscle strands causes the injection of the bulla into the flesh of the host. Figure 22 shows the head region of the first copepodid larva in contact with a portion of the gill, prior to attachment. Note how the second maxillae (Fig. 22, mx″) rasp the tissue of the gill (Fig. 22, g). Figure 23 is a drawing of the organism immediately after the injection of the bulla into the gill tissue. Here the animal is seen attached at the frontal margin (Fig. 23, f. m) to the foot (Fig. 23, ft) of the attachment filament. When the attached region between the foot and the frontal margin is examined under the high powers of the miscroscope the contracted foot muscles (Fig. 24, ft.m) can be seen quite plainly.

Another point which can now be cleared up, is the question of the permanent attachment of the organism. Wilson (1911, 1915) and the present writer (Fasten, 1913) came to virtually the same conclusion regarding this point; namely, that the maxillary attachment of the copepod serves as the final and permanent attachment for the female, while for the male it serves as a temporary attachment, until he is ready to seek out a female for copulation. However, a careful study of the copepod in all stages of attachment has led the writer to the conclusion that prior to sexual maturity, the maxillary attachment is quite as temporary for the female as it is for the male. The permanent attachment of the female takes place about the time when she is mature for fertilization. In order to get a comprehensive idea of these stages, it will be necessary to trace briefly the changes which occur in the first copepodid larva from the time of its initial attachment until it becomes sexually mature.

As already pointed out in the above citation, the larva hangs on for a short period at the frontal margin. Soon the second maxillae detach the proximal or foot end of the attachment tube from the frontal margin, and then their powerful claws are driven into this detached foot. Figure 26 shows the claws of the second maxillae (mx″) immediately after being driven into the foot (ft) of the tube of attachment (t). Degeneration

now sets in (Fig. 27), and soon the organism begins to lose its segmentation, while at the same time the feathered setae of the abdomen and swimming feet disappear. The swimming feet themselves become stub-like (Fig. 27, f′ and f″) and are ultimately absorbed entirely. The abdomen and the rest of the body round out and become more bag-like in appearance. The mouth appendages also change. The mouth tube grows more prominent (Fig. 27, m.t); the first and second antennae (Fig. 27, a′ and a″) become smaller and more stump-like in appearance. Perhaps the most interesting modifications take place in the second maxillae and maxillipeds. The former (Fig. 27, mx″), which are now attached by their claws to the foot of the attachment filament, lose their segmentation and begin to thicken considerably; also their terminal claws become smaller in size. The maxillipeds (Fig. 27, mxp) likewise thicken, grow somewhat in size and their terminal claws undergo modification.

Internally the digestive system (Fig. 27, d) has grown in size, specially the stomach. The glands may be quite easily traced. The sex-organs begin to shape themselves. The eye (Fig. 27, e) undergoes degeneration.

The changes just described occur during the first five or six days after attachment. From now on the copepod begins its active growth and development toward sexual maturity. This is completed in about another week and a half during which the mouth tube becomes very active, sucking abundant nourishment from the host. The body elongates and grows vigorously (Figs. 27 to 29, and 31), while the appendages, glands, digestive system, sex organs and other structures develop still more completely. Males and females may be found hanging side by side on the gills of the fish; however, the former are pigmies as compared with the latter.

Perhaps the most interesting changes which occur during this period are those which take place in the second maxillae and the maxillipeds. The second maxillae of both males and females are short, stout structures with their terminal claws driven into the foot of the attachment tube (see Figs. 28 to 31, mx″ and t). A careful examination of the region of attachment between the maxillae and the foot of the attachment filament reveals the fact that this attachment is not as yet permanent, and that the second maxillae may withdraw their claws in order to shift their position. This is specially well shown in Fig. 30, which is an enlarged drawing of the attached portions of the second maxillae shown in Figure 28. When figure 30 is examined it is seen that the foot (ft) has been pierced a number of times at the places marked x, by the claws of the second maxillae (mx″).

The maxillipeds (Figs. 28, 29 and 31, mxp; 32 and 33) remain jointed but transform completely. They become more conspicuous; their thickness and length increase, particularly that of the terminal segment.

This last named structure instead of bearing a single claw as it did in the free-living larvae (Fig. 8), now bears two claws (Figs. 28, 29 and 31, mxp; 32 and 33). The maxillipeds thus come to resemble chela with inner and outer claws. In the maxilliped of the female copepod (Fig. 32) the inner claw is curved into a pronounced hook, while the outer claw is straight and spine-like. In the maxilliped of the male copepod (Fig. 33) both the claws are slightly bent, but they are not nearly as prominently developed as in the female.

Very shortly before the copepods reach full sexual maturity (between two and three weeks after attachment) the maxillipeds function as aids in the permanent attachment of the organism. The male releases his maxillary hold on the attachment filament, and by means of the maxillipeds attaches himself to the abdomen of a mature female for copulation. For a fuller discussion of the attachment of the male to the female see Fasten, 1914.

In the case of the female, the maxillipeds (Figs. 28, 29 and 31, mxp) first work their way upward into the open space lying between the second maxillae (Figs. 28, 29 and 31, mx″), and then by means of the powerful claws they grasp the flesh of the gill (Fig. 31, mxp and g), thereby bringing the organism closer to the gill proper. As soon as the copepod is thus securely fastened, the second maxillae (Fig. 31, mx″) withdraw their claws from the proximal foot of the attachment tube (Fig. 31, t), and begin a sort of creeping process along this last named structure, until they reach the posterior region of the chitinized bulla implanted in the gill tissue. Here the claws of the second maxillae are securely driven in and the female becomes permanently attached for the rest of her life. The maxillipeds are soon withdrawn from the gill and undergo degeneration (compare Figs. 32 and 36). The second maxillae elongate, become intensely muscular and assume their adult appearance (Figs. 34 and 35, mx″). The tube of the attachment filament soon shrivels up and disintegrates, while the bulla transforms into a chitinized, funnel-shaped structure (Figs. 34 and 35, bu).

From the above discussion it is evident that there are three stages of attachment: (1), the initial stage, when the frontal margin of the organism is fastened to the foot of the attachment filament; (2), when the copepod is attached by its second maxillae to the foot of the attachment filament; (3), when the copepod becomes attached as an adult, either the male to the female, or the female to the chitinized bulla. The first two stages of attachment are temporary, while the third is permanent.

The investigators who have concerned themselves with the Lernaeopodidae, such as Claus (1862), Vejdovský (1877) and Wilson (1911 and 1915), claim that when once the female larva has attached the second

maxillae to the attachment tube, she remains thus attached until the completion of her life history. But it must be pointed out that prior to sexual maturity, the second maxillae of the organism (Figs. 27 to 31, mx″) in both male and female are attached to the proximal foot of the attachment tube (Figs. 28 to 31, t); whereas in the mature female these maxillae (Figs. 34 and 35, mx″) are in direct contact with the transformed chitinous bulla (Figs. 34 and 35 bu) imbedded in the gill tissue. Now a question which naturally arises is, how do the second maxillae of the adult females come to occupy their final position? An investigator who did not secure all of the intermediate stages between the time of the intitial attachment of the first copepodid larva and the period when it reached sexual maturity, might easily arrive at the same conclusion as Claus (1862), Vejdovský (1877) and Wilson (1911 and 1915); namely, that soon after the fusion of the second maxillae with the proximal end of the attachment filament, the slender tube of the attachment filament shrinks and shrivels up until the maxillae are brought into close contact with the distal bulla enclosed in the flesh of the host. However, the results set forth in the present paper do not confirm the observations of these investigators. As already pointed out, shortly before reaching sexual maturity the female grasps the gill with the chela-like maxillipeds, withdraws the claws of the second maxillae from the foot of the attachment tube, and then by a gradual creeping process along the attachment tube transfers these claws to the posterior margin of the bulla, where they become permanently attached.

5. SUMMARY

1. The first copepodid larva of *Salmincola edwardsii* (Olsson) Wilson is a complex, highly developed organism, possessing two pairs of antennae, a mouth tube, numerous paired mouth parts, and two pairs of swimming feet.

2. Internally the larva possesses an interesting attachment filament, a tripartite eye, various glands, and fully developed muscular, digestive and nervous systems.

3. The digestive system in the first copepodid larva of *Salmincola edwardsii* consists of a mouth tube with an upper and a lower lip, a pharynx, an oesophagus, a stomach, an intestine and an anus. It is completely developed and capable of functioning. In this respect it is in agreement with what Claus (1862) found for the digestive system of the first copepodid larva of *Achtheres percarum* Nordmann, but is not in accord with what Wilson (1911) found in the larva of *Achtheres amblopli-tis* Kellicott.

4. When the larva meets the proper host, the attachment filament is injected into the flesh of the host. This is accomplished by the contraction of numerous muscle strands which are inserted in the proximal foot of the attachment filament.

5. Prior to sexual maturity the attachment of the larva is temporary. There are really three stages of attachment: (1), when the animal first attaches itself and hangs on by its frontal margin to the foot of the attachment tube; (2), when the organism is attached by the second maxillae to the foot of the attachment tube; and (3), when the organism becomes permanently attached, either to the female as in the case of the male, or to the funnel-shaped bulla as in the case of the female.

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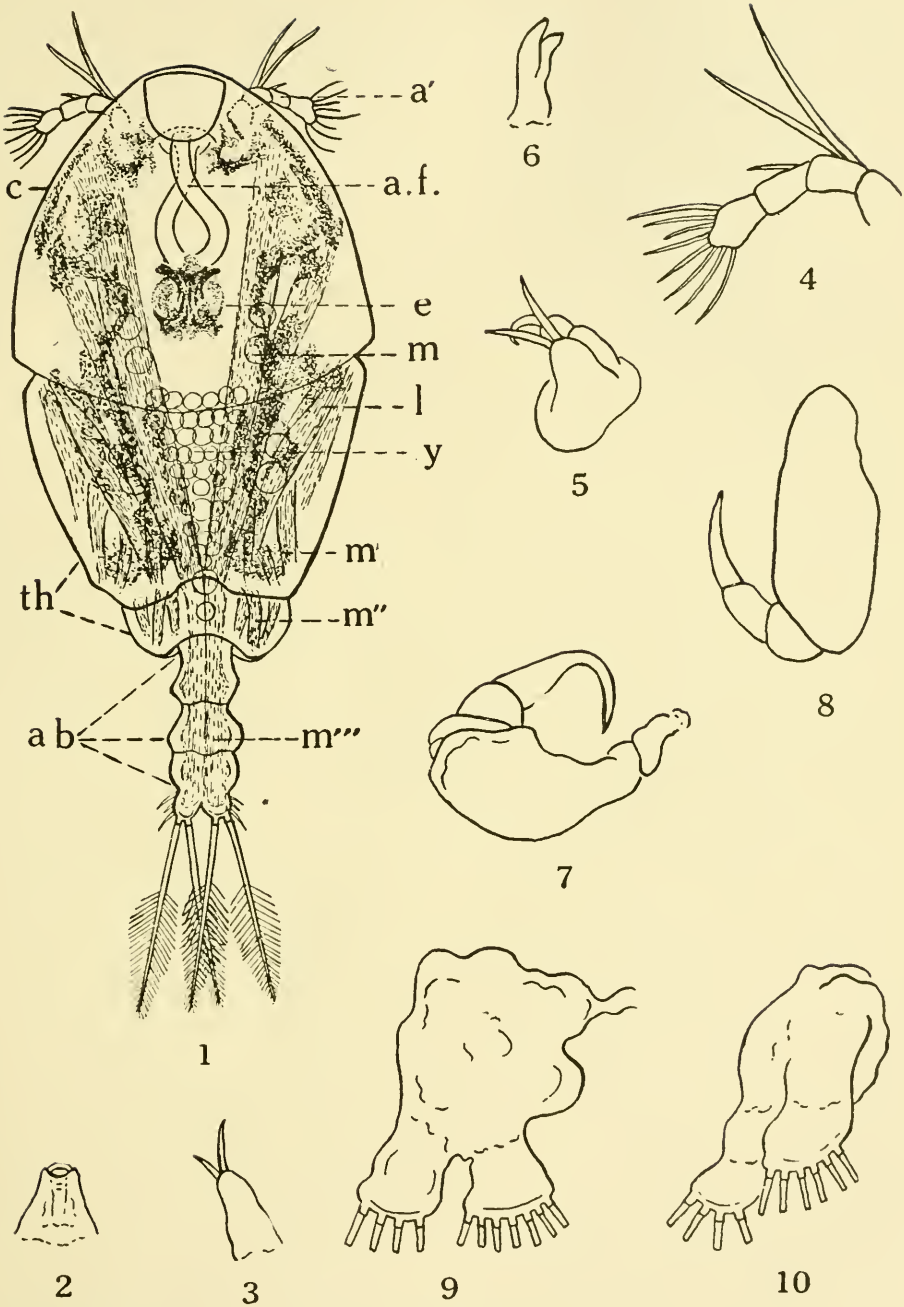
PLATE 21

a, first antennae.
ab, abdomen.
a.f, attachment filament.
c, cephalon.
e, tripartite eye.
l, lateral muscles
m, dorsal muscles.
m', muscles operating first pair of swimming feet.
m'', muscles operating second pair of swimming feet.
m''', dorsal abdominal muscles.
th, thorax.
y, yolk globules.

Fig. 1. Dorsal view of first copepodid larva. ×173.5.
Fig. 2. Mouth tube. ×347.
Fig. 3. Mandible. ×347.
Fig. 4. First antenna. ×347.
Fig. 5. Second antenna. ×347.
Fig. 6. First maxilla. ×347.
Fig. 7. Second maxilla. ×347.
Fig. 8. Maxilliped. ×347.
Fig. 9. Right swimming foot of first thoracic segment, showing exopod with four setae, and endopod with seven setae. ×347.
Fig. 10. Right swimming foot of second thoracic segment, showing expodod with four setae, and endopod with six setae. ×347.



N. Fasten, Del. PLATE 21

PLATE 22

a', first antennae.
a'', second antennae.
a''.g, second antennary glands.
an, anus.
b, brain.
bu, bulla.
ch, chitinous exoskeleton.
g.m, germinal mesoderm.
i, intestine.
l, lateral muscles.
l.l, lower lip.
m', muscles operating first pair of swimming feet.
m'', muscles operating second pair of swimming feet.
md, mandibles.
mxp.g, maxillipedal glands.
oe, oesophagus.
s.g, shell gland.
st, stomach.
u.l, upper lip.
w, body wall.

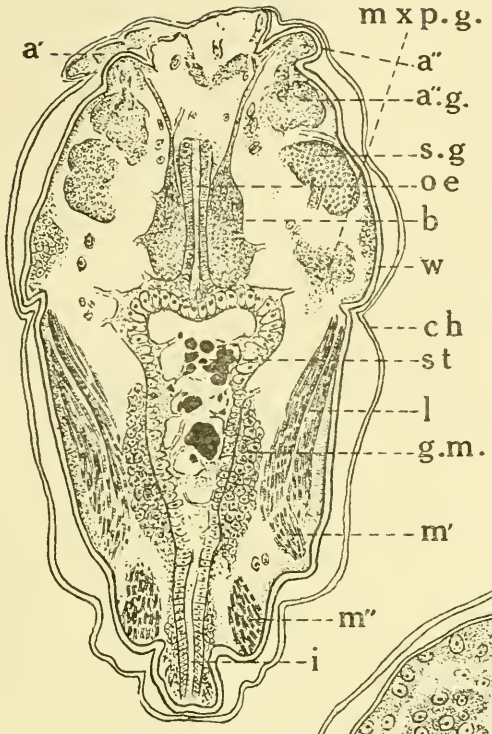
Fig. 11. Frontal section of first copepodid larva showing morphological details. ×258.3.

Fig. 12. Frontal section through abdomen of first copepodid larva showing intestine (i). ×750.

Fig. 13. Frontal section immediately following that represented in Fig. 12, showing the intestine (i) terminating in a distinct anus (an). ×750.

Fig. 14. Angular section through extreme lower region of third abdominal segment of first copepodid larva, showing the anal opening (an). ×1125.

Fig. 15. Transverse section of first copepodid larva in region of mouth tube. ×416.7.



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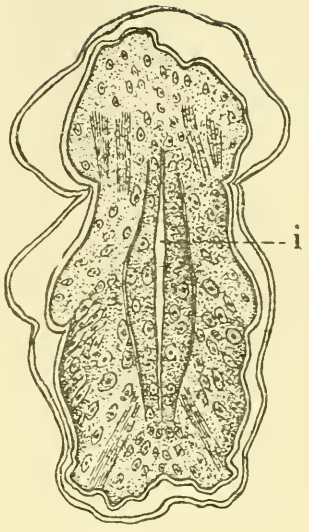

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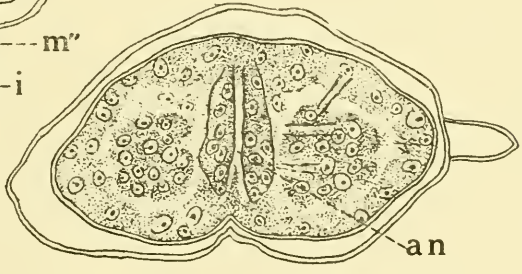

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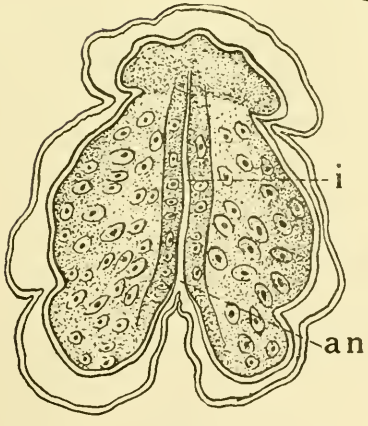

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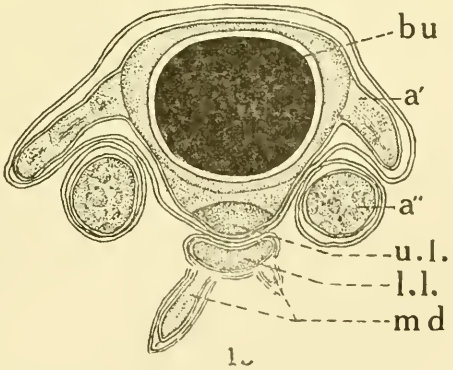


15

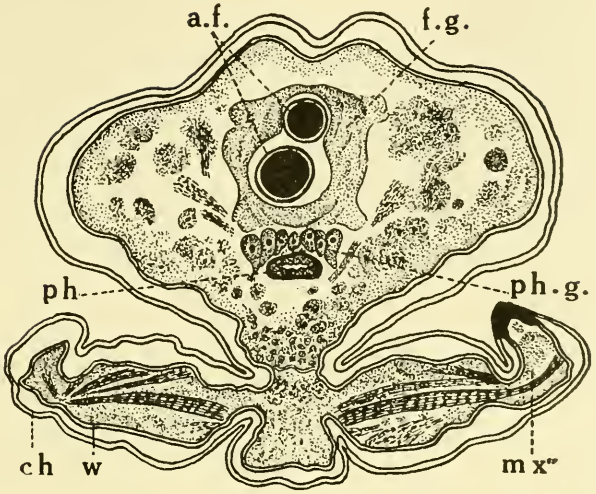
N. Fasten, Del. PLATE 22

PLATE 23

a.f, attachment filament.
ch, chitinous exoskeleton.
f.g, frontal gland.
mx″, second maxillae.
mxp, maxillipeds.
oe, oesophagus.
ph, pharynx.
ph.g, glandular cells of pharynx.
s.g, shell glands.
w, body wall.

Fig. 16. Transverse section of first copepodid larva in region of pharynx. ×500.

Fig. 17. Transverse section of first copepodid larva showing shell glands (s.g). The oesophagus (oe) can be seen as a circular tube fully developed. ×460.



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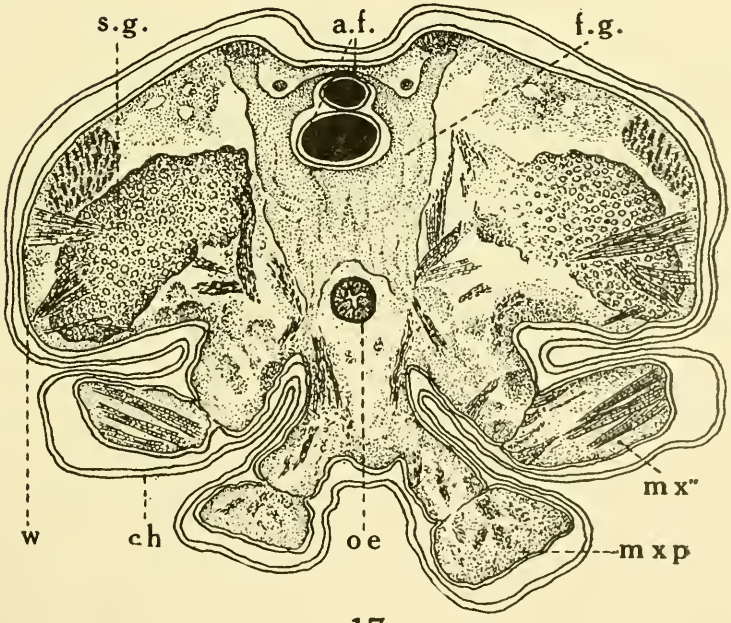


17

N. Fasten, Del.

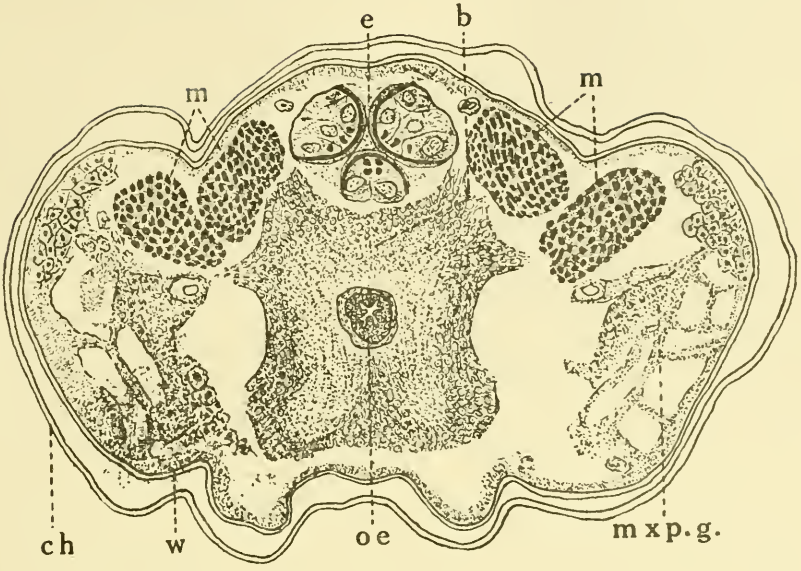
PLATE 23

PLATE 24

b, brain.
ch, chitinous exoskeleton.
e, tripartite eye.
g.m, germinal mesoderm.
l, lateral muscles.
m, dorsal muscles.
mxp.g, maxillipedal glands.
n, ventral nerve cord.
oe, oesophagus.
st, stomach.
w, body wall.

Fig. 18. Transverse section of first copepodid larva in region of tripartite eye (e). ×460.

Fig. 19. Transvere section of first copepodid larva in region of stomach (st). ×460.



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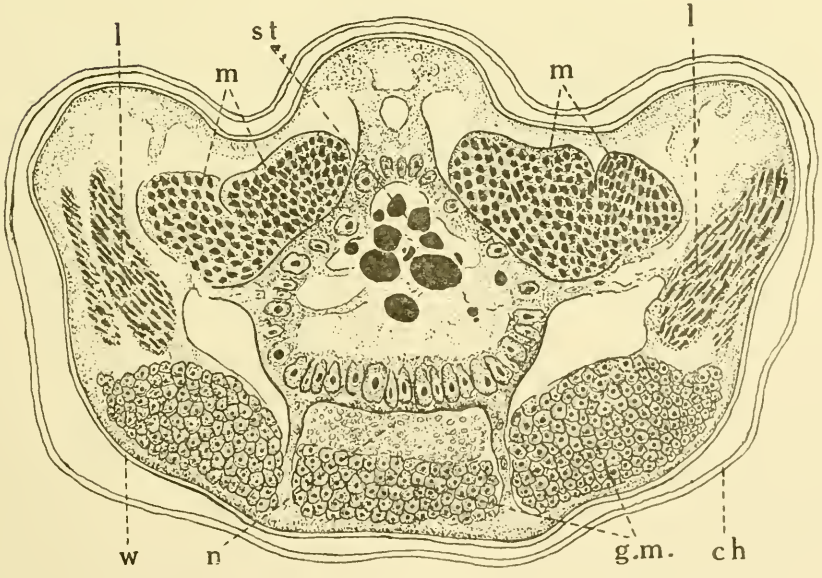

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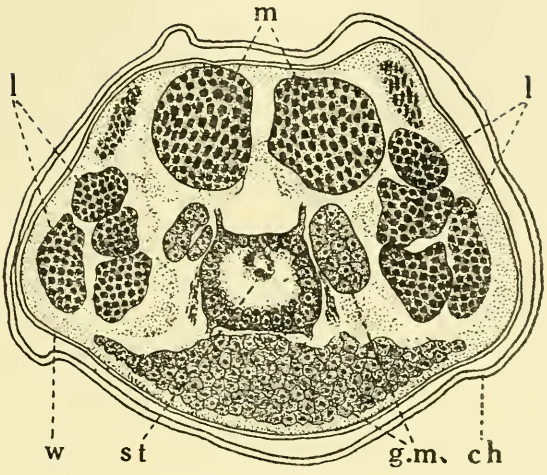
N. Fasten, Del. PLATE 24

PLATE 25

ab.l, first abdominal segment.
ch, chitinous exoskeleton.
f′, first pair of swimming feet.
f″, second pair of swimming feet.
g.m, germinal mesoderm.
i, intestine.
l, lateral muscles.
m, dorsal muscles.
st, stomach.
w, body wall.

Fig. 20. Transverse section of first copepodid larva through lower portion of stomach (st). The muscles (m and l) and the germinal mesodern (g. m) can be seen to good advantage. ×530.

Fig. 21. Transverse section of first copepodid larva through first abdominal segment (ab. l). Note the distinct intestine (i). ×530.



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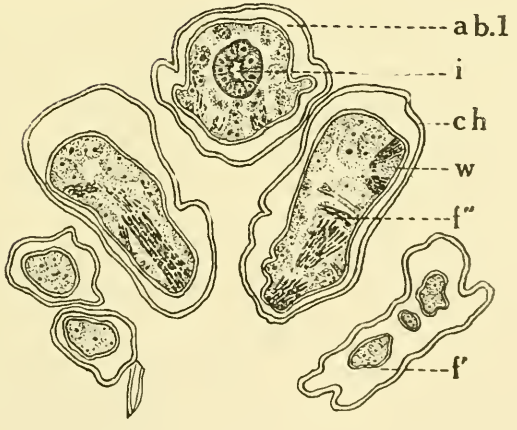


21

N. Fasten, Del. PLATE 25

PLATE 26

a', first antennae.
a'', second antennae.
a.f, attachment filament.
a.g, first antennary glands.
bu, bulla.
d, digestive tract.
e, tripartite eye.
f', first pair of swimming feet.
f'', second pair of swimming feet.
f.g, frontal gland.
f.m, frontal margin.
ft, foot of attachment filament.
ft.m, muscles attached to foot of attachment filament.
g, portion of gill.
l.l, lower lip.
mnd, mandibles.
m.t, mouth tube.
mx', first maxillae.
mx'', second maxillae.
mxp, maxillipeds.
t, tube of attachment filament.
u.l, upper lip.

Fig. 22. Head region of first copepodid larva in contact with a portion of the gill (g) prior to attachment. The second maxillae (mx″) are seen rasping the gill tissue (g). ×197.5.

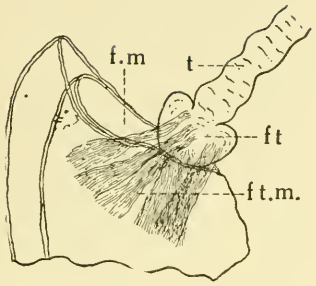
Fig. 23. Head region of first copepodid larva immediately after attachment to gill filament. Here the larva is seen attached at its frontal margin (f.m) to the foot (ft) of the attachment tube (t). The bulla (bu) is implanted within the tissue of the gill (g). ×197.5.

Fig. 24. Enlarged drawing of the frontal margin of the larva represented in Fig. 23, showing the contracted muscle strands (ft.m) attached to the foot (ft) of the attachment tube (t). ×415.

Fig. 25. Anterior head portion of a frontal section of the first copepodid larva, showing the natural location of the muscle fibers (ft.m) which are attached to the foot (ft) of the attachment filament. ×415.

Fig. 26. Drawing which shows the claws of the second maxillae (mx″) immediately after they have attached themselves to the foot (ft) of the attachment tube (t). The bulla (bu) is enclosed in the tissue of the gill (g). ×197.5.

Fig. 27. Attached larva undergoing degeneration. ×68.5.



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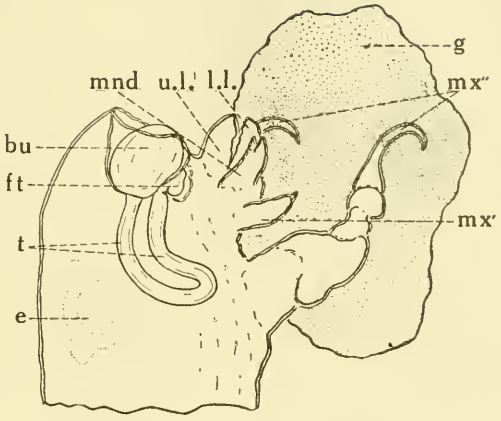

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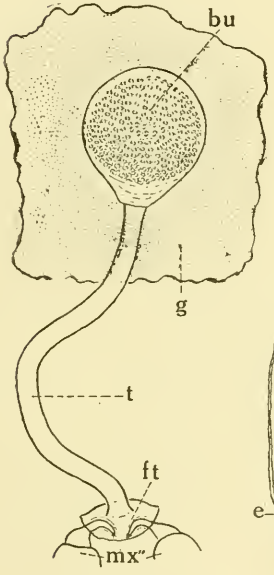

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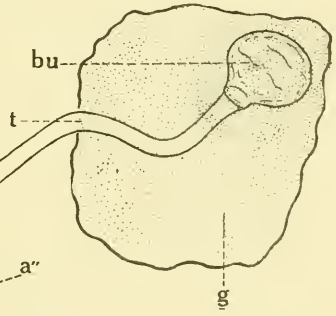

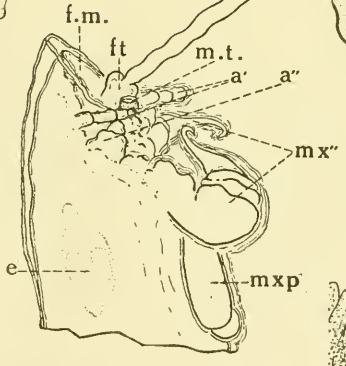

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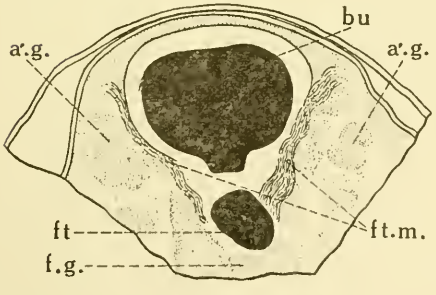

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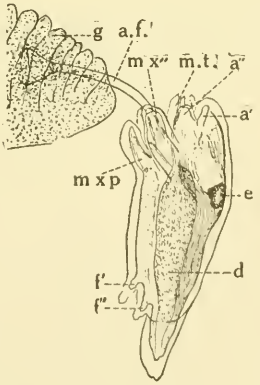


27

N. Fasten, Del. PLATE 26

PLATE 27

a', first antennae.
a'', second antennae.
bd, body of adult female.
bu, bulla.
d, digestive tract.
e.s, eggs sacs.
g, portion of gill.
m.t, mouth tube.
mx', first maxillae.
mx'', second maxillae.
mx.g, second maxillary glands.
mxp, maxillipeds.
mxp.g, maxillipedal glands.
r, reproductive organs.
s.g, shell glands.
sp, spermatophores.
t, tube of attachment filament.

Fig. 28. Immature female showing transformation of organs. The copepod is still attached by the second maxillae (mx'') to the proximal foot of the attachment tubs (t). The maxillipeds (mxp) can also be seen undergoing modification. ×90.

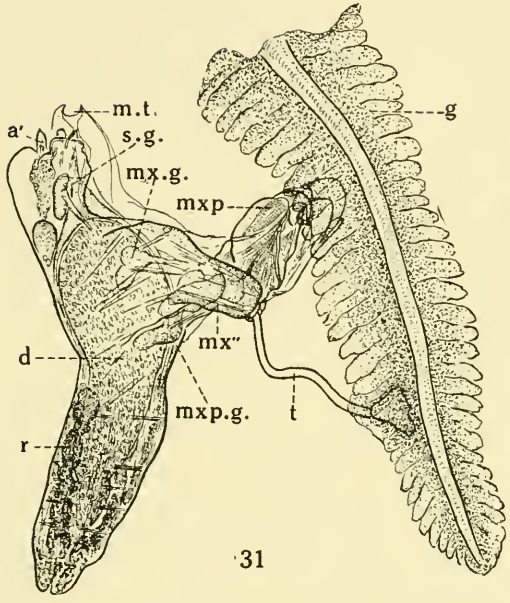
Figs. 29 and 30. See plate 28.

Fig. 31. Female nearly sexually mature with maxillipeds (mxp) fastened in gill filament (g), while second maxillae (mx'') are creeping along attachment tube (t) in order to attach themselves permanently to bulla. ×68.5.

Fig. 32. Maxilliped of female at time of attachment of second maxillae to bulla. ×163.5.

Fig 33. Maxilliped of male at sexual maturity. ×163.5.

Fig. 34. Adult female permanently attached by its second maxillae (mx'') to the bulla (bu). ×17.5.



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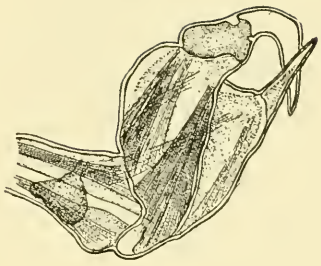

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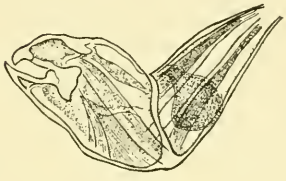

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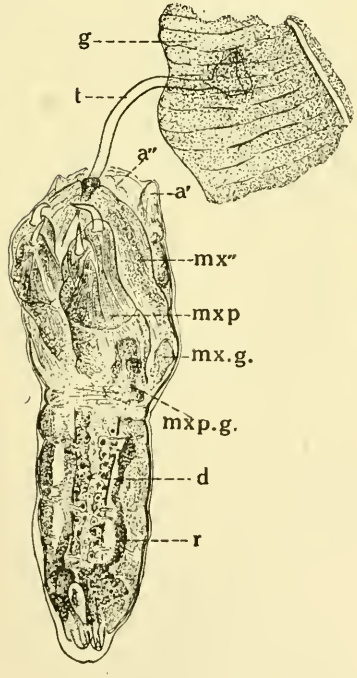

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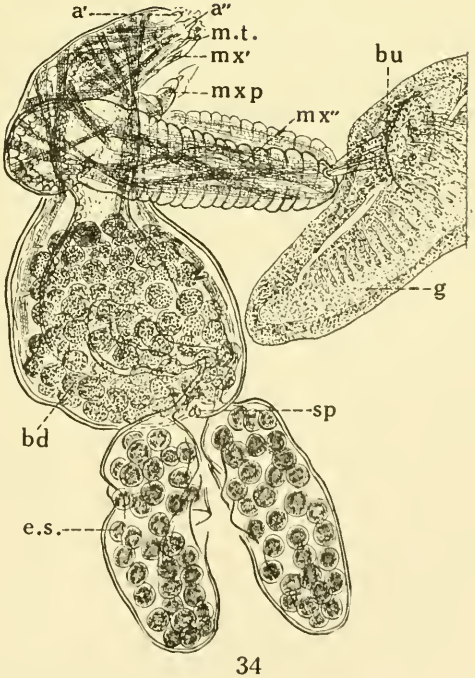

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N. Fasten, Del. PLATE 27

PLATE 28

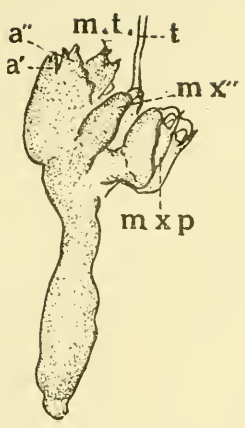
a' first antennae.
a'', second antennae.
bu, bulla.
ft, foot of attachment filament.
m.t, mouth tube.
mx'', second maxillae.
mxp, maxillipeds.
t, tube of attachment filament.
x, places where claws of second maxillae have punctured foot of attachment filament.

Fig. 29. Immature female with maxillipeds (mxp) completely modified into chela-like structures. ×70.4.

Fig. 30. Enlarged drawing of foot (ft) of attachment filament at about stage represented by Figs. 28 or 29, in which are seen marks (x) where the claws of second maxillae (mx'') punctured the foot (ft). ×400.

Fig. 35. Enlarged drawing showing region of permanent attachment between second maxillae (mx'') of adult female and the chitinized, funnel-shaped bulla (bu). ×80.

Fig. 36. Maxilliped of adult female copepod. ×148.8.



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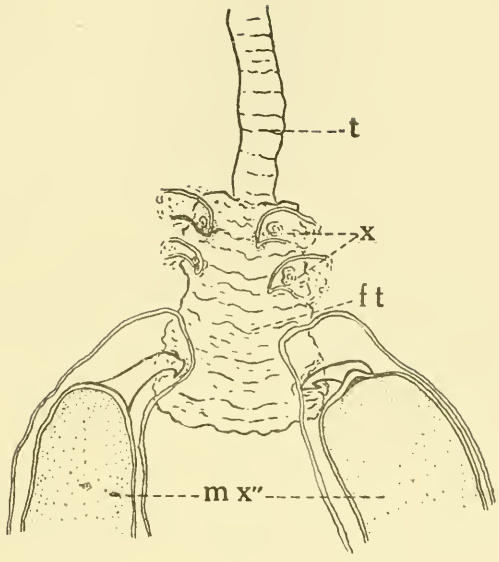

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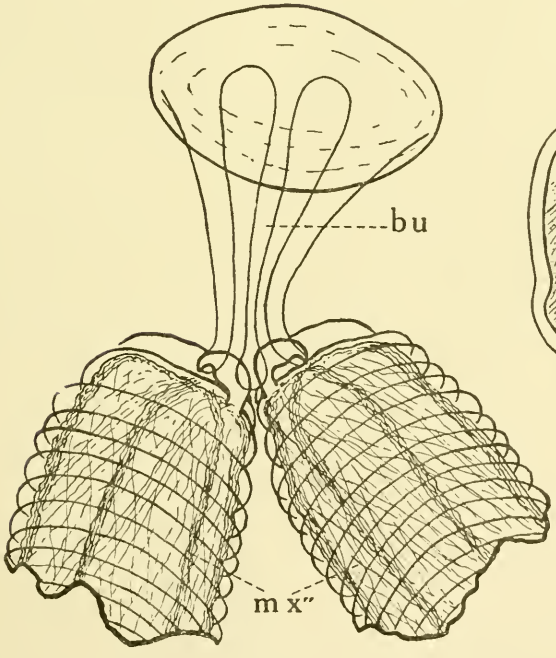

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36

N. Fasten, Del. PLATE 28

The Relation between the Osmotic Pressure of Nereocystis and the Salinity of the Water

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Bureau of Plant Industry, U. S. D. A.

1. INTRODUCTION

This investigation has to do with the relation between the osmotic pressure of a marine alga as determined by the freezing point method, and the salinity of the sea water. If variations in the salt content of the environment cause corresponding changes in the cell sap concentration, the question then arises as to how these are produced and whether the ability to so adapt itself is necessary to the plant's tolerance of fresh water. Therefore the problem finds its application in the study of the power of algae to adapt themselves to a change in the salinity of their environment. The experimental part of the work was done at the Puget Sound Biological Station at Friday Harbor, Washington, in the summer of 1918. Drevs (1895) has shown by the plasmolytic method that the osmotic pressure of certain marine algae can be increased by concentrating the sea water. The present investigation is concerned only with the adaptation to water of lowered salinity.

The plant chosen for experimentation was *Nereocystis luetkeana,* the most conspicuous and interesting plant of the Sound waters. Only young plants were used, because they could be easily grown in tubs in the laboratory. Unusual interest was attached to the problem in connection with this particular plant because of published differences of opinion as to whether it could grow in sea water diluted with fresh water from rivers. Preliminary experiments pointed to the fact that it would stand an astonishing amount of fresh water if it was gradually adapted to it but that any sudden change in salinity resulted in sudden death. The formation of the large blisters in such cases when the change was not gradual led to the idea that the extent of its adaptation was limited by its power of osmotic adjustment to the outside concentration.

Reports differ as to the occurrence of Nereocystis in sea water diluted by fresh water from rivers. Rigg (1915) and Setchell (1912) are led by their observations to believe that it requires water of normal salinity. But Frye (1915) found it growing near Point Couverden in Alaska in water of only 1.003 specific gravity, which was almost fresh enough to drink. The water from a creek empties there and "the affected kelps

are therefore about half the time in almost fresh water and about half the time in almost normal sea water."

The writer observed that when Nereocystis was plunged directly into fresh water it lost color and became soft in a few hours, the fronds being distorted by numerous blisters formed, evidently, by the rapid intake of water. But when the salinity of the water on the kelp was gradually decreased these effects were not produced and the plants were able to endure a considerable decrease in salinity without showing any bad effects. When a kelp was plunged directly into the same weakened concentration of sea water to which plants had gradually been accustomed and in which they were growing in good condition, blisters and other symptoms of ill health appeared. From this the explanation was offered that the ability of Nereocystis to tolerate fresh water is largely a problem of osmotic adaptation.

Osterhout (1906, 1906a, 1917) reports some striking instances of the endurance of sudden changes from salt water to fresh water and is led to the conclusion (1917) that "one who is inclined to attribute this remarkable tolerance of fluctuations in salinity to a process of gradual adaptation will meet with many difficulties." However, the following experiments show strong evidence that the adaptation of Nereocystis is quite largely a matter of gradual adjustment by a lowering of the concentration of the cell sap corresponding to the changed osmotic pressure of the sea water. However, the condition of the plants at the end of the process lends color to the view that death in fresh water may occur as the result of a loss of inorganic salts and other substances by diffusion.

2. METHODS

The experiment to determine the extent to which the cells of Nereocystis can adjust the concentration of their sap and produce equilibrium between the osmotic pressure within and that of the outside sea water consisted essentially of the following: (1) gradually decreasing the salinity of the sea water in the tubs in which the kelps were grown; (2) removing a sufficient number of plants after each change and extracting their juice; (3) getting the osmotic pressure of this juice by means of the depression of the freezing point as obtained with a Beckmann thermometer. The apparatus and technique necessary for making these determinations is described in any physical chemistry. Helpful details are given in papers reporting investigations involving such determinations (Garry 1904-1905; Harris 1914).

To subject the plants to different dilutions of sea water they were brought in from the shore with the rocks to which they were attached if possible; if not, they were weighted down by tying a small rock to the

holdfast end, and placed in large galvanized iron tubs painted on the inside. The tubs were placed in a deep trough built for the purpose on a shaded porch of the laboratory. A water faucet at one end and an outlet pipe at the other provided a way of keeping the temperature low on warm days by means of a stream of cold water flowing around the tubs. Not more than fifteen small kelps were placed in each of the five tubs. The tubs were emptied each evening and at once filled with water containing by volume 1/28 more fresh water. It happened that the tubs contained 28 times the volume of a small two-quart bucket which was a convenient measure of the increase in the amount of fresh water each 24 hours.

Before extracting the juice from the kelps for the freezing point determinations, the plants were washed three times in tap water to remove the salt water adhering to them, dried as well as possible by first wiping all surfaces with cheesecloth, then left spread out on cloths for twenty minutes. Any moisture left on the surface of the stipes or fronds produced an error in the freezing point reading. The fresh water, which of course cannot be all removed, dilutes the juice, as does some absorption of the fresh water during the washing. But at least the error is approximately the same for each extraction, whereas, if they were ground up unwashed, the salt left on the surfaces would cause too high a concentration of the extracted juice and the error would not be the same for all because the plants would come from waters of varying salinity.

After washing and drying them, the fronds, stipes and bulbs were ground up as finely as possible in an ordinary meat grinder. It was found more convenient to use the juice from this pulp than to use the pulp for the determinations. So the juice was squeezed out by means of a clean cheesecloth bag, and then transferred to the freezing tube of the Beckmann apparatus. Of course every precaution was taken throughout to keep absolutely clean the dishes and cloths coming in contact with the extract or pulp. Freezing point determinations were made without delay after the juice was extracted, to guard against any changes in it due to decomposition or evaporation.

In brief, therefore, the procedure necessary to obtain the effect of lowering the concentration of the sea water consisted in lowering the proportion of the sea water to fresh water by 1/28 each twenty-four hours. Then at the time the water in the tubs was changed each evening, four to six kelps were removed, the number depending on their size, and left over night in the same water from which they were taken; the juice from these was extracted and frozen the next morning. This meant that each bunch of kelps was exposed thirty-six hours to the final concentration from which it was taken, but that it was exposed twenty-four hours to each changed concentration preceding. This was simply to make sure that

there was sufficient time for the plant to become permanently adjusted to the last concentration before measuring the effect of this last dilution on its osmotic pressure. The freezing point of this water was then taken in order to compare it with that of the plants growing in it. This enables us to determine the osmotic surplus maintained by the cells throughout the adaptation process.

From the depression of the freezing point (Δ) the osmotic pressure is easily computed from the formula $P=22.4\times\Delta/1.85$. The explanation of this formula is simply that Δ for a gram-molecular solution of a non-electrolyte is 1.85° C., and this depression at 0° C. corresponds to an osmotic pressure of 22.4 atmospheres. For each 1.85° lowering of the freezing point, therefore, there is an osmotic pressure of 22.4 atmospheres. If the pressures are computed from the relation between molar concentration of cane sugar solutions and their osmotic pressures as established by Morse and Frazer (1905), different results are obtained. But, as has already been pointed out (Garry 1915), direct measurements of cane sugar solutions give pressures in excess of what they should be according to theory. Harris and Gortner (1914) have published a table of freezing point depressions and corresponding osmotic pressures in agreement with results computed from the formula $P=22.4\times\Delta/1.85$. The osmotic pressures given below are read directly from that table.

Owing to the fact that after removing a half dozen kelps or so from the tubs every day, the supply began to run short, the freezing point determinations were discontinued when the water was a little more than half fresh in order that the remaining kelps might be left and the experiment continued to see how much fresh water they would stand. The writer (1916) found that young kelps tolerated a proportion of 55% fresh water without extreme injury. In this later attempt one plant survived the 28 changes and remained alive several days in entirely fresh water. It was firm in texture and very evidently alive, but the color had

Table 1. *Showing the osmotic adaptation of Nereocystis to decreasing concentrations of surrounding sea water*

Number of 28ths fresh water	Δ for kelp juice	Osmotic pressure of juice in atmospheres	Δ for sea water dilutions	Osmotic pressure of water in atmospheres	Osmotic surplus of kelp juice in atmospheres
0	1.89° C.	22.72	1.62° C.	19.48	3.24
1	1.79° C.	21.52	1.57° C.	18.88	2.64
2	1.75° C.	21.04	1.52° C.	18.28	2.76
3	1.64° C.	19.72	1.46° C.	17.56	2.16
4	1.54° C.	18.52	1.40° C.	16.84	1.68
5	1.49° C.	17.92	1.34° C.	16.12	1.80
7	1.45° C.	17.44	1.21° C.	14.56	2.88
11	1.40° C.	16.84	.96° C.	11.56	5.28
13	1.32° C.	15.88	.84° C.	10.12	5.76
15	1.27° C.	15.28	.72° C.	8.67	6.61
17	1.03° C.	12.40	.60° C.	7.23	5.17

gradually faded as the salinity decreased until the fronds were light brown and somewhat misshapen, so that it did not present a healthy appearance. The extent of the adaptation which the cells of this plant had undergone could be shown very strikingly by placing kelps taken directly from the Sound in any of the sea water dilutions. Large blisters formed on fronds and bulbs within a few hours and the plants became soft and green. At no time were there blisters on the adapted kelps.

3. DISCUSSION OF RESULTS

Table 1 shows two very interesting facts. First, that the cells of Nereocystis adjust themselves fairly rapidly to the decreasing concentration of the water in which they are growing, the interval between changes to sea water containing 1/28 more fresh water being only 24 hours. There is an osmotic adaptation by virtue of which the plants are able to tolerate the fresh water in their environment. That the gradual adaptation brought about by a decrease in the concentration of the cell sap is necessary in this species for the tolerance of fresh water is shown by the blistering, deformity and softening, which result when kelps are plunged suddenly from normal sea water to the lower dilutions. In this Nereocystis differs from those species reported by Osterhout as able to endure sudden extreme changes in salinity. Second, this data shows that altho the osmotic pressure is steadily lowered in the process of adaptation, the cells maintain an average osmotic surplus of 3.62 atmospheres. This osmotic surplus or turgor pressure is the amount by which the pressure of the cell sap exceeds that of the water outside. It will be noticed that when the surrounding water was 11/28 fresh, that this surplus took a sudden jump. This may indicate an approach to a limit in the power to lower the osmotic pressure, or a period in the adaptation process in which the adjustment is not so completely made in 24 hours. In either case the pressure of the water would decrease more rapidly than that of the cell sap, giving rise to a higher osmotic surplus.

This osmotic surplus in Nereocystis of 3.6 atmospheres is low compared with that found in Cladophora, Enteromorpha and Chaetomorpha by True (1918). He shows a turgor pressure of 6.6 atmospheres, using the plasmolytic method with cane sugar. Duggar (1906) also gives data indicating similar turgors in marine algae; viz., Bornetia, Griffithsia and Pleonosporium, but they are reported in terms of salt concentrations causing plasmolysis instead of in atmospheres of pressure. Drevs (1895) found that certain algae maintain a constant osmotic surplus during changes through waters of increasing concentration. It is interesting to compare this relation between sap and sea water concentration in the case of algae with the results of similar work on sea animals. Garry

(1904-5, 1915) has made many comparisons of the osmotic pressure of the blood and body fluids of marine animals with that of the water in which they live, and with but few exceptions finds that the two are the same,—that there is no excess of inner concentration over that outside the organism. He succeeded in adapting animals to water of greater and of lower salinity, and in most cases the body fluids decreased or increased their concentration until it was the same as that of their environment.

4. OSMOTIC PRESSURE OF SEA WATER

It is interesting to note that the osmotic pressure of Puget Sound water is lower than that of waters from other regions of the earth in so far as reports have been made, except in a very few cases. From the measurements made at Friday Harbor it averaged 19.2 atmospheres, but of course it undergoes diurnal and seasonal variation. At the Golden Gate, San Francisco, Garry (1915) reports that on March 17, 1904 Δ=1.47° at high tide, 1.385° at low tide; on September 23, Δ=1.80°; so that from March to September the osmotic pressure varied from 16.6 atmospheres to 21.64 atmospheres. The water of the Atlantic coast where Δ=2.0° C. has a considerably higher salt content as does the water also at Pacific Grove, California (Δ=1.9°). The former's pressure would be 24.04, the latter's 22.84 atmospheres. I have found no figures for any other locality on the Pacific Ocean. The greatest depression reported is at Naples where Δ=2.29°, corresponding to an osmotic pressure of 27.51 atmospheres.

A more detailed comparison of the water at Friday Harbor with that of Woods Hole is interesting. In the following tabulations, the specific gravity and salt content of the water at Friday Harbor are obtained from the titration of the chlorine* content by means of Petterson's (1894) table; and the chlorine and total salt content of the Woods Hole water are obtained from Garry's (1915) density reading by using Petterson's table.

Table 2. *Tabulated comparison of the salinity of the sea water at Friday Harbor and Woods Hole*

	Friday Harbor	Woods Hole
Δ, in degrees centigrade	1.597	1.81
Osmotic pressure in atmospheres	19.204	21.76
Cl content in grams per liter	16.9	17.5
Salt content	2.95%	3.1%
Specific gravity	1.022	1.024

* The titration for chlorides gave the same Cl content as that reported by Shelford (1915) for Friday Harbor water.

5. DEMONSTRATION OF INTERCHANGE OF WATER AND SALTS IN OSMOTIC ADAPTATION

After it was shown that the osmotic pressure of Nereocystis decreases as the salinity of the water is lowered, the question naturally arose as to how it was brought about. The blisters which form so quickly on fronds and bulb when a plant is plunged suddenly into water containing less salt than does normal sea water, was evidence that it might occur as the result of the intake of fresh water and a consequent dilution of the cell sap. Then, too, it seemed possible that the change to an abnormal salinity might so change the permeability of the cell membrane as to allow a diffusion of salts from the cells out into the water, which loss would cause a decrease in pressure within the cell. Experiments were then undertaken to demonstrate whether either of these or both were responsible for the observed phenomena.

To determine whether fresh water in the environment causes a loss of salts small plants were placed in 250 cc. volumes of different dilutions each of known chlorine content; after a plant had remained in each for 24 hours the water was again titrated with silver nitrate (n/.0975) for chlorides. In all cases the kelps used were as nearly equal in size as possible. The results of these titrations are tabulated.

TABLE 3. *Showing the relation between the salinity of the water and the loss of salts from Nereocystis plants in it*

Sea water dilution in which kelp is left 24 hours	Amount of .0975N $AgNO_3$ required for each cc. of the dilution	Total Cl in g. per l. in dilution after containing kelp 24 hrs.	Cl supplied by sea water in the dilution in grams per liter	Excess Cl which must have come from kelp
Undiluted sea water	(1) 4.9 (2) 4.9 (3) 4.9	16.949	16.949	0.000
3 vol sea water: 1 vol. fresh water	(1) 3.775 (2) 3.700 (3) 3.775	12.966	12.712	0.254
2 vol. sea water: 2 vol. fresh water	(1) 3.216 (2) 3.316 (3) 2.650	10.567	8.475	2.092
1 vol. sea water: 3 vol. fresh water	(1) 1.900 (2) 1.730 (3) 2.075	6.559	4.237	2.322
Fresh water	(1) .985 (2) .850	3.174	0.000	3.174

We conclude from this table that the dilution of sea water with fresh water causes a diffusion of salts out of plants placed in it. This is shown by the fact that the amount of chlorides found in the diluted sea water in which kelp has been left 24 hours is greater than the amount in the water before the kelp was placed in it, the excess of necessity coming from the

plant. That this diffusion does not occur in normal sea water but is caused by a change in permeability of the cells to chlorides brought about by the fresh water is shown by the fact that the amount of chlorides in undiluted sea water is not increased by the presence of the kelps, but remains exactly the same as in normal sea water, viz. 16.949 grams per liter. As is to be expected, the preceding data shows that the amount of salts lost increases with decreased salt concentration of the water. Undoubtedly there is a very exact relation between the salinity of the water and the outward diffusion of salts from plants, but variations in size of the plants used preclude its determination here.

Having shown that a loss of salts is one reason for the lowering of the osmotic pressure of Nereocystis as the salinity of the water decreases, it remained to be determined whether an abnormal intake of water occurred at the same time which would so dilute the cell sap as to be a factor in changing its pressure. It was thought that as the larger proportions of fresh water had caused correspondingly greater losses of salts from the plants, so also the more dilute concentrations of sea water might be shown to cause the greater intake of water. The method followed was to cut the bulbs from plants of as nearly the same size as possible, being careful to include enough of the stipe so that the cut would come below the hollow part of the stipe, then to wipe these bulbs as dry as possible with a soft cloth and weigh them carefully. One was then placed in normal sea water, a second in an equal volume containing 10% fresh water, a third in 20%, a fourth in 40%, and so on up to entirely fresh water. After remaining in the various dilutions for 24 hours, the bulbs were wiped dry in exactly the same way as before, then carefully weighed. An increase in weight of those in diluted sea water above that occurring in those in water of normal concentration indicates the absorption of

Table 4. *Showing the relation between the salinity of the water and the increase in weight of Nereocystis bulbs left in it for 24 hours*

Sea water dilution; parts fresh water in 100 total volume	Original weight of kelp bulb in grams	Weight after 24 hours in grams	Increase in grams	% increase
0	16.922	17.157	.235	1.3
10	21.830	22.557	.727	3.3
20	21.835	22.942	1.107	5.0
40	24.492	26.187	1.695	6.9
60	16.072	16.417	.345	2.1
80	12.890	14.774	1.884	14.6
100	22.941	23.770	.829	3.6

water by the plant in its effort to bring about a state of equilibrium between its cell sap and the outside water. This therefore consists partly in equalizing the osmotic pressure inside and out by a dilution of the cell-sap. Table 4 shows the weights obtained.

We can safely conclude from this experiment that increased intake occurs in plants in water of subnormal salinity and so helps to enable them to adapt the pressure of their cells to the lowered pressure outside. In this they act as we would expect any living membrane to do. We might also expect that the amount of imbibed water as shown by increased weight would vary inversely with the concentration of the sea water. The preceding data apparently show this to be true; however, the evidence is weakened by the inconsistency in the results obtained in the 60% and 100% fresh water. The low percent of increase in these two cases may be due to the death of the bulbs and hence the failure of the osmotic exchange to occur, or to some other and unknown reason. On account of lack of time the experiment could not be repeated, so unfortunately the inconsistent figures must stand for the present unexplained.

6. CONCLUSIONS

1. The osmotic pressure of *Nereocystis luetkeana* is intimately related to the concentration of the sea water, decreasing as the water becomes less saline.

2. Nereocystis will not tolerate a sudden change to fresh water, but can be made to endure water 100% fresh if the adaptation is gradual enough to allow adjustment of the cell sap concentration to that outside the plant.

3. The osmotic pressure of Nereocystis in normal sea water as determined by the freezing point method is 22.72 atmospheres, and was lowered without killing the plants to 12.52 atmospheres in water 17/28 fresh, at which point the experiment was ended.

4. The osmotic pressure of Puget Sound water averaged 19.2 atmospheres.

5. Throughout the adaptation of the cells of Nereocystis to fresh water they maintain an average osmotic surplus, of 3.62 atmospheres.

6. The lowering of the osmotic pressure as the plant adapts itself to increasing dilutions of sea water is brought about (1) by the outward diffusion of salts and (2) by the increased intake of water.

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Early Stages in Bog Succession

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Introduction

It is well known that sphagnum bogs result from the abundant growth of Sphagnum in undrained places. The bog, of course, develops gradually, and it requires the lapse of a considerable period of time before the stage of physiographic succession is reached in which the surface is composed of the living moss underlaid with the products of various stages of its decay, supporting a bog association of xerophytic shrubs.

The climate and the topography of the Puget Sound country are well suited for the development of sphagnum bogs. In a region which has been at least twice covered with the debris of the advance and retreat of glaciers, naturally there are many depressions which are entirely undrained or whose drainage is at best very poor. The abundant winter rainfall, the lack of low winter temperatures and the high atmospheric humidity due to rain and fog are conditions favorable to the luxuriant growth of Sphagnum.

Naturally we should expect that in a region where Sphagnum bogs are so abundant as in the Puget Sound country there would be seen many instances of bogs in various stages of development. Such instances have been frequently observed by the writer. Cases have been seen in which a habitat is on its way toward development into a typical bog but has not yet arrived at that stage because not sufficient time has elapsed. In other cases one of the conditions is present, but no bog is developing because of the absence of the other condition. There are plenty of undrained habitats, but they do not become bogs unless Sphagnum grows in them to such an extent that the substratum, except at the surface, is composed of the products of various stages of the decay of vegetable matter (largely Sphagnum) in the absence of an adequate supply of oxygen. Likewise the moss is abundant in many places where its growth is not resulting in Sphagnum bogs because there is too much opportunity for drainage.

Sphagnum in drained places

An instance of the abundant growth of Sphagnum without the development of a bog is seen along the trial from Fairfax, Washington, to the ranger station. In September, 1917, this moss was found growing vigorously in a hollow along this trail at a distance of about 8 kilometers (5 miles) from the town. It was running over soil, logs, and roots. Fresh green stems of it a foot or more in length were readily found. All of the places where it grew were too well drained to allow te the development of a Sphagnum bog, and no Labrador tea (*Ledum groenlandicum*), swamp laurel (*Kalmia polifolia*), cranberry (*Oxycoccus*), *or sundew* (*Drosera rotundifolia*) were found. Of course, any physiographic change which would result in stopping the drainage from this place might result in the development of a typical Puget Sound sphagnum bog. We need then suppose only the continued growth and decay of Sphagnum, followed or accompanied by the introduction of the bog plants named above. The writer has no positive evidence in regard to the means by which these plants are transported to non-contiguous areas in the same vicinity. The writer has not tested the conditions necessary for the germination of the seeds of these plants, nor has he given special attention to possible means of their dispersal. It seems quite possible, however, that the cranberries, since they are readily seen on account of their color, may be eaten by birds and the seeds thus dispersed. The seeds of the other plants mentioned are all very small and light and are discharged by the opening of the capsules. It is possible that they may stick to the feet of the birds in wet weather and thus be carried a considerable distance.

Another instance of the growth of Sphagnum without the formation of a bog was observed in August, 1918, along the upper course of the north fork of the Skykomish river in Snohomish county, Washington. Several small areas were found in which this moss covered practically all of the surface but was not accompanied by any of the other plants commonly found in Puget Sound sphagnum bogs. None of these areas exceeded 150 feet square. They were flat but were at a sufficient elevation above the river so that drainage from the front edge of each was good. They were very wet because they were so flat. They were mostly swampy, and no doubt a considerable number of them had been depressions and had been filled by the accumulation of vegetation and by deposition of soil from the higher ground back of them. To what extent Sphagnum might have helped in this filling cannot be stated with certainty but the substratum where it was dug into had the appearance of swamp muck rather than that of sphagnum peat. The most characteristic plants occurring in these areas are marsh marigold (*Caltha palustris*) and scouring rush

(Equisetum sp.) although a sedge (Carex sp.) was rather common. Since these three species occurred in similar swampy areas in which there was no Sphagnum it does not appear that the presence of this moss had exerted any selective influence on the plant association. It does not seem that these areas are destined in the ordinary course of events to become sphagnum bogs at all.

Sphagnum on poorly-drained flat areas

The large "prairies" near the coast in Grays Harbor county, Washington, show many areas in which there is practically no drainage and in which small patches of sphagnum bog are numerous. Practically the whole area of these "prairies" is potential bog-forming habitat, and numerous early stages of bog succession are seen. In some cases a fairly typical bog stage has been reached. These forest openings or "prairies" lie between the towns of Carlisle and Pacific Beach. The largest one, about 1.5 kilometers (1 mile) in diameter, is near Carlisle. This area is extremely flat and very poorly drained, and is characterized mainly by swamp vegetation as sedges (Carex spp.) and rushes (Juncus spp.). Grasses were also found as well as marsh marigold and bunchberry (*Cornus canadensis*). The visit to this area was made on March 7, 1918.* At that time a large part of the area was covered with water from 2.5 to 30 cm. (1 to 12 inches) deep.

Two conifers are common in this forest opening. They are the lodge pole pine (*Pinus contorta*) and the Sitka spruce (*Picea sitchensis*). The former is much more common than the latter. Both are found in the neighboring forest but there the spruce is much more abundant than the pine. Both species are much smaller in the opening than in the forest, but the individuals are not misshapen and do not appear stunted. Apparently they are pioneers in the conquest of the swampy "prairie" by the forest. To what extent this present invasion by those conifers will result in the establishment of a forest depends largely upon the extent of bog development. The trees will evidently be unable to enter the areas on which Sphagnum is well established and should it surround growing trees in its progress it seems probable that the trees will be killed. Such has been the case elsewhere (Turesson 1916, Rigg 1917). No cedar (*Thuja plicata*) is found in these "prairies" except in a few swampy places. It is common in the neighboring forest. Hemlock (*Tsuga heterophylla*) is common in the forest but was not found in the "prairie" or the swamp. Douglas fir (*Pseudotsuga taxifolia*) is found in the forest but not in the prairies or swamps. Sweet gale (*Myrica gale*) is a common

* This visit was made by courtesy of the late Harry James Smith of the Bureau of Development, American Red Cross.

shrub on this "prairie". Much of the opening had a scattered growth of this shrub not more than 30 cm. (1 foot) in height. In other places there was a dense growth of it 15 dm. (5 feet) or more in height. Wild crabapple (*Pyrus rivularis*) occurred commonly along the edge of the lower places in which the water was a foot or more in depth.

Sphagnum was quite common in this opening. In some cases it occurred in areas 60-90 m. (several hundred feet) in diameter. In other cases it was in small bunches not exceeding 30 cm. (1 foot) in diameter. Very small hummocks of this moss were mostly associated with sedges although in a good many cases they were with rushes and in some cases with grasses. In some cases the succession had gone on to the typical bog stage in which nothing was present except Sphagnum, Labrador tea, swamp laurel, and cranberry. The latter plant was found only occasionally and then only in small areas, usually not more than 1.5 to 3 m. (5 to 9.8 feet). in diameter. In every instance it was found with Sphagnum. Cranberry is the only bog plant that the writer has not frequently found without Sphagnum. Bunchberry was frequently associated with this moss both with and without Labrador tea and swamp laurel.

In many cases Sphagnum was found in very swampy places, usually associated with Labrador tea and swamp laurel, and sometimes with cranberry. In some cases Sphagnum, swamp laurel, and the deer fern (*Lomaria spicant*) grew together. The writer has not elsewhere seen the deer fern associated with this moss. The growth of Sphagnum in pools of water in this area is common but has not progressed far enough to fill the pools and enable the bog succession to occupy them.

In this "prairie" then, small areas of bog are already established, and are quite evidently spreading. The drainage is so poor that in the normal course of development it seems probable that large portions of the prairie will become sphagnum bog. The bog may even kill portions of the forest in the course of its development. The course of events as elsewhere observed (Rigg 1917), however, indicate that the forest will finally invade the bog, and that the climax vegetation of the area will be the climax coniferous forest (probably hemlock) of the region.

It may be of interest to call attention to the striking similarities between this so-called prairie at Carlisle, Washington, and the "prairie" at Yakutat, Alaska. The resemblance between these two extensive forest openings consists mainly in that both are flat and poorly drained, and are characterized mainly by swamp vegetation such as sedges and rushes, together with grasses and marsh marigold, and that both have small coniferous trees and patches of Sphagnum. In both the bog succession has gone on to the typical bog of the Northwest in many small patches, while earlier stages are common. In the Yakutat area there are numerous

dead trees, probably killed by the advance of Sphagnum, while such a condition was rarely found at Carlisle and the cause of death seemed less evident. The layer of organic matter on the Yakutat prairie is about 30 cm. (1 foot) deep and rests upon very coarse gravel, evidently glacial outwash. The soil of the prairies of Grays Harbor county (Mangum 1912) is Montesano silty clay loam (the mucky phase in several areas). The soil owes its origin to the decomposition of sandstone, basalt and shale gravels, which were elevated during Pleistocene time. The original material has been considerably modified by weathering and the accumulation of much organic matter which has resulted from restricted drainage conditions. The flora of the Yakutat prairie has been previously described (Rigg 1914.)

Sphagnum in a hardhack swamp

In some cases there occur depressions which are undrained and the major portions of which are carpeted with an extensive growth of Sphagnum, and yet no bog has been formed, since the layer of decayed and decaying moss beneath that which is living is insignificant, and none of the other plants that characterize the bog stage are present. Sphagnum is there, the lack of drainage is there, but sufficient time for the formation of a sphagnum bog has not yet elapsed.

An undrained depression about 2 hectares (5 acres) in extent near Covington, Washington, illustrates this early stage in bog development. Water finds no outlet whatever from this depression until it is perhaps 60-90 cm. (several feet) in depth. It is stated by those familiar with the region that water is commonly 60 cm. (2 feet) or more in depth in this depression in winter, and that it does not disappear until August. The soil (Mangum 1911) in which this depression occurs is derived from glacial drift and has been more or less worked over by glacial waters. It is Everett gravelly loamy sand. This soil "is derived mainly from the coarser glacial deposits of sand and gravel which were laid down by the waters of the melting ice, but some of these gravelly areas probably represent the glacial drift, modified by the action of glacial waters, which have removed the finer material and left the coarser sand and gravel, deposited in beds, pockets, and strata of various degrees of thicknes. The drainage of this type of soil is too thorough."

The visits of the writer to this depression were made on October 21 and November 17, 1917. There was no water in the depression on either of those dates though the substratum was thoroughly watersoaked on the latter date. It seems probable that this depression is not free from standing water for more than three months of the year on the average. The time that it is free from water is not the normal growing season for any-

thing but fungi. The warmth and moisture of autumn in the Puget Sound region greatly favor the growth of these plants.

There are four distinct plant associations in this area. In the central portion of the area, approximately at its lowest point is an open portion containing a few vigorous bunches of sedge. The remainder of this

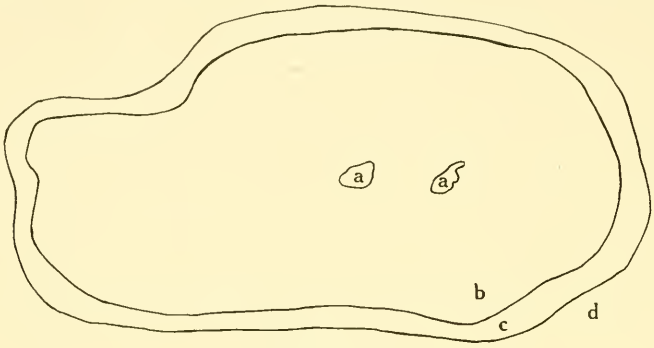

PLATE 29

Diagram of plant associations in the Covington depression: a, Carex-Sphagnum; b, Spiraea-Sphagnum; c, Populus-Polytrichum; d, Pseudotsuga-Gaultheria.

zone had no vegetation, except a thin but continuous carpet of Sphagnum and an occasional mushroom. This is designated as the Carex-Sphagnum association on the accompanying diagram. By far the larger portion of the depression is covered with a dense growth of hardhack (*Spiraea douglasii*). These bushes are mostly from 1.5 to 3 meters (5 to 10 feet) high and grow so close together that it is difficult for one to make his way through the dense thicket that they form. This shrub is quite characteristic of swamps and of the marginal ditches of bogs in the Puget Sound region. It flourishes well where the soil is covered with water much or even all of the time. Its stems have a tendency to become prostrate, and when this occurs they send up new erect branches in great numbers, thus forming a dense tangle of woody stems. It is thus well fitted to continue its growth into a swamp. Since a dense growth of Sphagnum covers the ground thruout this zone in this depression it may be called the Spiraea-Sphagnum association. While the hardhack thicket is continuous over the larger part of this zone, there are occasional openings in it. The growth of Sphagnum was more vigorous in these openings than in the thicket. The Sphagnum everywhere covers the prostrate hardhack stems and extends 15 to 25 cm. (6 to 10 inches) up the bases of the erect stems. In many cases it has formed little mounds 10 to 20 cm. (4 to 8 inches) high over stubs of broken hardhack stems. A few mushrooms were found in this zone also. On a few slightly elevated areas in the less dense portions of

the hardhack thicket occurs a thick growth of *Polytrichum juniperinum* instead of Sphagnum. Two specimens of aspen (*Populus tremuloides*) grow in the central portion of the Spiraea-Sphagnum association near the Carex-Sphagnum association.

There were a few small cedar logs in this zone and the Carex-Sphagnum zone. Aulacomnium sp. and two other species of moss grew on these logs. The logs evidently float in winter and this fact accounts for their flora. They had evidently floated to the situations in which they were seen and their presence furnishes no evidence that a forest had ever occupied this depression. The second zone occupied the portion of the depression only slightly elevated above the first.

A very well marked zone of aspen 10 to 20 m. (2 or 3 rods) in width, occupies the beginning of the upward slope of this depression. Those trees are mostly from 2.5 to 15 cm. (1 to 6 inches) in diameter. The larger ones have a dense growth of foliaceous lichens on their trunks and larger branches and many of the branches are dead. The younger trees seem healthy. There is a good deal of hardhack among the aspen and a few scattered aspens grow in the outer portion of the Spiraea-Sphagnum association so that the line of demarcation between the two does not seem so sharp when one is in the area. It appears perfectly well marked, however, on looking down from the road near by.

Sphagnum grows to a limited extent in a good many places among the aspen trees, but the ground is much more generally covered by *Polytrichum juniperinum*. We may designate this as the Populus-Polytrichum association.

On the higher and comparatively level ground above and surrounding the Populus-Polytrichum zone is what may be called the Psuedotsuga-Gaultheria association, since the principal tree is the Douglas fir (*Pseudotsuga taxifolia*) and the commonest shrub is salal (*Gautheria shallon*). Young trees and even seedlings of fir are common right down to the Populus-Polytrichum zone but not in it. There are a few young trees of red alder (*Alnus oregona*) associated with the fir. The soil of this zone is glacial deposit and is gravelly and, in places, stony. In some of the more open places there is a good deal of kinnikinnick (*Arctostaphylos uva-ursi*).

If this region remains undisturbed it is easy to foresee its natural development into the sort of sphagnum bog that is typical of the Puget Sound region. Indeed one may wonder why it has not already developed into one. Three possible explanations occur to the writer as to why this depressions is still in such an early stage of bog development. One is that comparatively recent changes may have occurred in the drainage of the region. Another is that the Sphagnum got a late start here. The third is

that the rate of development of both swamp and bog in this depression has been limited by the fact that the water runs off rapidly through the gravelly soil. The fact that the area is dry in late summer must have limited the coming in of many plants, and undoubtedly was a factor in the rather slow growth of Sphagnum before it was thoroly shaded by hardhack. The fact that this depression is flooded in spring and early summer also keeps out a spring flora of any sort except aquatic vegetation, and the fact that it dried up in late summer tends to kill out the aquatic plants. The result has been very slow development of vegetation. There is a well developed bog many acres in area only 1.6 kilometers (1 mile) from this depression. While the soil survey shows the same type of soil underlying both the bog and the depression, it is not known what local differences there may be in the soil that may influence the water-holding capacity in these two areas.

The bogs of the Puget Sound region overlie several different types of soil. Many of them occur on glacial till. In some of these the sphagnum peat lies directly upon the material deposited by glacial action. In other cases a layer of swamp muck lies between the glacial material and the sphagnum peat, indicating that a swamp stage preceded the bog stage. In some cases there is also a layer of inwash material consisting of sand and clay. The following bogs occur on glacial till, little if at all modified by stream action: Green Lake bog, Henry bog, Maltby bog, and Fauntleroy bog. Turesson (1916) has made a section of the Green Lake bog and finds the following layers: (A) 60 cm. of *Sphagnum teres* peat; (B) 15 cm. brownish black mud; (C) 20 cm. *Sphagnum palustre* peat; (D) 90 cm. forest peat; (E) 150 cm. Carex peat; (F) Clay.

Other bogs occur on residual soil. The bogs on the prairie in Grays Harbor county are examples of this. Other bogs south of Olympia, Washington, are known to occur on fine sand deposited by glacial streams. Other conditions being equal, development of bogs must undoubtedly be slower on very sandy or gravelly soil than on clay, because water would run away through the soil more rapidly. Undoubtedly some of the bogs near the coast overlie sand that has been deposited by wave action, and in some cases worked over by winds. In no case has the writer found a bog in the Puget Sound region directly overlying rock. The whole subject of the soil underlying the peat deposits of this region needs further investigation.

The bogs of the Puget Sound region are not typical "raised" bogs such as occur in Newfoundland and some parts of eastern Canada and Maine (Nichols 1918). Our bogs are usually slightly higher in the center than at the margin, but the difference in level is at most 1 meter or less, while in the "raised" bogs such as have been described by Ganong,

Transeau, Nichols, and others, the center in some cases is as much as 6 meters (20 feet) higher than the margin. Our bogs do not usually have a uniform surface, but are characterized by hummocks of Sphagnum often 6 dm. (2 feet) or more in height. Some, however, have a rather uniform surface over a good deal of their area. Our bogs are usally surrounded by a marginal ditch which in winter may contain 3-6 dm. (1 to 2 feet) of water while no water at all stands on the bog itself.

In the development of this depression at Covington into a bog we need suppose only the lapse of time and the introduction of the ordinary bog plants—Labrador tea, swamp laurel and cranberry. The continued growth of Sphagnum and the accumulation of the products of its decay under the anaerobic conditions will gradually kill out the few bunches of sedge present as well as the hardhack except that in the Populus-Polytrichum zone. Hardhack is the most characteristic shrub of the marginal ditch of Puget Sound bogs, but it is not commonly found where the growth of Sphagnum has progressed far enough to form a layer of decayed material of any considerable depth beneath it. The occasional aspens in the lower two zones will likewise be killed by the Sphagnum. When the sedge, the hardhack and the aspen, now the only three species of spermatophytes occupying the main portion of this depression, disappear, we shall have, in the Sphagnum and the products of its decay, ideal conditions for the establishment of bog plants. Since there is a large bog only a mile distant we need suppose only the means of transporting these this short distance.

The Populus-Polytrichum association at present marks the portion of this depression which in the course of natural development would evidently become the marginal ditch of the bog. It was noted that where the leaves of the aspen had fallen upon the substratum the Sphagnum covered by them was either dead or dying. This was especially evident where a few leaves lay close together forming a continuous covering, but was clearly noticeable even where only a single aspen leaf rested upon the Sphagnum. In one case an abundant growth of the mycelium of a fungus was found upon the dead moss under a single aspen leaf. The leaves of the hardhack are much smaller and lighter, and it requires a large accumulation of them to cause serious injury to the growth of the moss. Very few places could be found where they had caused any injury at all, while the injurious effect of the aspen leaves was evident wherever this species was abundant. It seems probable that the conditions above described are a considerable factor in the formation of a marginal ditch around so many Puget Sound bogs, as Turesson has pointed out.

The flora of the marginal ditch varies a good deal in different bogs. Hardhack is, however, usually abundant, forming a dense thicket. The

wild crab apple is common. A very dense growth of bush honeysuckle (*Lonicera involucrata*) is found in several cases. The western dogwood (*Cornus occidentalis*) is rather abundant in a good many cases. Willows (Salix spp.) and the red alder are frequently found. A few marginal ditches are invaded by cascara (*Rhamnus purshiana*); and conifers, such

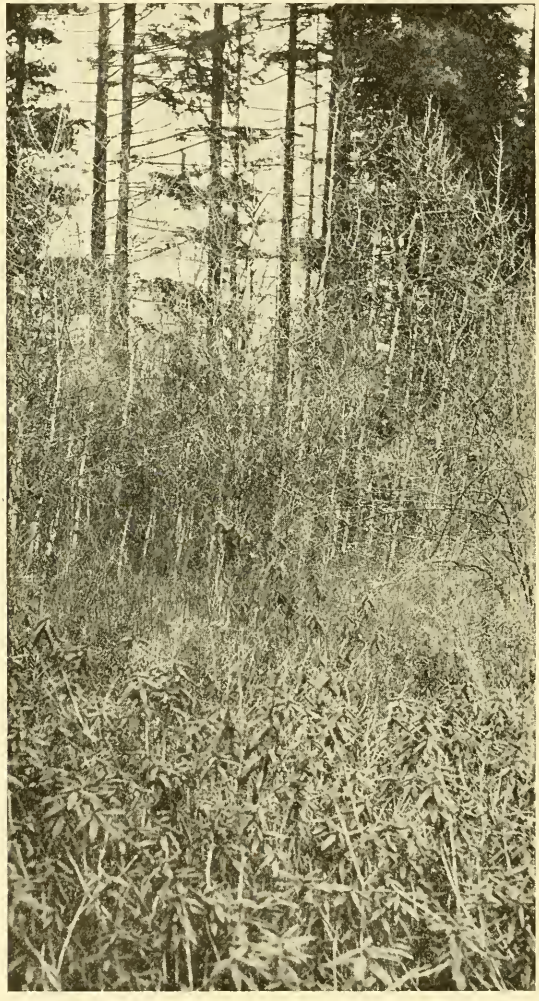

PLATE 30

View of margin of a typical Puget Sound sphagnum bog (Henry bog). Labrador tea is prominent in the foreground. Back of this is the zone of aspen in the marginal ditch. In the background is the fir forest outside of the bog.

as Douglas fir, western hemlock and giant cedar are fairly common in them.

We may look still further ahead to the time when this bog will, if nature has her way undisturbed, reach a stage late enough so that the forest will invade it as it has other Puget Sound bogs; and to the final occupation of the area by the climax forest (probably hemlock) of the region, the sphagnum bog being merely a temporary stage.

The advance of bogs on sedge swamps

Another type of bog development in this region is that in which Sphagnum is encroaching upon a swamp characterized by an abundant growth of rushes. Many Puget Sound bogs are bordered on one or more sides by such swamps and the tension line between the two types of vegetation commonly represents an early stage of bog development. Such a condition is found at the north side of Fauntleroy bog* just south of Seattle. It was also present in several portions or the Mud Lake area just north of Seattle before the draining of this lake, and is still found in several portions of the bog at Poulsbo, Kitsap county, Washington.

In these cases the Sphagnum may grow upon the ground among other vegetation or in some cases it may find partial support from the rushes and sedges. In the swampy northern portion of Graham lake, Maltby bog is progressing by the growth of Sphagnum at the surface of the water in and around the dense clusters of rushes.

The fact that bunches of skunk cabbage (*Lysichiton camtschatcense*) are common in bogs in this region seems to indicate that these bogs are a later succession upon swamps. Each bunch of skunk cabbage occupies a narrow but rather deep pit in the Sphagnum. Its survival from the swamp stage to the bog stage and its final elimination by the latter with the aid of mosses other than Sphagnum has been well described by Turesson (1916).

The advance of bogs on open lakes

Another type of bog development in this region is that in which the bog is advancing upon the open water of a lake. In some cases the Sphagnum with its typical flora of ericaceous shrubs borders directly upon the lake. In a good many cases there is a lake in the center of the bog, Sphagnum being dominant around its entire margin. Two such cases have been examined by the writer. The largest of these is Crystal lake in Maltby bog. This lake is somewhat circular in shape and has an area of about 4 hectars (10 acres). Its border is not entirely regular

* Nearly all of the Puget Sound bogs mentioned in this paper have been described (Rigg, 1913, 1917).

since the bog has encroached more rapidly in some places than in others. The water is 1.8 m. (6 feet) or more in depth at the very margin of the lake. This was tested by standing on the margin of the Sphagnum (a none too secure footing in many cases)and thrusting a pole down. Where bottom was reached it was, of course, of very soft material into which the pole could be pushed with little effort. In some places it was found that the surface of the bog projected out over the lake so that the pole could be thrust backward under the mat of vegetation on which the observer was standing.

Labrador tea, swamp laurel and cranberry were all observed as pioneers in the advance of Sphagnum on this lake. The first two are naturally more effective than the last on account of their stronger stems. Sometimes all three were found together, sometimes one alone, and in other places two of them together provided the means for the advance of the Sphagnum. All possible combinations of the three were found. The stems of these shrubs grow out into the lake, and their growing tips rise above the surface. Sphagnum keeps growing out on the surface of these and weighting them down. To a certain extent the growth of these tips keeps pace with the weighting down of the stems by the growing Sphagnum, and the woody stems of these species thus becomes deeply imbedded in the bog. The lake is finally filled up to the surface mat largely by organic matter. A good deal of this undoubtedly drops from the bottom of the mat while the continued growth of vegetation on top replaces the material thus lost. To what extent plankton material may contribute to the filling of this lake has not been investigated.

Another illustration of the advance of a bog on a lake is found near Sunnydale just south of Seattle. This lake is considerably smaller than Crystal lake, and is much shallower at the margin. In the main, the bottom of this lake slopes very gently down from the surface of the bog. No place was found where the water was more than 30 cm. (1 foot) deep at the edge of the bog. It has a zone of water lily (*Nymphaea polysepala*) and purple marshlocks (*Comarum palustre*) around it, and these are largely the forerunners of the bog. The water lily seems to function largely by filling the lake margin with organic matter. It does not act as a direct support for Sphagnum. Purple marshlocks on the other hand, functions in the shallow water here somewhat as the woody bog plants do in Crystal lake. Its stems form a rather dense mat beneath the water, while its leaves rise above the surface. This mat forms the support for the advancing moss. Labrador tea, swamp laurel and cranberry are found to a limited extent as bog pioneers, but their rôle is a minor one.

A bog borders the south side of Kitsap lake in Kitsap county, but does not seem to be making much progress upon it, since large coniferous

trees are found in the bog very close to the lake margin. One spruce tree (*Picea sitchensis*) approximately 30 cm. (1 foot) in diameter was found less than 1 meter from the open water of the lake. The cause of this practically static condition of the bog margin was not determined.

The bogs around Mud lake, prior to the lowering of the lake by drainage in 1916, furnished illustrations of both of the above types of the filling of lakes by the advance of Sphagnum. Where Labrador tea, swamp laurel and cranberry were pioneers in bog advance, the peat bog birch (*Betula glandulosa*) was commonly associated with them. The water here was not nearly so deep at the advancing margin of the bog as it is at Crystal lake. This birch sometimes grows to a height of 4.1 meters (15 feet) and its stems are much stouter than those of Labrador tea and swamp laurel, and thus furnish a much stronger support for the advancing Sphagnum. This species is not confined to the situation just described. It occurs commonly in various moist habitats around advancing bogs, both around the Mud lake bogs and several other bogs, notably those at Cottage lake in King county, and at Milton in Pierce county. The situation at Mud lake described above is the only one seen by the writer, however, in which it is a factor in the advance of Sphagnum upon open water. Smaller specimens of it are sometimes found in a fairly mature stage of the bog but it does not seem to flourish well there. The evidence, so far as the writer has seen it, seems to indicate that this species belongs in the succession preceding the bog and neither in the bog succession itself nor in forest stage which succeeds it.

On the south side of Mud lake the conditions were somewhat similar to those around the bog lake at Sunnydale described above. Purple marshlocks functioned in somewhat the same way in both cases. In some cases in the Mud lake region it was preceded by water lilies, but in other cases the shallow margin of the lake was being filled by a dense growth of the rhizomes of the buck bean (*Menyanthes trifoliata*), and at a later stage Sphagnum was advancing upon this by the aid of the purple marshlocks. This plant was also found as a pioneer in the advance of the bog upon Echo lake before conditions were changed there by improvements.

In several bogs, notably those at Mud lake and Sunnydale, the water lily was found in the same sort of pits as those in which the skunk cabbage is found. Like the latter plant, it has large leaves, and it seems probable that it has survived from the lake stage much as the other has from the swamp stage. The common presence of these two species in our bogs furnishes ready evidence as to the plant successions that have preceded the bog stage.

The advance of bogs on shallow ponds

Still another type of bog development is that in which Sphagnum grows submersed and thus encroaches directly upon the open water of shallow pools and ponds without the direct aid of other plants. This has been found frequently where the water was 45 cm. (18 inches) or less in depth. It has been seen by the writer in the following places: Mt. Rainier; upper valley of north folk of Skykomish river; Yakutat, Alaska; Carlisle, Washington. This type of bog advance is often seen, too, in pools within a large bog. Such instances have been observed in Henry bog north of Seattle and in a bog near Covington, Washington. It is a factor also in the gradual encroachment of the later stages of bogs upon the marginal ditch, as is illustrated in a bog near Seabeck, Washington.

Puget Sound bogs have, then, three ways of advancing upon open water. The first is by the aid of the woody plants that characterize the later stage of the bog itself, so that the mature bog stage advances fully organized, without outside aid. In this case the development stage of the bog is omitted. The second is by the advance of Sphagnum upon herbaceous plants that are not characteristic of the mature bog stage at all, but function in connection with bogs as pioneers only. In this case the bog later develops upon the Sphagnum that has grown upon these herbs. The third method is by the growth of Sphagnum in water, without the aid or support of other plants. In this case the bog develops upon the moss when it has grown until the combined bulk of decaying material and living material comes above the surface of the water.

Floating mats

It seems quite possible that bogs sometimes form the later succession on free-floating mats of vegetation, but no definite evidence of this was found. Several patches of Sphagnum bog, unconnected with the shore, were found near the extreme southeast side of Mud lake. There were numerous floating mats of other vegetation around them. The insecurity of the footing on many portions of these small bog areas seemed to suggest that they might be a later succession on these floating mats, but no intermediate stages were found to establish evidence that this was the case. The lake was drained before time could be found for a detailed study of the situation.

Species of sphagnum

The writer has not yet made a sufficient examination of the part played by the various species of Sphagnum in the different types of bog develop-

ment in the Puget Sound region to make any detailed statements on this phase of the subject. It is, however, easy to distinguish in the field in this region two groups of Sphagnum based on very obvious characters, the distinction being readily confirmed by microscopic characters (Frye 1918). The first group contains the stouter more leafy ones (4 species in all) which were so commonly used for surgical dressings during the late war (Hotson 1918, Nichols 1918). The second group comprises all other species of Sphagnum found in the region, some 20 in number. They are characterized by being more slender in appearance and having much fewer leafy branches. The mosses taking part in the bog development on the Carlisle prairie, except in rare instances, all belong to the first group. Those in the Maltby bog, the Mud lake bogs, the Sunnydale bog, and the ones growing near Fairfax and along the north fork of the Skykomish river all belong to the second group. The bogs at Poulsbo and Kitsap lake comprise both groups, frequently growing side by side.

Summary

1. Numerous instances of early stages of bog succession are to be seen in the Puget Sound region.
2. Sphagnum often grows in drained places and thus does not form bogs unless drainage is obstructed. Examples are seen at Fairfax and on the north fork of the Skykomish river.
3. Hummocks of Sphagnum grow on poorly drained prairie and thus form bogs. Examples of this are seen around Carlisle.
4. An instance is seen at Covington in which Sphagnum is abundant in an undrained depression but no bog is formed. Probably time is the element lacking.
5. Sphagnum advances upon swamps, growing among or upon sedges and rushes, and the bog thus succeeds the swamp. Illustrations of this are found in Fauntleroy bog, Maltby bog and Poulsbo bog.
6. Bogs gradually fill lakes, the Sphagnum advancing by the support of woody bog plants. Instances of this are seen at Crystal lake and Mud lake.
7. Bogs also fill lakes by the aid of herbaceous plants which are not characteristic of later bog stages. Instances are seen at Sunnydale and Mud lake.
8. Sphagnum often advances directly into shallow water without the aid of other plants. Examples are seen in Henry bog, the Mud lake bogs, at Yakutat in Alaska, and on the Carlisle prairie.

9. Possibly free-floating mats of vegetation may also be a means of bog establishment.

10. Sphagnum bogs develop on different types of soil, the development being slower on excessively drained types.

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Sphagnum from Bog to Bandage[1]

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Contents

Introduction

Sphagnum is the only moss for which any important economic use has been reported, and the more it is studied and experimented with the more varied and extensive are found to be its uses. In a former article the writer (Hotson 1918a) endeavored to give a brief history of sphagnum as used for surgical dressings both in Europe and America, especially in its relation to the World War. A brief consideration was also given to its distribution, habitat, structure and uses together with the results of some original work on absorbency of species best adapted to surgical work. It was further shown that it is on account of the great absorptive power due to the pressure of large, empty, perforated cells in the stem and

[1] Editor's Note—This work was done before the signing of the armistice and met an urgent necessity. While the urgency is gone, the call for sphagnum dressings is likely to continue in a lesser degree on their merits.

[2] Superintendent of Sphagnum Dressings for the Northwestern Division of the American Red Cross.

specially in the leaf that sphagnum is so highly desirable for surgical dressings, which in many respects have proved superior to those made of absorbent cotton.

"According to Professor Porter (1917) sphagnum pads surpass cotton pads in the following important particulars: (1) They absorb liquids much more rapidly, about three times as fast. (2) They take up liquids in much greater amounts; a cotton pad will absorb only five or six times its weight of water, as compared with sixteen, eighteen, and even as high as twenty-two times for a sphagnum pad. (3) They retain liquids much better, which means, of course, that the dressings need be changed less frequently. (4) They distribute the absorbed liquids more uniformly throughout their mass. (5) They are cooler and less irritating, yet at the same time fully as soft. (6) They can be produced at much less expense." (Nichols 1918a). So acceptable have these dressings proved that they have been used on practically all the allied fronts as well as in most of the base hospitals in Great Britain, France, Italy, Egypt, and to some extent in the United States.

Of the 40 species of sphagnum found in the United States there are only four that are commonly used for surgical dressings, though other species may be used to a limited extent. In the light of our present experience, it is probable that species hitherto rejected may be utilized for this purpose. The essential qualities of desirable sphagnum are softness, flexibility, elasticity and absorbency. Other things being equal, any species that will hold at least ten times its dry weight of liquid might well be considered among those suitable for surgical dressings. But as long as plenty of material that has an absorbency of 16 to 20 can be obtained, it is obviously undesirable to use an inferior quality.

It is estimated that fully 90% of the sphagnum in the United States suitable for surgical dressings is located on the Pacific Coast, from Oregon to Alaska. Up to the time the armistice was signed the Northwestern Division was entrusted with practically all allotments for sphagnum dressings asked for by the National Red Cross for overseas.

During the first two years of the war there was considerable opposition by British surgeons to the use of the sphagnum dressings on the ground that it was an "unnecessary makeshift." The opposition gradually disappeared, and in February 1916, they were made "official" dressings by the British War Office.

When the United States entered the war there was a similar indisposition on the part of American surgeons in France to use these dressings, a condition that one would naturally expect, as surgeons as a rule are extremely conservative, especially with the material they use in operations. Later this opposition had apparently disappeared, judging from

the orders received for these dressings. At the time the armistice was signed the British were making over a million of these pads a month. The Canadian Red Cross under the direction of Dr. J. B. Porter, was working on an order of 20,000,000, and turning out between two and three hundred thousand a month; while the American Red Cross, having completed an order for half a million, had just nicely started another allotment of 1,000,000 sphagnum dressings under the direction of the writer.

Although considerable work has been done in determining the characteristics of usable moss and the species possessing them, the more difficult problems have arisen in connection with handling the moss most effectively after it has been gathered. The object of this article is to present some of the methods and devices used in handling sphagnum from the time it is gathered until it is made into dressings.

Collection of Sphagnum

The main collecting ground in Washington has been in Pacific county, while in Oregon the supply has come from the vicinity of Tillamook, Newport, Marshfield and Florence. All the moss used in the Northwestern Division has been collected by volunteer labor. "Moss drives" occurred at more or less regular intervals. These were announced through the local papers and by means of posters. In each case simple directions for collecting were printed on the back. Similar advertising was done at other

PLATE 31

Carrying Sphagnum from the bog to the road.

centers. At first, when "moss drives" were announced, the whole town would go out in a body — men, women and children — making a picnic of it. In some instances holidays were proclaimed, all places of business closed, and the autos, delivery wagons, etc., requisitioned for the "drive." In most cases, however, these events occurred on a Sunday. As time went on and the novelty wore off, fewer responded to the call, but a goodly number of the "faithful few" were always on hand. The moss is usually collected by hand, but sometimes a fork is used as shown in plate 32.[3]

On these drives one of the hardest tasks confronted South Bend and Raymond, where they had to start at 7:30 A. M., load autos and auto trucks on barges which were taken about ten miles (16 km.) by steam tugs to Tokeland. After unloading they drove eight or ten miles to the bog, gathered the moss and returned. On such a trip they would gather from 500 to 1500 gunnysacks of moss, depending upon the size of the crowd and the accessibility of the moss. All of this was volunteer work — the barges, autos, tugs, etc., all donated. The success of these drives was largely due to the energies of Captain L. L. Darling, who planned and organized them; and Mr. L. L. Bush, who located the bogs and gave the instructions in collecting.

The difficulties presented to the Red Cross workers at Tillamook were almost on a par with those just mentioned. They had to drive 20 miles (32 km.) to the bog, 10 of which were over corduroy roads. Even under

PLATE 32

Collecting Sphagnum with forks in a bog near Ilwaco, Washington.

[3] The writer wishes to acknowledge his indebtedness to Prof. A. R. Sweetser for the photographs used in plates 31 and 33; to Dr. W. Haydon for those in plates 35 and 41; to the Seattle Chapter of the Red Cross for those in plates 37, 38, 39 and 44; to Miss Evelyn Gill Klahr for that in plate 42; to the engineering department of the University of Washington for the drawing in plate 43; and to the Northwestern Division of the Red Cross for making it possible to obtain many others.

these difficulties, sufficient moss was collected for nearly 200,000 pads. Plate 33 illustrates one Sunday's work which aggregated 2000 sacks of moss. Great credit is due to the people who live near the bogs and have coöperated in this work, many of them doing so at a real sacrifice for their sons, brothers and sweethearts at the front. It is these people who have made it possible for pads to be made in such large numbers and at comparatively little cost. It counts for little to work out the method of making an acceptable pad and to instruct women in large cities like Seattle and Portland in making them, if the raw material is not forthcoming. Never has a chapter in the Northwestern Division of the Red Cross had to stop making pads for lack of moss. They may have had to wait on the sorter or drying apparatus, but the raw material was always on hand, thanks to the coöperation of the people living near the bogs.

The following are a few of the salient points to be kept in mind while gathering sphagnum:

(1) Know exactly what you want before you begin. No collection should be undertaken until samples of the moss have been submitted to headquarters and approved. Until fairly familiar with the work, carry an approved sample of sphagnum with you and compare it frequently with the material you are collecting. If you collect the wrong moss you waste your own time and strength, the shipping expenses, and the time of the workers sorting.

(2) Gather clean moss. Collectors are often anxious to fill their sacks quickly. It is more important to fill the sacks carefully. Sphagnum badly mixed with other plants, roots and rubbish requires infinite labor in the sorting room and discourages the workers there. Aim at making a record for the quality and not for the quantity of the moss you collect.

(3) Work the moss bed as deep as possible. Sometimes suitable moss extends a foot or more below the surface. In general, whenever the plants begin to break up as a result of the first stages in decay, they must be discarded, but as long as they remain intact and the stem fairly well crowded with lateral branches, they may be used, the color playing little or no part in determining the suitability.

(4) Gather the moss in double handfuls. If wet, squeeze out as much water as possible before putting it in the bag but do not wring it, as that will break and injure the stem. Hasty collections often leave the moss dripping wet, and this not only adds needlessly to the weight of the sacks but increases the difficulty of drying later on.

(5) As you work your way through the bog collecting moss, take all the good material as you go. Do not pick a little here and a little there, thus making later work in the bog difficult and unattractive. *Be thorough.*

PLATE 33

The results of one Sunday's gathering at Tillamook, Oregon; 2000 sacks.

(6) Sacks used in storing or shipping moss must first be boiled or sterilized with formaldehyde in order to prevent mildewing.

Storing Sphagnum

The demands for sufficient moss to supply the daily needs where a large number of work rooms is concerned necessitates storing it for a considerable length of time, specially where the difficulties of gathering in winter are great, and the collections are entirely dependent on volunteer labor as is the case in the Northwestern Division. How best to store the moss so as to insure a constant supply is one of the difficult problems connected with sphagnum work. It is readily conceded that sphagnum keeps best in its native haunts, and if it is at all possible to obtain it during the winter directly from the bog and in sufficient quantities, that is by far the best place to keep it. This indeed may be possible to some extent in the Pacific Northwest if only small amounts are required, but it is impossible to obtain several carloads on short notice during the winter months. During the summer the bogs are comparatively dry, so that the moss may be obtained with half the energy and inconvenience that it necessitates during the rainy season. Moreover, along the Atlantic Coast and in Alaska the bogs are frozen up during the winter, making it impossible to obtain the moss under any conditions.

In considering this problem several questions presented themselves: Will the moss mildew? If so, can it be prevented? If stored wet, will it heat? If so, how may this be overcome? If wet, will it decay? These are some of the points that presented themselves at the outset. Others cropped up as time went on. In order to arrive at some solution to the problem, a series of experiments was undertaken.

(a) Moss was stored in bulk in different ways: on the floor of a well ventilated room; on the floor in the basement of large buildings poorly ventilated; and in the open exposed to the weather. When stored exposed to the weather, the moss is practically always saturated, which not only makes it unpleasant to handle but adds materially to the expense of freight if it has to be shipped. The outer portion of the pile also becomes so bleached, weathered and broken that if the moss is not entirely spoiled its usefulness is at least greatly impaired. Moreover, there is considerable opposition to sorting moss when it is excessively wet. Storing moss in a cement basement proved quite satisfactory, except that there is more tendency to mildew than where there is good ventilation. In some instances a small amount of formaldehyde was sprayed between the layers of moss as they were spread down. But this precaution was seldom necessary. The moss never heats and seldom mildews except when infected from the sacks or other things with which it comes in contact.

If the method of storing in bulk is adopted the best results are obtained by piling the moss in a heap in an unoccupied building where the floor has previously been cleaned and sprayed with a 4% solution of formaldehyde to destroy any mildew spores. It should be borne in mind that the moss will retain its moisture a long time, consequently it may seriously injure a valuable floor by warping the boards.

(b) Moss was stored in sacks as it comes from the bog. This method proved satisfactory provided the sacks were sterilized before they were used, otherwise the moss was apt to mildew. The most serious objection, however, to using sacks is the expense. When stored for a considerable time 75% to 80% of the sacks could not be used a second time on account of partial decay due to constant moisture. Moreover, the scarcity of sacks made it almost impossible to obtain sufficient quantities for shipping purposes without using any for storage.

(c) Moss was stored in bales. The shortage of sacks suitable for shipping and the necessity for storing the moss became so acute that it was necessary to devise some method of handling it which would not require sacks. Experiments were conducted in baling the moss. Through the courtesy of the University of Washington a mechanic was put at the disposal of the writer to assist in making a baler for this purpose. Here, too, the question of whether sphagnum would heat or mildew when baled

moist had to be considered; whether the moss could be baled as wet as it comes from the bog or whether it must be partially or completely dried first; what effect would the pressure have on the absorbency of the moss; etc. These questions have been answered quite satisfactorily by the experiments with the baler.

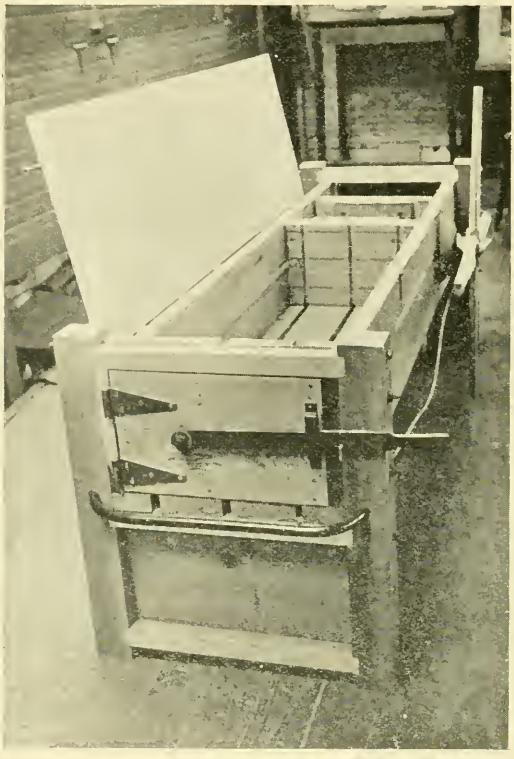

PLATE 34

The baler used for baling moss for shipping, in the Northwestern Division of the Red Cross.

Baling Sphagnum

In making a baler it was thought best to obtain one that was inexpensive and simple, so that any carpenter might easily reproduce it. With this thought in mind, several types of balers were considered, such as those used in baling hay, paper, hops, etc. The one finally decided on was simply a modification of the principle underlying all of these.

As the accompanying photograph (plate 34) indicates, the baler is a horizontal trough 9 feet long 17 inches wide and 12 inches deep (2.74 m. x 43.2 cm. x 30.5 cm.). The length is sufficient to hold at one

time enough moss for a single bale. The pressure for baling is obtained from a side wheel with four spokes for handles. The size of the bale is 12 x 17 inches and may be as long as is thought best. After making bales of different lengths it was decided that one 16 inches (40.6 cm.) long was the most convenient to handle. The bales are thus made 12 x 17 x 16 inches (30.5 x 43.2 x 40.6 cm.), bound by wire with three strips of ordinary lath on each end as well as on the bottom and top, to keep the wire from cutting into the moss. The sides are usually left unprotected, since it is found that if the bales are piled on the side while damp they pack sufficiently to prevent the loss of any great amount of moss by handling.

Three horizontal grooves are made in piston, the inner surface of the door and on the bottom where the bales are to be made. Into these grooves the pieces of lath to protect the sides of the bale are put and held in place by brads. The trough is then filled with moss which has previously been looked over to remove any material that is useless for making pads, the lid is clamped down and the pressure applied by means of the windlass. When the moss is pressed sufficiently the piston is held in place by a dog and rachet. The lid is then raised and three additional pieces of lath are put on top of the bale in a similar position to those on the ends and bottom. The bale is wired with No. 15 double looped annealed baling wire, which is about 9 feet (2.75 m.) in length. With the size of the bale used, one of these strands will make two by cutting in the middle, leaving

PLATE 35

Baling moss with an ordinary Pacific Coast paper baler.

a loop at the end of each. The cut ends of three wires are pushed down through vertical grooves on the inner surface of the door and up through corresponding grooves in the piston, drawn tightly with strong pincers, caught in the loop and fastened on top of the bale. The pressure is then released, the door opened and the bale removed. Clean paper is spread out and the bales are piled on it with the unprotected surface down. They should be turned from time to time.

The first bales were made early in July, some of dry moss, some with the moss slightly dampened, and others with it saturated. Several of each kind were made and stored in a well ventilated room. After two months one of each of these was opened and examined, with the most satisfactory results, specially with the moderately moist and wet bales. The dry moss, however, was broken up too much, and after further experiments it was finally abandoned as being impracticable. At the end of four months the baled, damp moss was just as fresh and sweet as when it was first baled, with no sign of heating or mildewing.

In order to safeguard against mildew, in some of the bales a small amount of 4% solution of formaldehyde was sprayed when about half of the moss for the bale was in the trough. The rest of the moss was then put in and baled in the usual manner. In most cases, however, this was found to be unnecessary, and in some cases even a disadvantage, because the moss kept moist so long that the gas did not evaporate from the center until the bales were opened, and then it was irritating to the sorter. At the writer's request, Dr. Walton Haydon, of Marshfield, Oregon, carried on similar experiments, using an ordinary Pacific Coast paper baler (plate 35), which makes a bale 24 x 18 x 18 inches (61 x 45.7 x 45.7 cm.). In these practically the same results were obtained, except that they were rather too large to handle conveniently.

The above experiments show that the principle of baling sphagnum for surgical dressings is an entirely satisfactory method of handling the moss as it comes from the bog. As has been said it never heats and seldom mildews except when infected by the container. If the bales are covered with burlap that has not been sterilized, they almost invariably mildew. Experience has shown that after the bales are made they should be kept in a well ventilated room, not piled too closely together and never covered with anything that will likely contaminate them. The practicability of baling sphagnum for surgical dressings is also in accord with the experience of Dr. Porter in dealing with the Nova Scotia moss.

Sorting Sphagnum

There must at all times be a close relation between the process of gathering moss and sorting it. Experience has shown that carelessness on

the part of the collector may greatly reduce the efficiency of the sorter, frequently rendering otherwise useful moss absolutely worthless. The amount of moss sorted is thus proportional to the care of the gatherer, and yet it is readily seen that with the difficulties confronting the collector, too much time cannot be spent on that end of the work, as there are a hundred persons available for sorting where there is one for collecting. There is a happy mean to which we should strive in order to obtain the greatest results with the least labor. Picking over or sorting the moss is usually one of the tedious phases of making sphagnum dressings. The sorting may be done either in some central place or at several centers. Good results have been obtained by distributing the moss to women's clubs, who sort and return it to the place where it is dried. In some places a great deal of assistance has been rendered by high school teachers and students. In such cases a small portion of the regular school time may be allotted to this work, or if there is objection to that, volunteers may be asked to remain half an hour or even an hour after the school is dismissed.

The following are a few suggestions for the sorter to keep in mind:

1. Moss to be sorted should be spread out several hours in advance, preferably late on the previous day. If it is in a damp condition the large lumps may be slightly shaken apart and separated; but if dry and packed, as is more likely to be the case, it should be sprinkled lightly with water and an oilcloth thrown over it for several hours, or over night if possible.

2. In cleaning the moss it is not necessary to remove every particle of foreign matter such as tiny bits of leaves or grass, but, of course, all the larger leaves, all bits of stick, any sharp pine needles, stiff grass, etc., must be removed. As the moss is spread out in the drying frames it should be looked over a second time as a matter of precaution.

3. After the moss is sorted, do not leave it in sacks for any length of time but spread it out as soon as possible. If it is allowed to dry in the bag it frequently forms a hard lump which when pulled apart breaks up the moss.

4. When dry, handle the moss just as little as possible, as in handling it the small leaves are broken off. It is these leaves that do the work of absorption, hence the more that are lost the less efficient the moss. Never sort dry moss. If, inadvertently, it becomes too dry, moisten it.

Drying Sphagnum

The fact that sphagnum absorbs such a large amount of water and holds it with such tenacity makes the process of drying one of the most

PLATE 36

A drying rack used in the work rooms at the University of Washington. A working drawing is shown in plate 43, Fig 1.

difficult steps in the preparation of the moss for dressings and one that has required a great deal of careful thought and experimentation. This is particularly true along the Pacific Coast during the wet season when the atmosphere approaches saturation for months at a time.

Experiments have shown that artificially dried sphagnum is harsher and far more brittle than moss that is dried in the open air under ordinary conditions. This is due to excessive drying in which not only the water contained in the reservoir cells is removed but also that in the green cells and the walls of the plant. It is the removal of this excessive amount of moisture, depriving the plant of its elasticity, that makes it brittle and harsh. In ordinary air-dried moss a portion of this moisture is retained by the plant for a longer time, and if removed at all it is so gradually that the walls are left flexible. Dr. Porter suggests that "it is probable that the controlling factor is the relative humidity of the circulating air rather than the rate at which the sphagnum is dried. For example, I see no difference between moss dried in summer by placing it

near a stove and that dried in trays in an ordinary Montreal workroom in winter. In the first place, the temperature is probably over 100° F., whereas, in the second, it is only about 65° F., but in each case the relative humidity is very low, probably ten or fifteen per cent. The moss is thus almost completely desiccated. We have gone so far as to recommend that in winter drying rooms should be kept just as cold as possible for the work of the people. We have even discussed the advisability of humidifying them somewhat after the method employed in certain branches of cotton spinning."[4] There seems to be no objection to using artificial heat, or any other means of drying, provided the drying is not carried too far. For instance, it would be perfectly in accord with the facts thus far known, to use artificial heat, say up to 90° or 100° F. at first, and then finish the drying without artificial heat.

In considering any apparatus for drying the greatest precaution should be taken not to handle the dry moss any more than is absolutely necessary, and any contrivance that stirs or shakes it while drying should be avoided as far as possible. Rough handling tends to break off the minute branches and leaves which are the parts of the plant that make it valuable for surgical work.

PLATE 37

The blower with 2 H.P. motor that supplies the air for drying the moss in the trays in plate 38. This is installed in the Seattle Chapter of the Red Cross.

[4] From a communication to the American Red Cross.

A convenient means of drying moss on a small scale suitable for most of the smaller Chapters is a rack such as is used at the work rooms at the University of Washington (plate 36). Each shelf is four inches deep (10 cm.), the bottom covered with chicken wire which, in turn, is covered with unbleached muslin. In removing the dried moss it has been found most convenient to draw out the muslin with the moss on it, one person holding each end and emptying all of it at once, thus avoiding much handling.

The most elaborate plan for drying sphagnum in the Northwestern Division is that installed in Seattle and is illustrated in plates 37, 38 and 39. This was planned and constructed by Mr. Harding Gow, who is a full-time volunteer at the Seattle Chapter. The drier is located on a balcony six and a half feet (2 m.) wide and about seventy feet (20.3 m.) long, above the main workroom. The moss is placed in trays 30 x 32 inches and 1⅝ inches deep (76.2 x 81.3 x 3.13 cm.). The trays have galvanized fly screen bottoms and are arranged in racks of which there are 22 in all, each rack holding 18 trays, giving a total of 396. The racks (plate 38) are placed at the outside edge of the balcony, leaving a space of 4 feet (1.2 m.) between the trays and the wall. The balcony floor is 14 feet (4.27 m.) below the ceiling. This space is divided into two stories by a light floor, having nine tiers of trays on each floor. The air is heated by two stacks of six tiers, Vento radiation, 9¾ x 60 inches (24.8 cm. x 18.3 m.) enclosed in a sheet iron case. The air is forced through this case by a double 11-inch (27.9 cm.) blower driven by a two horse-power motor. It is delivered from the case by two outlets 12 x 24 inches (30.5 x 61 cm.) in cross section, which are located at the end of the balcony near the back wall and also as near the floors of the passages as the radiators will allow, as experiments have shown that the heated air will travel farther along the passage when the inlet is near the floor. The opposite end of the passage is closed by a light door, so that all the heated air delivered into the passage is forced through the trays.

The blower (plate 37) runs at 860 revolutions per minute, delivering between 5000 and 6000 cubic feet (424-510 cbm.) of air. The maximum temperature of the air actually in contact with moss is about 90° F. The trays when filled level full will dry in 24 hours. Each tray has a capacity of ½ pound (227 g.)of dry *Sphagnum palustre,* or ¾ pound (340 g.) of dry *Sphagnum imbricatum,* the two species commonly used. If run at its full capacity, therefore, this drier will insure 200 to 300 pounds (91 to 136 kg.)of dry moss per day, which should make a minimum of 2100 dressings 12 x 24 inches (30.5 x 61 cm.) or 6300 dressings 8 x 12 inches (20.3 x 30.5 cm.).

The moss, after it has been sorted is hoisted in large clothes baskets to the balcony and emptied into carts, each of which holds three baskets. The carts are wheeled down the passage and the trays filled. The dry moss is stored temporarily in a large bin extending from near the main floor up to the balcony (plate 39). As it is needed it is drawn off by means of a chute that leads to the main floor.

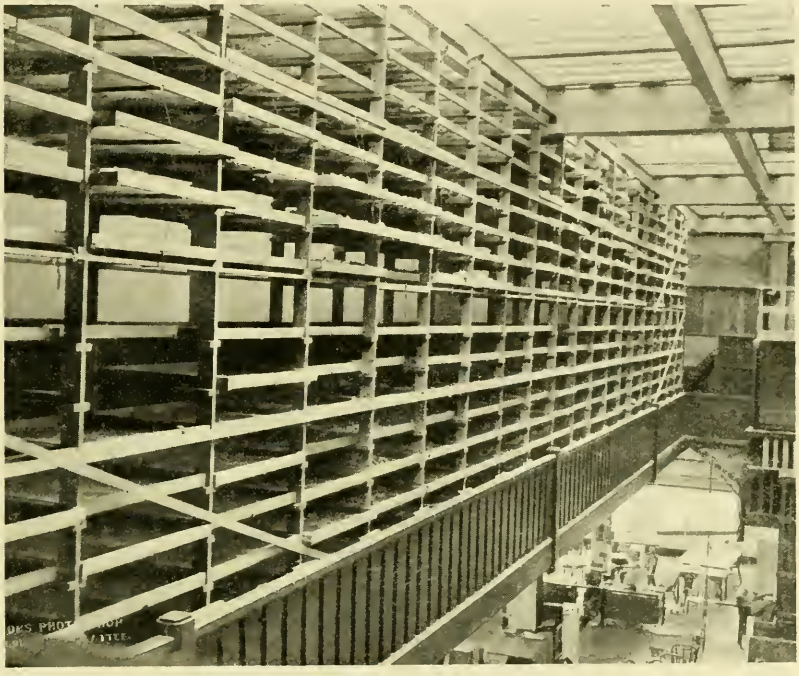

PLATE 38

Arrangement of the trays for drying moss in the Seattle Chapter.

A third type of drier has been suggested by Mr. L. L. Bush, Divisional Field Representative in Pacific County. The device consists of a box or tank which is air tight, except that it is open at the top. The air-tight feature is such that the box will stand a slight pressure from the inside without leaking save as the air escapes above. It is constructed of ¾-inch (1.9 cm.) lumber lined on the inside with stiff paper and sealed at the joints with some quickly drying material. About a foot (30.5 cm.) from the bottom is a false floor consisting of fine chicken wire similar to that used above. Through an opening near the bottom a shaft connects the air chamber with a small fan and motor. The moss is laid evenly on the screen floor to a suitable depth and packed well against the sides so

PLATE 39

A portion of the trays shown in plate 38 and the bins for storing the moss; also the chute for carrying the moss to the ground floor.

that the air has no special channels for passage but is distributed equally through the moss. The rate of drying depends upon the amount of circulation. A large and strong inflow of slightly warmed air (about 90° F.) will produce fairly rapid drying. The value of this method lies in the forced circulation through the moss.

A device (plate 40) used for drying moss has been designed by Mrs. Austin R. Baldwin of the New York County Chapter of the American Red Cross. This is constructed on the plan of a Ferris wheel which is 11 feet (3.35 m.) high with eight pairs of arms, each pair supporting two trays of moss, making sixteen in all. Each tray is 33 x 22½ x 2½ inches (83.8 x 57.2 x 6.4) with ¼-inch (6.3 mm.) wire screen for the bottom

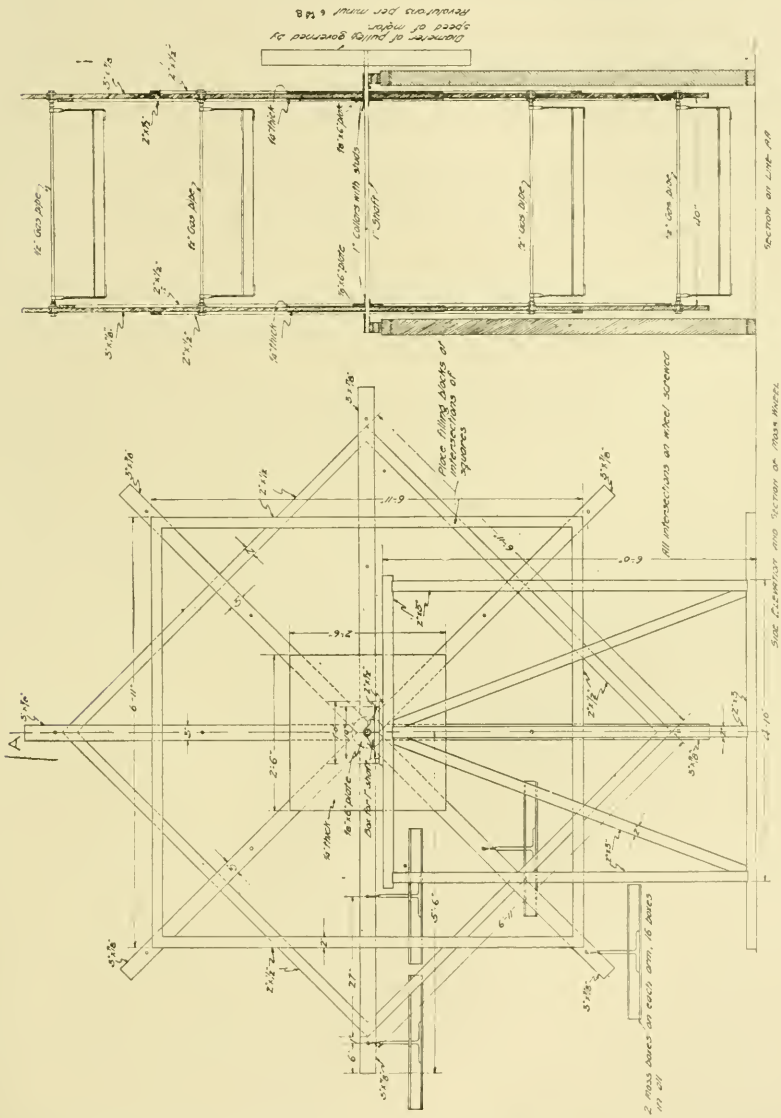

PLATE 40

Side elevation and section of the "Ferris" wheel used in the New York County Chapter of the Red Cross. Designed by Mrs. Austin R. Baldwin.

and top. The trays are filled with moss of about the same degree of dampness, and the wheel is kept in motion by a one horse-power electric motor.

In connection with this drying apparatus some valuable experiments were undertaken to ascertain if humidity is a factor to be considered in drying moss artificially.[5] To this end an attempt was made to make the atmosphere of the drying room similar to that which might be called a good drying day, with special care as to the relation of temperature and humidity. Artificial heat was furnished by a gas heater. The temperature was regulated by means of gas burner rings of different diameters, three in all, controlled by stop cocks as in a gas range. Air was forced through the heater by an electrically driven motor (½ H.P.) making 2700 revolutions per minute. The temperature maintained in the drying room was made to conform to the percentage of relative humidity desired. In any event, the temperature was not allowed to rise about 105° F., as a higher temperature seemed to be deleterious to the moss. On days of low humidity moisture was provided for by live steam, by a pan of water on the heated pipe from the heater, or by passing intake air over water. Wet and dry bulb thermometers were used to determine the condition of the room air and outside air.

For the first one or two hours of drying, depending on the moisture content of the moss, the relative humidity was held between 50% and 60% to avoid too rapid drying. When the sphagnum appeared to be about half dry, the percentage of relative humidity was lowered, and the drying was finished with a relative humidity of about 30%. If the moss was half dried in the racks before being put in the wheel the relative humidity was at once lowered to about 30%.

The wheel, when originally installed, made six revolutions per minute. This was increased to eight revolutions per minute, increasing the mean velocity of the outer trays from 188.5 feet (57.5 m.) per minute to 251 feet (76.5 m.) per minute, the mean velocity of the inner trays from 94 feet (27.7. m.) per minute to 125.6 feet (38.3 m.) per minute, with beneficial results.

The moss in the outer trays dries more rapidly than that in the inner trays, furthermore, the moss at the edges of the outer trays exposed to a larger volume of air, dries more rapidly than that at the edges exposed to a smaller volume. It was found advisable not only to exchange the inner trays with the outer ones during the process of drying, but also to turn the trays half way round to get uniformity in drying.

Sometimes it was necessary to run the wheel all night in order to obtain sufficient moss, but in such cases the heater and blower were shut off at 10 P. M. It requires from 10 to 12 hours for all the moss on the

[5] The writer is indebted to Mrs. Austin R. Baldwin for the facts concerning these experiments.

wheel to become dry, while moss drying naturally in trays in an adjoining room would require 48 to 72 hours, according to the weather. The reasons for this were: first, the higher temperature of the drying room, which at 10 P. M. had risen to 95° to 100° F., but fell to within two or three degrees of the temperature of the adjoining room by morning; and second, the motion of the moss in the trays on the wheel through the air of the drying room. Air circulation seemed to be an important factor. When these conditions became apparent three electric fans were installed. These also accelerated the changing of the air in the drying room between drying periods.

Absorbency tests of five pads made from naturally dried moss and a similar number from artificially dried moss show that the latter absorbs within three per cent as much water by capillarity as the former. Although perhaps this investigation has not been carried on long enough to come to a definite conclusion yet these preliminary experiments suggest that moss artificially dried under proper relations of temperature and humidity might be of great value in making surgical dressings.

As has been stated above, the American Indians used sphagnum quite commonly as a dressing. Their method (plate 41) was to throw the moss upon bushes or string it along poles. This is one of the best and most effective ways of drying if the weather is suitable, but it is extremely

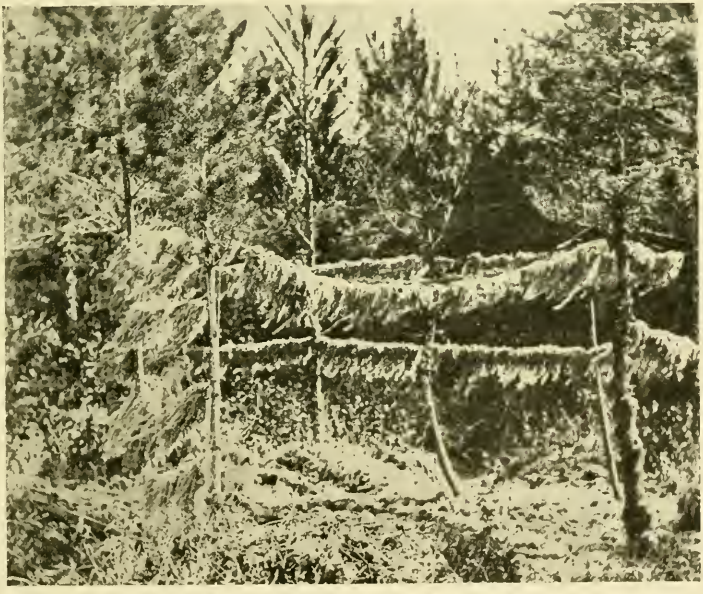

PLATE 41

The Indian method of drying Sphagnum.

PLATE 42

A method used in Nova Scotia for drying Sphagnum. It consists of a frame-work with galvanized wire screen for bottom, and muslin or cheese cloth around the edges to prevent the wind from blowing the moss away.

wasteful. One of the methods used by Dr. Haydon for drying in the field is fashioned after the Indian method, and consists of a frame-work with crossbars on which the long strands of *Sphagnum palustre* are strung. When dried the moss is spread out in the center of a sheet 6 x 9 feet (1.8 by 2.7 m.), the sides folded over the moss and then rolled up from the end and roped. Another method of drying, used by Dr. Haydon consists of a framework made of two by two inch (5.5 cm.) scantling with ordinary lath one inch (2.54 cm.) apart as a bottom, on which the moss is spread. The moss is covered by a piece of fish net to prevent it from being blown off the rack.

In Nova Scotia sphagnum is sometimes dried in the field on racks (plate 42), the sides of which are closed in by strips of unbleached muslin

PLATE 43

Part of the equipment used at the University Auxiliary of the Red Cross, Seattle.

Fig. 1. A drying rack; length made to suit the room; bottom of galvanized chicken wire with small mesh; made of dressed ¾ x 3 inch (1.9 x 7.6 cm.) lumber. Photograph shown in plate 36.

Fig. 2. A convenient table for a work room either for making pads or sorting moss; length of table to suit size of room; may be made of common lumber 1 inch (2.5 cm) thick planed on one side; top and edges protected with building paper and covered with 54-inch (1.34 m.) pebble oil-cloth.

Fig. 3. A drying tray; made so that several can be stacked on one another; bottom similar to that in Fig. 1.

Fig. 4. A tray for dry moss; for holding material while pads are being made; composed of ½-inch (1.3 cm.) dressed lumber.

Fig. 5. A moss frame for an 8 x 12 inch (20.3 x 30.5 cm.) pad. Used as a guide in determining the appropriate amount of moss for the pad.

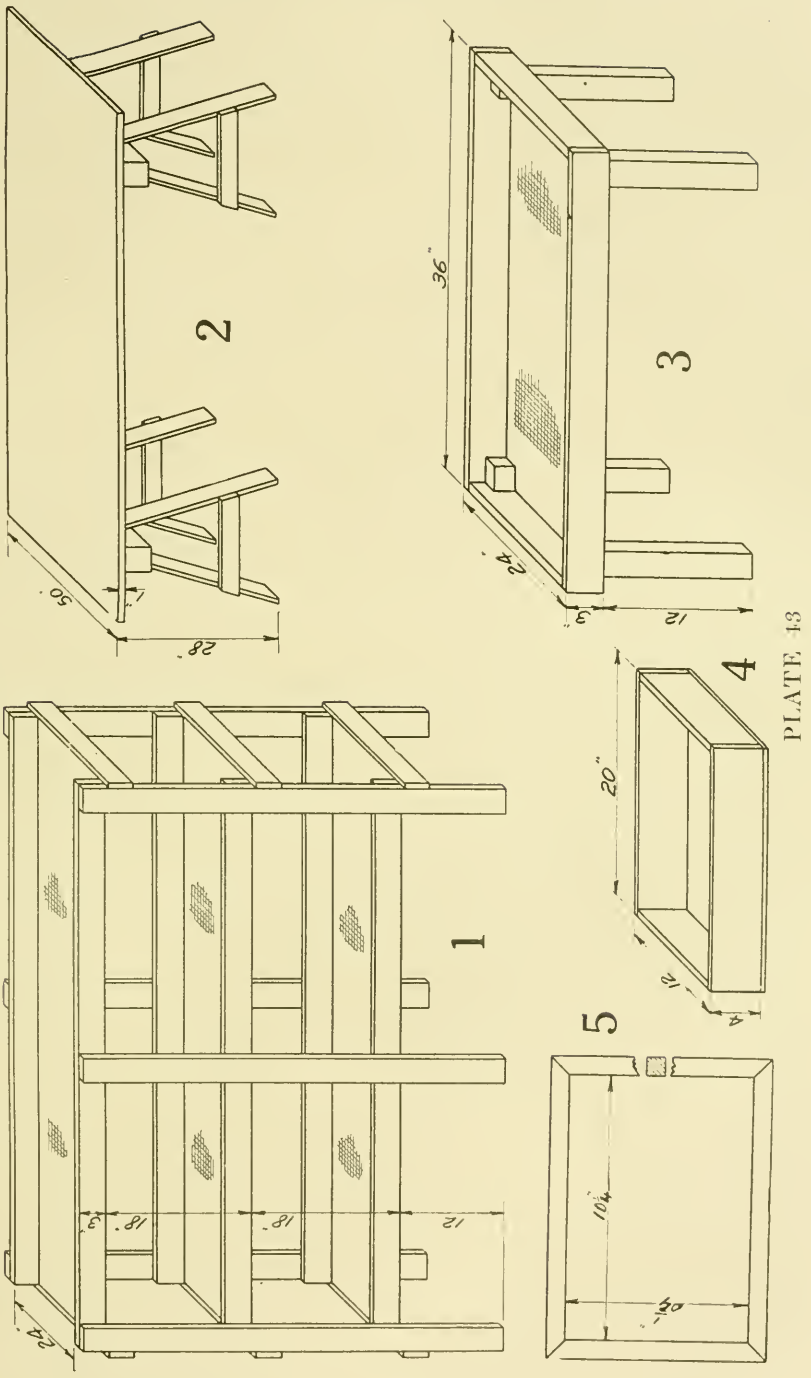

PLATE 13

or cotton to avoid the loss of moss by the wind, while the bottom is made of galvanized chicken wire similar to that used in indoor drying rack.

After the moss has been dried, no matter in what way, it is stored loosely in bins, if the quantity is large, but in small Chapters pasteboard cartons have been found convenient and usually sufficient for storing the excess of dry moss.

Equipment of a Sphagnum Workroom

The equipment for making sphagnum surgical dressings need not necessarily be elaborate. The ideal condition is to have three separate rooms conveniently located with respect to each other, one for sorting, one for drying the moss after it has been sorted and one for making the pads. The room space might be reduced by having certain days for sorting and others for making the pads so that these two processes might be carried on in the same room. In such a case the same tables, a convenient type of which is shown in plate 43, Fig. 2, could be used for both.

In the drying room one of the methods of drying described above should be installed. The racks (plates 36; and 43, Fig. 1) have proved

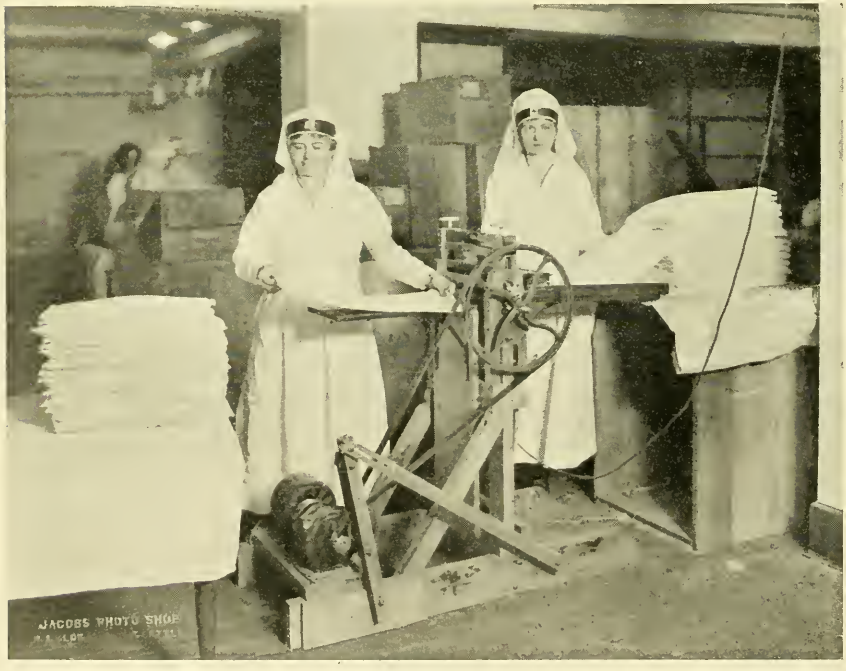

PLATE 44

Passing the completed pads through a clothes wringer run by electricity.

the most satisfactory for small Chapters where allotments do not run more than 10,000 pads a month. The drying trays (plate 43, Fig. 3) are convenient where floor space is a consideration. In the room for making the pads, besides the ordinary tables and cutting table to be described shortly, there should be the following: (1) a sufficient number of moss frames (plate 43, Fig. 5) of appropriate size for gauging the amount of moss; (2) dry moss trays (plate 43, Fig. 4) for holding moss while pads are being made; (3) spring clothes pins for holding the zorbik envelope in place; (4) a few yard sticks; (5) a polished foot ruler (3 dm.) for each worker; (6) large clothes basket for carrying pads and moss; (7) a convenient place for storing the sorted dry moss; (8) a clothes wringer (plate 44).

Making Sphagnum Pads

When the moss is perfectly dry it is ready to be made into dressings which are composed of gauze, a thin sheet of wood pulp paper (known as zorbik or Scot tissue), non-absorbent cotton and Sphagnum. Plate 45 is a photograph of a class of University of Washington girls working on the first 50,000 sphagnum pads made by the American Red Cross for overseas.

PLATE 45

A class of University of Washington women working on the first 50,000 sphagnum pads.

In cutting the gauze care should be taken so as not to cut it on the bias. In order to avoid this many smaller Chapters draw the thread and cut by hand. This, however, is slow and tedious when pads are called for by the hundred thousands.

(a) *A gauze cutting table*

If a considerable quantity of cutting is required, either of gauze or zorbik,[6] it is best to have a special table which should be marked with parallel longitudinal lines, the two outside ones spaced the width of the gauze; while between these should be lines parallel to them along which the gauze is to be cut. Cross lines are drawn where the cross cuts are to be made. The two end guides are placed half the space from two selected cross lines, as the gauze is doubled at the ends. They are usually about 8 feet (2.44 m.) apart and fastened to the table with wing nuts. The

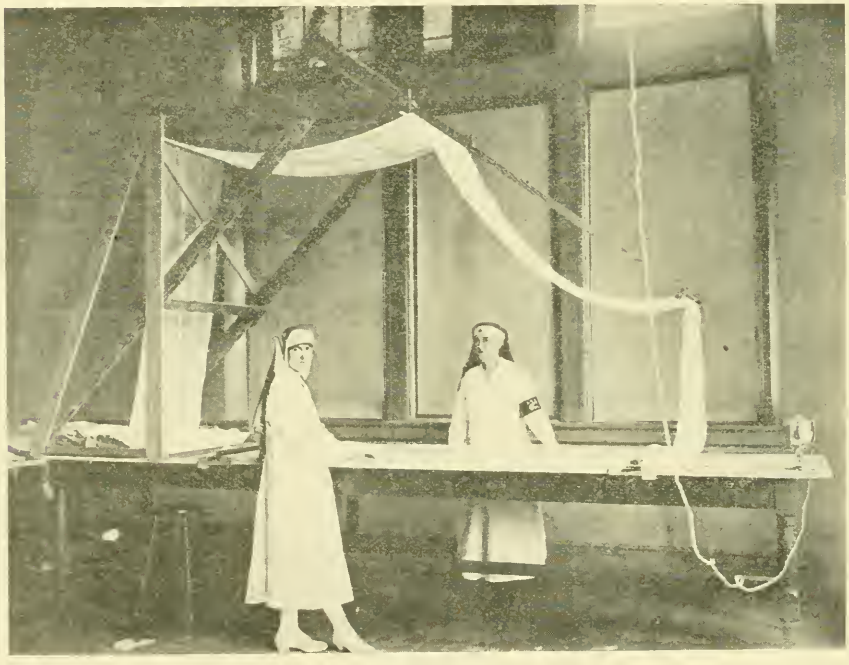

PLATE 46

A gauze-cutting table with an overhead spreading and foldin device.

straight edges, which are each one-eighth of an inch (3.2 mm.) thick, three inches (7.62 cm.) wide, and 3½ feet (1.1 m.) long, holds the folds up to the end guides.

[6] Zorbik or Scot tissue is a very thin wood-pulp paper used to envelop the sphagnum and prevent it from sifting out.

To hold the gauze along the sides No. 1 harness needles with the temper drawn, or pieces of knitting needles about three inches (7.6 cm.) long, may be used. These needles are forced into the table along the outside lines three and one-thirty-second inches (7.86 cm.) from the cross lines, so that when one of the straight edges is against them, a pencil will mark along the edge directly over the cross line on the table. When cutting gauze the bolt is laid on the end of the table beyond where the folding is to take place. A layer of gauze is stretched out on the table and the edges kept straight by putting the selvage over the needles. The straight edge is slipped between the layers, thus holding the folds up to the guide. One of the weights is placed on the straight edge to keep it from slipping. Each of these weights is made of two strips of wood ⅛ inch (3.2 mm.) thick, 1¾ inches (4.4 cm.) wide, and 2 feet 2 inches (81.3 cm.) long; with lead strips between them 1¼ inches (3.2 cm.) wide and weighing 6 or 8 pounds (2.7 — 3.6 kg.). The whole is covered with cloth.

After the weight is placed on the straight edge the gauze is stretched back to the opposite end and another straight edge and weight used in a similar way there. This process is continued back and forward, each time securing the selvage over the needles. When the four straight edges have been used, the lower one is drawn out and used again. When a bolt has thus been stretched out on the table, light lines are drawn where the cuts are to be made. This cutting may be done with an electric cutter or by hand.

(b) *A spreading and folding device*

A device (plate 46) for spreading and folding gauze has been designed by Mr. W. H. Durham, superintendent of buildings and grounds at the University of Washington, where it is installed. It has proved very convenient in avoiding confusion and delay that often arise in spreading the gauze by hand. This device consists of a rather inexpensive framework that is fastened to one end of the cutting table with a swinging arm to carry the gauze back and forth. The bolt of gauze is laid between the upright frames at one end of the table. The loose end of the gauze is drawn over the glass tube directly above, then over the other upper glass tube and down between the two glass tubes at the lower end of the swinging arms. These glass tubes, it should be remembered, have a ¼-inch (6.3 mm.) iron bolt running through them for support.

The gauze is then spread on the table as already indicated. As the arm is swung back and forward the gauze is spread and is always kept up out of the way. An extra board put on the upper end of the swinging arm is just sufficient to balance the long arm so that it remains wherever it is placed, and can easily be pushed up out of the way when not in

actual use. With this device a bolt of gauze containing 100 yards (91.4 m.) can easily be spread and cut in half an hour by two people.

(c) *General directions for making sphagnum dressings*

In making a sphagnum dressing a piece of zorbik or Scot tissue of appropriate size is placed on the table and on it a wooden frame corresponding to the particular size to be made. The frame used for the 8 x 12 inch (20.2 x 30.5 cm.) pad is 6¼ x 10¼ x ½ inches (15.9 x 26 x 2.3 cm.); that for the 12 x 24 inch (30.5 x 61 cm.) pad is 10¼ x 22 x ¾ inches (26 x 55.9 x 1.9 cm.), and the one for the 14 x 20 inch (35.6 x 50.8 cm.) pad is 12 x 18 x ¾ inches (30.5 x 45.7 x 1.9 cm.). Some prefer using a piece of pink cardboard the same size as the inner measurements of the frame, placing it under the gauze to indicate the limits of the moss, but the frame is generally found more satisfactory. It is immaterial which device is used so long as the results are obtained. The frame is filled evenly with moss but not packed. Over this a thin layer of cotton is placed and the frame removed. The margins of the zorbik are then folded over the cotton and sphagnum. It is usually convenient to use spring clothes-pins to hold the ends in place, although this is not absolutely necessary. In order to keep the outside covering free from particles of moss it is best to remove this incomplete pad to another table where there is no moss. Here it may be finished by the same worker or by another. A piece of gauze of appropriate size is spread out on the table and the incomplete pad on the center of it, with the non-absorbent cotton up. A thicker layer of cotton is then put over the pad, extending about ½-inch (1.3 cm.) beyond the edges. The gauze is folded over the pad so that the long fold is on the back, that is, on the side next the non-absorbent cotton. The open ends are folded in "muff-wise," first folding the under side up over the zorbik envelope, then folding the upper side to correspond and adjusting the"muff-end" carefully. The pad is patted lightly to make sure the sphagnum is evenly distributed throughout, and then passed through a clothes-wringer (plate 44). The object of putting the pad through the wringer is partly to reduce its thickness, thus saving shipping space; and partly to press the moss into the thin layer of cotton and zorbik thus preventing it from shifting when handled. This is extremely important specially in large pads. The zorbik which envelops the sphagnum and the thin layer of cotton keeps the moss from sifting out, the thicker layer of non-absorbent cotton on the back prevents the discharge from soaking through the bandages, and the gauze on the outside holds everything in place, exposing a soft absorbent surface to the wound. A photograph showing the steps in making a standard sphagnum pad 8 x 12 inches (20.3 x 30.5 cm.) is shown in plate 47.

(d) *Directions for making dressings for overseas*

The following directions give in detail the method of making certain sphagnum pads. These directions are based on the original ones received from Dr. John A. Hartwell, director of surgical dressings for the National Red Cross. Even after nearly a year of experimentation with them they are published with some degree of hesitation. They are given in detail mainly in order to preserve a record of them. For overseas three sizes of pads are made: 8 x 12 inches (20.3 x 30.5 cm.), 12 x 24 inches (30.5 x 61 cm.), and 14 x 20 inches (35.6 x 50.8 cm.). The specific directions are given below for each of these:

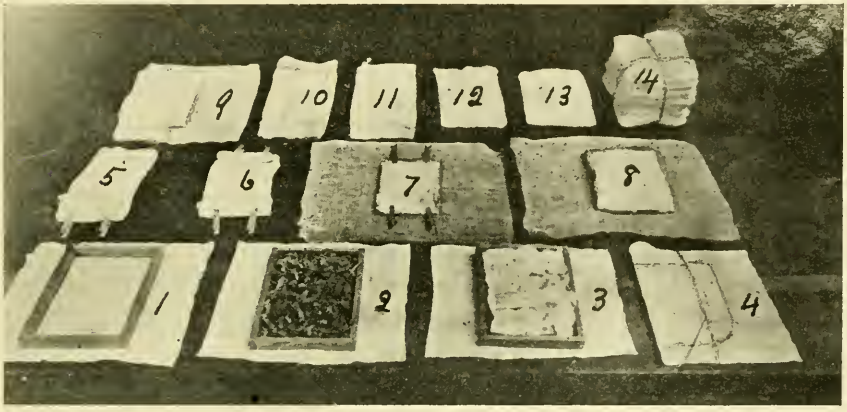

PLATE 47

The successive steps in the making of a sphagnum pad. (1) Frame on zorbik. (2) Moss in frame. (3) A thin film of non-absorbent cotton on moss. (4) Frame removed; zorbik folded from left. (5) Zorbik folded from right and the near end; spring clothes pins holding zorbik in place at the near end. (6) Far end of zorbik folded and held with spring clothes pins. (7) Zorbik envelope with moss side next the gauze. (8) Clothes pins removed. (9) Gauze folded in from left. (10) Gauge folded in from right. (11) Pad turned over. (12) Near end muffed. (13) Both ends muffed and patted even. (14) Ten pads tied for packing.

8 *x* 12 *inch* (20.3 *x* 30.5 *cm.*) *pads*

(A)

1. Cut the gauze 20 x 18 inches (50.8 x 45.7 cm.).
2. Cut zorbik 15 x 13½ inches (34.3 x 38.1 cm.) and stretch to 16 inches (40.6 cm. Cut twice as many pieces 12 x 7⅔ inches (30.5 x 19.5 cm.) and stretch to 9 inches (22.9 cm.).
3. Cut non-absorbent cotton 8 x 12 inches (20.3 x 30.5 cm.). Make thick enough to weigh ½ ounce (14.2 g.). This weight will vary a little as cotton varies in quality and uniformity.

(B)

1. Place one large sheet of zorbik with 15-inch (38.1 cm.) side parallel to edge of table. Place in center of this two face-pieces with 9-inch (22.9 cm.) side parallel to edge of table. If the zorbik is of poor quality two large sheets may be necessary.
2. Place wooden frame in center of zorbik with 6¼-inch (15.9 cm.) side parallel to edge of table. Frame should measure 6¼ x 10¼ x ½ inches (15.9 x 26.7 x 1.27 cm.), inside measurements. Place sphagnum in frame filling it evenly but not packed.
3. Cover sphagnum with thin layer of non-absorbent cotton. Remove frame.
4. Fold zorbik from sides over sphagnum and cotton, then fold ends over pad. (When pressed down the zorbik envelope should now measure 6½ x 10½ inches, or 16.5 x 26.7 cm.).
5. Pass zorbik envelope to separate table for gauze.

(C)

1. Place gauze on table with selvage at top.
2. Place zorbik envelope in center of gauze with 6½-inch (16.5 cm.) side parallel to edge of table.
3. Place the backing of non-absorbent cotton on pad, with a margin of cotton extending beyond the zorbik envelope on all sides.
4. Fold gauze from left to right over pad; turn in and cut edge at right about 1½ inches (3.81 cm.) and then fold from right to left over pad. Turn pad over and muff ends on face of pad, i.e. on paper side of pad.
5. At this point, if gauze has been wrapped snugly, the pad should measure 7¾ x 11¾ inches (19.5 x 29.8 cm.) or a little less; and if all measurements have been carefully followed, there should be a margin of non-absorbent cotton on all sides of zorbik envelope, varying from a little less to a little more than ½ inch (1.27 cm.) in width, depending upon the firmness of the zorbik envelope and the stretchiness of the zorbik.
6. Pass for careful inspection, then sew with medium sized stitches along both ends and the back fold.
7. Pass through wringer. Pad will now measure 8 x 12 inches (20.3 x 30.5 cm.) and weigh from 1¼ to 1¾ oz. (35.4 to 49.6 g.). Weight must be kept within these limits.
8. Tie in packages of ten with the face side up, except the top one which should be face down; thus a cotton side is exposed on top and bottom of the package.
9. Label: "10 Sphagnum Absorbent Pads 8 x 12 inches (20.3 x 30.5 cm.). N. B. Cotton side does *not* go next to the wound."

12 x 24 inch (30.5 x 61 cm.) pads

(A)

1. Cut the gauze 30 x 36 inches (76.2 x 91.4 cm.).
2. Cut zorbik 26 x 27 inches (66 x 68.6 cm.) and stretch to 29 inches (71.1 cm.) with the 26-inch (66 cm.) edge parallel with the grain

of the zorbik. If the zorbik does not come in the large sheets, cut 26 inches (66 cm.) by width. Also cut twice as many sheets 26 x 13 inches (66 x 33 cm.).

3. Cut cotton 12 x 24 inches (30.5 x 61 cm.). Make thick enough to weigh 1½ oz. (42.5 g.) if a good uniform cotton is used, or 2 oz. 56.7 g.) if using Burton-Dixie cotton.

(B)

1. Place one large sheet of zorbik with the 26-inch (66 cm.) side parallel to the edge of the table, and in the center of this two face-pieces slightly larger than the wooden frame, with the grain running at right angles to that of the large piece. If the zorbik is of poor quality two large sheets may be necessary.
2. Place wooden frame in center of zorbik. (Frame should be 10½ x 22 x ¾ inches, or 26.7 x 55.9 x 1.9 cm., inside measurements). Place sphagnum in frame filling it evenly but not packed.
3. Cover sphagnum with a thin film of non-absorbent cotton. Remove frame.
4. Fold zorbik from sides to center over the sphagnum and cotton: then fold ends over pad.
5. Pass the zorbik envelope to a separate table for gauze.

(C)

1. Place gauze on the table with selvage at the top and bottom.
2. Place the envelope in the center of the gauze with 10½-inch (26.7 cm.) side parallel to the selvage of gauze.
3. Place a backing of non-absorbent cotton on the pad, with a margin of non-absorbent cotton extending well beyond the zorbik envelope on all sides.
4. Fold the gauze from left to center, turn in cut edge at right and fold from right to center. Muff ends of gauze on face of pad, i. e., on paper side of pad.
5. Pass through wringer.
6. Tie in packages of five with the face side up, except the top one which should be face down; thus a cotton side is exposed on top and bottom of the package.
7. Label: "5 Sphagnum Absorbent Pads, 12 x 24 inches (30.5 x 61 cm.). N. B. Cotton side does *not* go next to the wound."

14 x 20 inch (35.6 x 50.8 cm) pads

(A)

1. Cut the gauze 24 x 36 inches (61 x 91.4 cm.).
2. Cut zorbik 32 x 23 inches (81.3 x 58.4 cm.) and stretch to 25 inches (63.5 cm.). Cut twice as many face pieces 21 x 10⅘ inches (53.3 x 27.4 cm.) and stretch to 15 inches (38.1 cm.).
3. Cut non-absorbent cotton 14 x 20 inches (35.6 x 50.8 cm). Make thick enough to weigh 1½ oz. (42.5 g.) if using a good uniform cotton, or 2 oz. (56.7 g.) if using Burton-Dixie cotton.

(B)

1. Place one large sheet of zorbik with 32-inch (81.3 cm.) side parallel to edge of table. Place two face-pieces in center with 15-inch (38.3 cm.) side parallel to edge of table. If the zorbik is of poor quality two large sheets may be necessary.
2. Place wooden frame in center of zorbik with 12-inch (30.5 cm.) side parallel to edge of table. (Frame should be made 12 x 18 x ¾ inches or 30.5 x 45.7 x 1.9 cm., inside measurements.) Place sphagnum in frame filling it evenly but not packed.
3. Cover sphagnum with a thin layer of non-absorbent cotton. Remove frame.
4. Fold zorbik from sides to center over sphagnum and cotton; then fold ends over pad. (When pressed down the zorbik envelope should measure approximately 12¼ x 18¼ inches, or 30.9 x 46.3 cm.).
5. Pass zorbik envelope to separate table for gauze.

(C)

1. Place gauze on table with selvage right and left.
2. Place zorbik envelope in center of gauze with 12¼-inch (30.9 cm.) edge parallel to edge of table.
3. Place the backing of non-absorbent cotton on the pad with a margin of cotton extending well beyond the zorbik envelope on all sides.
4. Fold gauze from left to right over pad; then from right to left over pad. Turn the pad face upward and muff ends of gauze on face of pad, i. e., on paper side of pad.
5. At this point, if gauze has been wrapped snugly, the pad should measure 13¾ x 19¾ inches (35 x 50.1 cm.) or a little less. And if all measurements have been carefully followed there should be a margin of non-absorbent cotton on all sides of the zorbik envelope, varying from scant ¼ to ¾ inch (.6 x 1.9 cm.) in width depending upon the firmness of the tissue envelope and the stretchiness of the zorbik.
6. Pass for careful inspection, then sew both ends and along the back with medium sized stitches.
7. Pass through wringer. Pad will now measure 14 x 20 inches (35.6 x 50.8 cm.) and weigh 4 to 4½ oz. (113 — 128 g.). Weight must be kept within these limits.
8. Tie in packages of 5 with the face side up, except the top one, which should be face down; thus a cotton side is exposed on top and bottom of the package.
9. Label: "5 Sphagnum Absorbent Pads 14 x 20 inches (or 35.6 x 50.8 cm.). N. B. Cotton side does *not* go next to the wound."

(e) *Directions for making dressings for base hospitals in U. S.*

Base hospitals in this country seldom, if ever, need dressings as large as those just described. Consequently the sizes of sphagnum pads for their use have been greatly reduced to meet their particular needs. The

commonest ones called for are 7 x 7, 4 x 6, 4 x 4, and 2 x3 inches (17.8 x 17.8, 10.2 x 15.2, 10.2 x 10.2, 5.08 x 7.6 cm.).

7 x 7 inch (17.8 x 17.8 cm.) pads

(A)

1. Cut the gauze 17 x 12 inches (43.2 x 30.5 cm.).
2. Cut zorbik 15 x 10 inches (38.1 x 25.4 cm.).
3. Cut non-absorbent cotton 7 x 7 inches (17.8 x 17.8 cm.).

(B)

1. Place one or two sheets of zorbik, according to the quality, on the table with the 15-inch (38.1 cm.) side parallel to edge of table.
2. Cover area of 6 x 6 inches (15.2 x 15.2 cm.) in center of zorbik with thin layer of sphagnum. A piece of stiff colored paper 6 x 6 inches (15.2 x 15.2 cm.) placed under zorbik is useful as a guide in making this pad.
3. Fold zorbik from sides over sphagnum. Then fold ends over pad.
4. Pass zorbik envelope to separate table for gauze.

(C)

1. Place gauze on table with selvage at top.
2. Place zorbik envelope in center of gauze with end parallel to edge of table.
3. Place the backing of non-absorbent cotton on pad with a margin of cotton extending well beyond the zorbik envelope on all sides.
4. Fold gauze from left to right over pad. Turn in cut edge at right about 1 inch (2.54 cm.) then fold from right to left over pad. Turn pad over muff ends on face of pad.
5. When pad is complete, if all measurements have been carefully followed, there should be a margin of non-absorbent cotton about ¼ inch (6.3 cm.) wide on all sides of the zorbik envelope.
6. Sew along back and ends with medium sized stitches if necessary.
7. Tie in packages of ten with the face side up, except the top one which should be face down; thus a cotton side is exposed on top and bottom of package.

4 x 6 inch (10.2 x 15.2 cm.) pads

(A)

1. Cut the gauze 11 x 9 inches (27.9 x 22.9 cm.).
2. Cut the zorbik 9 x 9 inches (22.9 x 22.9 cm.).
3. Cut the non-absorbent cotton 4 x 6 inches (10.2 x 15.2 cm.).

(B)

1. Place one or two sheets of zorbik, according to the quality, on the table; under zorbik place a piece of stiff colored paper 3 x 5 inches (7.6 x 12.7 cm.).
2. Cover the paper with a thin layer of sphagnum.
3. Fold the zorbik from the sides and ends over the sphagnum, making a complete tissue envelope.
4. Pass the zorbik envelope to a separate table for gauze.

(C)

1. Spread out the gauze with the long side parallel with the edge of the table; place on it the tissue envelope with the long edge parallel with the short side of the gauze, and about 2 inches (5.08 cm.) from the right side.
2. Over the tissue envelope place a thin layer of non-absorbent cotton and adjust it so that it extends beyond the tissue envelope, making about ¼-inch (6.3 mm.) margin on all sides.
3. Fold the gauze from right to left and left to right over the cotton.
4. Muff the ends of gauze on face of pad similar to other sphagnum pads. Stitch the back and ends if necessary.
5. When the pad is complete it should be 4 x 6 inches (10.2 x 15.2 cm.).
6. Tie in packages of 10 with face side up, except the top one which should be face down; thus a cotton side is exposed on top and bottom of package. Pads 4 x 4 inches (10.2 x 10.2 cm.) are made in a similar way. The zorbik is cut 8 by 7 inches (20.3 x 17.8 cm.) and the gauze 10 x 7½ inches (25.4 x 19 cm.). Pads 2 x 3 inches (5.08 x 7.6 cm.) are also made similar to the 4 x 6 inch (10.2 x 15.2 cm.) pads. The zorbik is cut 4½ x 5 inches (11.5 x 12.7 cm.) and the gauze 6 x 6 inches (15.2 x 15.2 cm.).

   In all cases a margin of non-absorbent cotton of about ¼-inch (6.3 mm.) is left.

The above method of making sphagnum dressing is quite different from that employed by the British who make about ten different sizes, according to the special use they wish to make of them. The encasements for these pads consist of a flat bag made of long cloth with fine enough weave to prevent the moss from sifting through. This bag is filled with the appropriate amount of moss and sewed up.

The Canadian Red Cross has adopted three types of sphagnum dressings: the British type just mentioned; a "standard dressing" similar to that made by the American Red Cross; and a bed pad made of second grade moss. During the year 1918 the Canadians concentrated most of their energy on the standard dressing, making less of the British type and comparatively few of the bed pads.

From October 1917 up to the time the armistice was signed on the 11th of November, 1918, the Northwestern Division of the Red Cross had made 10,000 of the British type and 540,000 of the American type of sphagnum pads. Of this number 60,000 were made at the University of Washington, partly by the students and partly by interested persons in the vicinity of the University. In addition to these there are standing orders which (Feb. 1919) have not been countermanded, from the base hospital at Camp Lewis, for 600 pads a month of the following sizes: 4 x 6, 4 x 4 and 2 x 3 inches (10.2 x 15.2, 10.2 x 10.2, and 5.08 x 7.6 cm.); and from the base hospital at Vancouver for 1600 pads a month, half 7 x 7 and half 4 x 6 inches (17.8 x 17.8, 10.2 x 15.2 cm.). The making of sphagnum

dressings, however, was suddenly curtailed when hostilities on the western front in France ceased. The order for a million pads which had just been begun was immediately reduced to 40,000, which were practically completed a month later. In making these pads 18 Chapters as conveniently located to the source of supply of the moss as possible were selected. In the state of Washington these were Seattle, Tacoma, Bellingham, Everett, Thurston County, Pacific County, Aberdeen, Hoquiam, Montesano, Chehalis and Vancouver; in Oregon, Portland, Astoria, Lane County, Willamette County, Corvallis, and Marshfield; and in Alaska, Juneau.

The Atlantic Division has also been doing sphagnum work but has been somewhat hampered on account of the lack of suitable moss conveniently located. In spite of this handicap, however, the model workroom in New York City, where practically all this work for the Division was done, turned out for overseas 45,540 pads. This work was carried on under the direction of Mrs. Austin R. Baldwin.

Inspection of Sphagnum Pads

Sphagnum pads should be made under the personal supervision of a qualified instructor. It is of the greatest importance to have the most rigid inspection while the pads are being made, as it is easier to detect errors at that time than it is after they are completed. The following suggestions are offered to assist in the supervision and inspection:

1. At least two persons should be present when any box is packed to verify the count and see that all the directions are followed. The Chapter records should show the names of the packers of each box.

2. Dressings must be tied in definite numbers 8 x 12 inch (20.3 x 30.5 cm.) pads in packages of ten each; 12 x 24 and 14 x 20 inch (30.5 x 61 and 35.6 x 50.8 cm.) pads in packages of five each. These should be arranged with face upwards except the last one which is placed face down so that the cotton side is exposed on the top and bottom of the package.

3. Do not tie the packages very tightly, as the string may cut into the pads and break the tissue envelope, thus making the dressing useless.

4. There should be at least ½-inch (12.7 mm.) margin extending all around the pad beyond the edge of the zorbik envelope. This is absolutely essential for a successful pad, because when the zorbik envelope approaches the margin of the pad it acts like a lamp wick, carrying the water to the back of the pad, thus nullifying the usefulness of the non-absorbent cotton.

5. In the inspection of sphagnum dressings the main point to be kept in mind is to have a pad that will do the work required of it. If it fails to do this the pad must be made over in the Chapter, but do not become fussy about minute non-essential details.

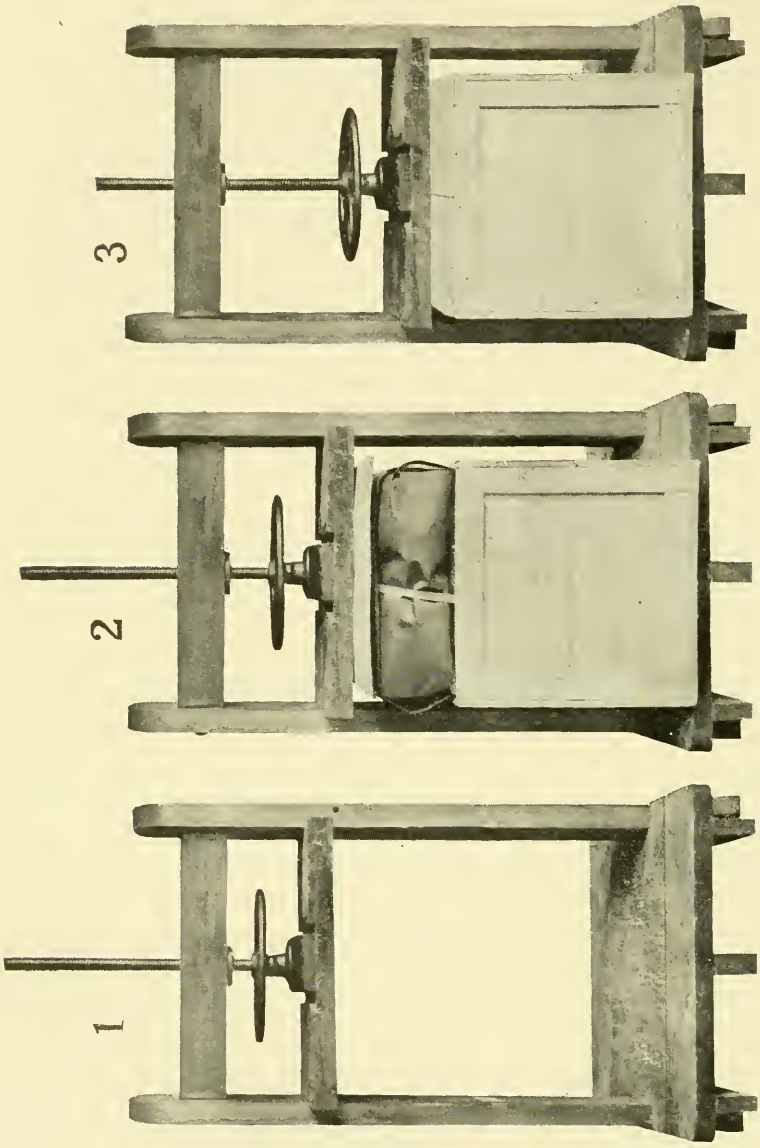

PLATE 48

Mode of packing sphagnum pads at the Portland Chapter of the Red Cross, a saving of labor and shipping space. Figures 1 to 3 are successive steps in the process.

Labeling

1. The labeling should be clear, concise and accurate so that anyone unpacking the case of dressings at its destinatiion can see at a glance the type of dressing and the number in each package.

2. Each package should be labeled. If tied with pieces of selvage, stamp the label on the selvage. If tied with cord, use manila tags.

3. The label should give the following information:
   (a) 10 Sphagnum Absorbent Pads 8 x 12 inches (20.3 x 30.5 cm.).
   N. B. The cotton side does *not* go next to the wound.
   (b) 5 Sphagnum Absorbent Pads 14 x 20 inches (35.6 x 50.8 cm.).
   N. B. The cotton side does *not* go next to the wound.
   (c) 5 Sphagnum Absorbent Pads 12 x 24 inches (30.5 x 61 cm.).
   N. B. The cotton side does *not* go next to the wound.

Packing

1. Line the box carefully with standard waterproof paper. This must be overlapped at least 2 inches (5.08 cm.) at all corners and edges. In order to prevent the waterproof paper soiling the pads, the box should be innerlined with common wrapping paper. Kraft paper is not necessary.

2. The packages of pads may be placed flat or on their edges but *must not* be doubled or crushed.

3. In case a press is used in packing, the pads must all be laid flat as otherwise they will be broken. In order to save shipping space and, incidentally, manual labor in packing sphagnum dressings, the Portland Chapter adopted a method of pressing their pads, taking their cue from an old cider press. A photograph of this press is shown in plate 48.

The sterilization of sphagnum pads is done after they reach their destination just before they are used. The high heat and pressure used in sterilization by an autoclave tend to make the moss brittle and to lessen the absorbency, but not to such an extent that its usefulness is seriously impaired.

Conclusion

The great demand for sphagnum has largely been the outgrowth of the world war as a result of the tremendous need for surgical dressings and the acute shortage of cotton for explosives. Now that the war is over that pressing need has been removed, and for the time being the making of this kind of dressing for war purposes is almost completed. However, the inexpensiveness of the moss, its high absorbency, its abundance in certain parts of the country and its undoubted superiority over gauze and absorbent cotton for some purposes, clearly indicate that it is too important a dressing to let die with the war. If, however, sphagnum is used for dressings in a commercial way, quite different methods of handling must be employed. If the labor of collecting, sorting and making of sphagnum pads as carried on by the Red Cross had to be paid for at a living wage, it would make the cost of them practically prohibitive; so if these pads are to be commercialized a different and less expensive method of handling the moss must be employed,—more machinery and less hand work. Considerable experimentation has been done pointing to a solution of

some of these problems, and preparing the way to some extent for a commercial enterprise. In the first place progress has been made, specially in Scotland and Canada in using a fan-like apparatus for cleaning the moss. From figures obtained from Rev. Adam Forman, Beattock, Scotland, the machine used there for cleaning moss will do 16 times as much work as the people who run it can do by hand. This is the result of several actual trials. It thus appears probable that sorting or cleaning the moss can be done at least to some extent, by machinery.

In the second place, in connection with drying the moss, experiments carried on in Montreal, New York, and Seattle, all more or less independently of each other, have led to practically the same results, namely, that a successful system of drying sphagnum must take into account the proper adjustment of temperature, humidity and circulation of air. It would appear that artificial heat may be used to advantage and without seriously impairing the usefulness of the moss for surgical dressings if these factors are carefully guarded.

In the third place if these dressings are manufactured commercially, a modification in the mode of making the pads is essential. It would never pay to make them in the elaborate fashion that the Red Cross has done where free expert assistance was almost unlimited. The writer has experimented sufficiently at least to satisfy himself that the moss can be prepared quite inexpensively in large sheets which may be varied in thickness according to the probable demand on the absorbency of the pad. These sheets can easily be cut into the particular size required and covered in an appropriate manner.

Finally, the writer wishes to express his appreciation of the efforts of all who have so loyally supported the cause of sphagnum in the Northwest. He is particularly indebted to Mrs. Henry Brakel and Miss Evelyn Gill Klahr for giving instruction in making sphagnum pads in the Northwestern Division; to Prof. Albert Sweetser of the University of Oregon, Special Field Agent for the Northwestern Division, for the location and collection of sphagnum in Oregon; to Mr. L. L. Buch, Special Field Agent in Pacific County, Washington, for locating moss and directing the collection; and to the University of Washington for making it possible for many of the experiments herein recorded to be carried on.

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During the last four years a considerable number of articles have appeared on sphagnum in its relation to surgical dressings, many of them as newspaper articles or local news items, both in Great Britain and

America. The following are the more important articles that have come to the writer's attention:

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A Study of Susceptibility in Some Puget Sound Algae

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In the course of the summers of 1917 and 1918, spent at the Puget Sound Biological Station, the axial susceptibility relations of a number of the Puget Sound algae were examined as opportunity offered, thus extending the work done earlier at Woods Hole (Child, 1916a, 1916c, 1917a, 1917b). The observations were mostly made on common species which were readily obtainable in fresh condition, and there was no attempt to obtain a full representation of the algal flora of the region. I am deeply indebted to the Director of the Station for the facilities afforded for this and other work, and also to Miss A. M. Hurd for the identification of most of the species examined, which include the following forms:

Chlorophyceae

Cladophora sp.

Phaeophyceae

Desmarestia aculeata
Desmarestia ligulata
Nereocystis luetkeana

Rhodophyceae

Callophyllis variegata
Callymenia phyllophora
Agardhiella tenera
Euthora fruticulosa
Fauchea laciniata
Nitophyllum ruprechtianum
Bonnemaisonia hamifera
Odonthalia lyallii
Polysiphonia californica
Polysiphonia urceolata
Dasyopsis plumosa
Callithamnion sp.
Ptilota pectinata
Ceramium sp.

Some observations were also made upon a diatomaceous pseudothallus of the *Navicula* group.

METHODS

The agents chiefly used in determining the susceptibility relations were KNC m/500—m/100, HCl m/500—m/100, ethyl alcohol 5% and in certain cases higher concentrations, the 'vital' dye neutral red, either alone or followed by some other agent, e.g., KNC. In a few special cases KOH or NaOH m/100 was used, the penetration and action of methylene

blue was determined for several species and some data were obtained on color gradients resulting from the reduction of $KMnO_4$. In several species the course of death in standing water was also observed.

The methods of procedure have been described in earlier papers (Child, 1916a, 1916c, 1917a, 1917b, 1919a) and need only brief consideration here. The thalli, or portions of them, are simply immersed in sea water solutions of the agents used and the course of the death changes in the cells is followed along the axes. These death changes may be determined in various ways: for example, the smaller, less opaque forms may first be stained to some degree with neutral red and then placed in some other agent, e.g., KNC. Neutral red is orange red or light red in solutions near neutrality and becomes purple in acid and yellow in alkaline solution. The living cell stains light red with the dye, but as it approaches death the color deepens, indicating a change toward increased acidity and the coagulating masses of protoplasm may become deep blackish-purple as if markedly acid, but soon after the break-up and coagulation of the protoplasm the color changes to yellow, i.e., the dead cells become alkaline. When the cells are killed with KNC following staining with neutral red, this change occurs very rapidly, but essentially the same changes occur when the cells are killed in neutral red alone, this so-called vital dye being lethal in sufficient concentration and with sufficient time. The death changes in neutral red and in other agents following neutral red are fully described in the papers referred to above. In the use of neutral red as a stain preceding other agents care must be taken not to stain too deeply or too long a time, since the toxic effect of the neutral red may alter or even reverse the susceptibility relations to the other agent (Child, 1916c, 1917a).

In the green algae the death changes in the various agents, KNC, acid, alcohol, can often be directly observed as changes in appearance and color of the protoplasm, but they usually appear more clearly if the plant is first stained with neutral red and then treated with some other agent. When the agent is not strongly acid, death is very soon followed by the loss of red color, while in acid agents the cells may become opaque black.

In the brown and red algae the pigment of the plant may be used as an indicator of the entrance of the agent and the death of the cell, since death is accompanied by a change in color as well as by coagulatory changes in the protoplasm. In the brown algae the color changes to yellowish green or dull olive green, according to the species and the agent, this change being evidently due to the more or less complete loss of the pigment (phycophaein, phycoxanthin) from the cells, and where the quantity of the pigment is sufficient, the solution may become colored by it.

In the red algae the pigment (phycoerythrin) is to some extent an acid-alkali indicator. When the plants are killed in alkaline solution, the color changes to yellow, while in acids the change is first to purple and then usually to whitish, and in neutral agents a gradual loss of the red or brownish red color occurs, the plant becoming successively light red, pinkish and whitish or pinkish white. Evidently in acid and alkaline solutions the pigment is first changed in color and then, at least in acids, gradually diffuses out into the surrounding fluid, while in neutral agents the diffusion from the cell occurs, but with little or no change in the color of the pigment.

In the forms with large cells it can readily be seen that the pigment changes are accompanied by break-up and coagulation of the protoplasm. The exact moment among these changes when death occurs cannot of course be determined, but the course of these changes and the times at which they occur in different parts of the plant or of single cells can be determined, and these data show the differences in susceptibility.

Methylene blue in the concentrations used (1/100000, 1/10000) is apparently not toxic or only slightly toxic to some species of red algae, although it enters the cells very readily and accumulates there, but some interesting data on penetration were obtained with it.

Potassium permanganate is reduced by protoplasm to MnO_2 which stains the protoplasm brown. It has been noted elsewhere that with this agent the physiological differences along the axis in animals appear very distinctly as color gradients, the differences in color depending upon the rate of reduction of the agent at different levels of the axes (Child, 1919a). With certain concentrations gradients in disintegration of the protoplasm also appear. This agent was used in concentrations from m/10000—m/1000 with a number of algae and gave beautiful color gradients, and in certain forms with large, elongated cells, the disintegration gradients in the single cells could also be clearly seen.

The development of the susceptibility method is based upon the conclusion from various lines of evidence that susceptibility to certain agents, such, for example, as the cyanides, which are powerful reducing agents and inhibitors of oxidation in living protoplasm, shows a certain general relation to the rate of oxidation in protoplasm. In the course of experimental work with a large number of animal forms ranging from the protozoa to the vertebrates, evidence has been accumulated along many different lines to show that susceptibility, not only to the cyanides but to at least many other agents, including anesthetics, acids, alkalies, salts, and "vital" dyes, and even to such conditions as extremes of temperature and lack of oxygen when no chemical agent is used, conforms to the same

general laws. The data thus far at hand indicate that in concentrations or intensities of an agent which are too high to permit the organism to acquire tolerance or become acclimated, the susceptibility varies directly, though by no means necessarily proportionally, with the rate of general metabolism, probably with rate of oxidation. On the other hand, capacity to acclimate or acquire tolerance to low concentrations or intensities of the same agents also varies directly with rate of metabolism or oxidation.

It is impossible to consider at this time the evidence on which these conclusions are based. A part of it has been published in the writer's earlier papers and in those of Dr. L. H. Hyman,[1] but some of it is still unpublished, and work is still going on to make the evidence more complete. If these conclusions are correct it is evident that differences in susceptibility may be used to some extent as a means of determining quantitative differences in physiological condition. In the present paper the method is used in this way with killing concentrations. It has been found, moreover, that the differences in rate of staining in permanganate parallel the differences in susceptibility, so that this method also serves to show something of differences in physiological condition. With these facts and conclusions in mind, the significance of the observed differences in susceptibility with reference to physiological condition will be clear.

The susceptibility relations in forms with apical growth

Since the susceptibility gradients of various species have been described in earlier papers (Child, 1916a, 1916c, 1917a) and since most of the Puget Sound species examined show similar gradients, the general results may be briefly stated, but certain species and certain methods not previously used require more extended consideration. In all forms examined except Nereocystis and Callymenia, which are discussed below, each axis of the vegetative thallus shows with all agents used a distinct gradient in susceptibility decreasing basipetally. Such a gradient is called for convenience a basipetal gradient. In all forms showing such a gradient the axes are of the usual type with apical growing region, consequently the susceptibility gradient indicates a graded difference of some sort in physiological condition from the apical cell or cells basipetally. This susceptibility gradient is most distinct and regular in the younger portions of the thallus, while in older parts irregularities sometimes appear, a cell or region showing greater or less susceptibility than would be expected from its level in the axis. Some of these irregularities are evidently due to injury, while others are doubtless due to what may be called chance or incidental conditions. For example, when the stems or branches of a

[1] See for example Child 1913, 1915a, 1915b chapter IX, 1916a, 1916b, 1916c, 1917a, 1917b, 1919a; Hyman 1916, 1917 and references given in these papers.

thallus are closely crowded together, as is often the case, irregularities are more frequent than in cases in which an axis has been free on all sides. Probably differences in oxygen content of the water among the crowded stems and differences in illumination, possibly also differences in hydrogen ion concentration in the water among the crowded stems, are factors in determining these irregularities. It should also be noted that in the more sensitive forms even an hour or two in standing water, particularly if any marked rise in temperature occurs, is sufficient to produce very marked irregularities in the gradients, or, since the axis shows a gradient in susceptibility to these conditions as well as to chemical agents, to obliterate the gradient almost or quite completely. In Desmarestia, for example, fresh specimens of both species examined show very distinct and regular gradients, but after a few hours in the laboratory very marked irregularities are found and in many axes the gradient is absent or reversed, the apical regions being less susceptible than the basal. Moreover, these changes occur before the plant shows any change in color or any other visible indications of injury or death as a result of the altered environment. In fact, distinctness and regularity of gradient is a very satisfactory criterion of good physiological condition.

In forms with branching thallus among those examined not only does a basipetal gradient appear in each axis, but the thallus as a whole, or each system of main stem and branches shows a gradient corresponding to the growth-form of the species. In forms in which the distinction between main stem and lateral branches is strongly marked and particularly in cases in which lateral branches are short, the apical ends of the most apical axes are the most susceptible regions of the plant and the susceptibility of the different axes decreases basipetally in regular order. In forms in which the distinction between a chief and a subordinate axis is less marked and in forms in which all axes grow equally or almost equally there is little or no distinction between them as regards susceptibility. In the plant as a whole, then, as well as in the single axis, the differences in susceptibility indicate a graded difference in physiological condition and such difference is very clearly related to the growth-form of the species.

The monosiphonous hair-branches of Dasyopsis and various other forms which themselves represent axes with apical growth, show basipetal susceptibility gradients like other vegetative parts of the plant. Moreover, the hair branches nearer the apical end of the axis from which they arise are more susceptible than those farther basal. In Dasyopsis particularly this gradient in the hair-branches is very distinct and uniform.

The fact that in the vegetative portions of the thallus differences in susceptibility exist not only in each axis but between the different axes and that these differences in susceptibility are definite in character and corre-

lated with the growth-form of the species is significant. The following case of *Ptilota pectinata* is discussed somewhat more fully as illustrating the significance of the relation between susceptibility and growth-form.

In the thalli of *Ptilota pectinata* examined the branching of the primary axes is at first alternate in a single plane, but later a second branch arises opposite each of the first branches. These branches of later origin are more susceptible than the older branches opposite them. The secondary branches on the primary lateral branches, or at least those of later origin, are opposite, but when a lateral branch becomes converted into a main axis by increasing distance from the primary growing tip or by injury to or removal of the latter, it changes its form of branching from opposite to alternate, and this is afterward again transformed into the opposite type by the later appearance of a branch opposite each of the earlier branches. In general the apical regions of main axes with alternate branching are more susceptible than those of lateral branches with opposite secondary branching. Moreover, a general thallus gradient appears among the lateral branches on a single axis, those nearer the apical end being more susceptible than those farther basal.

Here the differences in growth-form and in susceptibility confirm and supplement each other. The apical regions of the main axes are in different physiological condition from those of the doubtless somewhat inhibited lateral branches, and all the facts, including the differences in susceptibility (see discussion below) indicate that the difference is primarily a difference in rate or intensity of fundamental metabolic reactions, the apical regions of the main axis possessing a higher rate than those of lateral branches. If this conclusion is correct, it follows that in these regions of higher metabolic rate branches can arise only alternately, doubtless because each new branch or more specifically its growing tip, inhibits the development of other growing tips within a certain distance. Later, however, when the branch has attained a certain length and the distance between this tip and the axis from which it arose has increased to a certain amount and perhaps also the growing tip of the branch has become physiologically older and less active, a new branch arises opposite.

Again, on the primary lateral branches which are evidently somewhat inhibited in their growth-activity by the growing tip of the main axis, the secondary branches are opposite from the beginning, because the lower metabolic rate in these growing tips makes the range of their inhibiting action on other growing tips less, i. e., their inhibiting action does not extend so far as that of the more active tips.

The case of this species is considered at some length because I believe it affords a striking example both of the manner in which growth-form and

metabolic condition are associated and of the value of the susceptibility method as an indicator of quantitative difference in physiological condition. In this form the differences in susceptibility of the different apical regions and axes afford a picture of physiological conditions in the different parts of the plant which makes it possible to interpret to some extent the growth-form in physiological terms. Many facts of experiment and observation indicate that the inhibiting influence of a more active growing tip is effective through a greater distance in the plant than that of a less active tip, and when we find that the axes with higher susceptibility show alternate branching, those with lower susceptibility opposite branching we are able to assert that the differences in growth-form are associated with quantitative differences in physiological condition, and second, that from what we know of susceptibility, these differences are in all probability differences in the rate of fundamental metabolic reactions.

Of course, the general thallus gradient in susceptibility, its differences in different species, and the relations between these differences and different growth-forms point to the same conclusions. Invariably, so far as observations have gone, it has been found that where main axes and lateral branches are distinguishable in growth-form, they are also distinguishable as regards susceptibility, and vice versa. However we may interpret the relation between susceptibility and metabolism, the significance of these facts cannot be doubted.

While no special attempt has been made to determine the susceptibility relations of other than the vegetative parts of the plant, some observations have been made on the susceptibility of cystocarps in Bonnemaisonia, Polysiphonia and Dasyopsis, and of the stichidia of Dasyopsis. In general the cystocarps, or at least the earlier developmental stages, show a basipetal gradient like the vegetative axes, and the earlier stages are more susceptible than the later. The earlier stages of cystocarp development show about the same susceptibility as vegetative axes from the same region of the plant, but fully developed cystocarps are markedly less susceptible than the vegetative axes. The stichidia of Dasyopsis likewise show a distinct basipetal susceptibility gradient. The earlier stages of stichidium development are more susceptible than those regions of the thallus from which they arise and also more susceptible than the later stages. In well developed stichidia the basal half, more or less, is distinctly less susceptible than adjoining vegetative regions.

It is of interest to note that these specialized reproductive axes, the stichidia, make their appearance first in the more basal regions of the thallus, where the susceptibility is lowest, and from this region of first appearance their region of origin gradually ascends the axis. Evidently

the more basal regions attain before other parts the physiological condition which determines the formation of stichidia, and as the plant axis grows longer or physiologically older or both, and each level attains this same physiological condition, stichidia arise successively at higher and higher levels. In other words, there is a gradient represented by a time sequence in the appearance of stichidia on the axis, beginning basally and progressing toward the apical end. That this order of development of stichidia along the axis is in some way associated with the differences in physiological condition indicated by the susceptibility gradient is at least highly probable, though of course it does not follow that the quantitative differences in physiological condition indicated by the susceptibility gradient along the vegetative axes are themselves directly responsible for the origin of stichidia. These quantitative differences may be accompanied by, or may bring about qualitative differences in condition, and these latter may be the immediate determining factors. But whatever the actual situation may prove to be, there is every reason to believe that the gradient in origin of stichidia is related in some way to the conditions of which the susceptibility gradient is an indicator.

Observations made with the vital dyes neutral red and methylene blue show certain points of interest with respect to the question of the passage of these substances into and out of the cell. All of the algae examined can be stained with neutral red, and in most cases the dye enters all cells of the plant readily and accumulates in the cells until its concentration becomes much higher than outside and is finally toxic. In most species, however, an axial gradient in staining with neutral red appears which is in the same direction as the gradient in susceptibility, i. e., the rate at which the neutral red enters the cells decreases from the apical end of the axis basipetally. Usually this gradient is distinct only for a few moments at the beginning of staining and all cells become uniformly red. The most strongly marked staining gradient observed appeared in a Cladophora thallus in which the staining of successive cells from the apical region occurred very slowly, so that the apical cell became opaque black by the time the third or fourth cell was markedly red, and death and decoloration began in the apical cell soon after this. Here the gradient in death and decoloration followed the staining gradient closely down the axis. In other Cladophoras examined at Woods Hole the staining gradient in neutral red was slight and evanescent as in other forms and the death gradient much more clearly marked. The very strongly marked staining gradient in this case may have been associated with very slow growth, in consequence of which the difference in condition between successive cells was unusually great.

When neutral red has once entered an algal cell it usually does not

come out again to any appreciable extent until the cell is killed, by accumulation of the dye, by environmental conditions, or by the action of some other agent following neutral red. In all forms except the Cladophora mentioned above the death gradient in neutral red is much more strongly marked than the staining gradient, and in forms with elongated cells, e. g., Callithamnion is very distinct within the length of a single cell, although a staining gradient is rarely distinguishable within the cell. Death in neutral red, as indicated by the coagulation of the protoplasm into irregular masses which at first become black or purple and then gradually lose their color as the dye turns yellow, begins at the apical end of such a cell and may take fifteen to thirty minutes to traverse the length of the cell and several hours, in some cases a day or two, to pass over a few centimeters of axis, while the staining gradient is slight and visible for only the first few moments of staining.

Since neutral red may attain a much higher concentration inside the cell than outside and since it is not given up until death occurs, it is evident that it must be adsorbed or held in some other way within the cell, and that the ability of the cell to hold it is in some way associated with the living condition. However the neutral red is held within the cell, it evidently interferes with the physiological activities, since it becomes toxic and kills in sufficient concentration.

The relation of methylene blue to at least some algal cells differs in certain respects from that of neutral red. Bonnemaisonia, for example, in a 1/10000 solution of methylene blue takes up the dye readily and shows at first a beautiful basipetal staining gradient as in neutral red. In 10—15 minutes the whole plant is stained deep blue, but on return to water the dye begins to come out at once; also with a basipetal gradient, the apical ends of the axes becoming completely decolorized, while lower levels are still deeply blue. In 1½ hours the whole is completely decolorized and apparently quite uninjured, and the process of staining and decoloration may be repeated many times. After staining one hour in 1/10000 methylene blue complete decoloration requires 6—8 hours. Evidently the stain passes out less readily than in. In Callithamnion much the same relations appear, a distinct staining gradient and complete decoloration in water with a similar gradient. Here, however, decoloration is almost as rapid as staining. For these two forms methylene blue is apparently not toxic with the concentration and times of staining used, because, although it readily enters the cells, it is not held to any marked degree and so is readily given up again when the concentration gradient between inside and outside becomes sufficiently steep. That it is held within the cell to some extent is indicated, first, by the fact that its concentration within the cell may become much greater than that outside, and second by its less rapid passage outward than inward.

Odonthalia shows somewhat different conditions. Here, as in the other forms, methylene blue enters readily and a distinct staining gradient appears, but on return to water the cells do not give up the dye. Moreover, for this form methylene blue is dictinctly toxic and kills when it reaches a sufficient concentration in the cells, and when the cells die the dye comes out, together with the plant pigment, leaving the dead cells green. The death gradient is basipetal like the susceptibility gradients to other agents, but the differences in time of death along the axis are very much greater than the differences in rate of staining.

The work with methylene blue was not begun until late in the season of 1918 and was done chiefly with the three forms mentioned. These few observations are, however, sufficient to show that the cells of different species, although all highly permeable to methylene blue, differ widely in their relation to it. Its toxicity apparently depends, not on the permeability of the mebrane to it, but upon the degree to which it reacts physically or chemically with some constituent or constituents of the cell. With methylene blue, as with neutral red, the staining gradient, i. e., the gradient in permeability, may play some small part in determining the death gradient, but it seems clear that other conditions within the protoplasm rather than the permeability of its limiting surfaces are the chief factors in determining the course and rate of progress of death along the axis. When, for example, all parts of an elongated cell stain uniformly so far as can be seen, as is usually the case in these two dyes, but the death changes begin at the apical end of the cell and progress in an orderly way, reaching the basal end only after several minutes, it seems obvious that other factors than permeability are chiefly concerned in determining the course and rate of progress of death.

The results with these vital dyes enable us to distinguish to some extent between susceptibility and permeability. The permeability gradient and the susceptibility gradient show similar axial relations, but the permeability gradient is much less strongly marked. The logical conclusion from these facts is that differences in permeability are associated with other differences in physiological condition in the cells, but that the permeability varies much less widely than the conditions on which susceptibility depends.

Observations were made on a few species with $KMnO_4$. In all cases the staining gradient resulting from the reduction of the permanganate corresponded to the susceptibility gradient observed with other agents. In monosiphonous forms with more or less transparent cells, e. g., Callithamnion, not only the change in color, but the changes in aggregation of protoplasm can be followed within the single cell and, as with other agents a basipetal intracellular gradient in these changes appears. Moreover, the

differences in susceptibility of main and lateral axes are similar to those observed with other agents.

In various species the course of death along the axis in standing water was observed and was found to be basipetal like the other susceptibility gradients, provided the axes were not too closely crowded. When they were crowded, regions where different axes lie in contact are likely to die earlier than would be expected from their level in the axis, a fact which suggests that under these conditions lack of oxygen may be a factor in death. The death gradients were observed in standing water in Desmarestia, Agardhiella, Euthora, Bonnemaisonia and Callithamnion. The criterion of death used in these cases is the change in color in consequence of loss of the plant pigment. It is of interest to note that the course of death under these conditions is similar to that observed with the various agents used.

Nereocystis and Callymenia

Among the species examined which show peculiarities either in growth-form or in other characteristics, the kelp *Nereocystis luetkeana* is first considered. It has already been shown that in this species the stipe and the frond differ as regards the position of the chief growing region, that of the stipe being at the upper (apical) end (Sheldon, 1915), while that of the frond is near the basal end (Fallis, 1915). In my susceptibility observations young complete plants ranging in length from 30—150 cm. and separate stipes and fronds up to 100 cm. from more advanced plants were used. In HCl $m/200$ a change in color from the normal to a dirty green, also a decrease in turgor of the cells begins in about one hour. In the stipe the changes appear first 1-3 cm. below the float and progress rapidly in both directions, i. e., toward and over the float and basipetally over the upper ¼—⅓ the length of the stipe and from that point on more slowly to the base. In these young plants the float is somewhat less susceptible than the upper region of the stipe, but the difference is not very marked. The upper ¼—⅓ of the stipe, the region where growth is occurring most rapidly, is much more susceptible than more basal levels.

In the frond the change in color and loss of turgor begin at or within a few millimeters of the base of the flat portion at about the same time as in the upper portion of the stipe and progress rapidly along the frond for a distance of 5—6 cm. in fronds 20—30 cm. long and over a greater distance, e. g., 20—25 cm. in fronds 100 cm. or more in length. This region represents the most rapidly growing region of the frond. Progress of the death changes farther toward the tip of the frond is slower, except in the distal third more or less, the region of lowest susceptibility, where the differences in susceptibility at different levels are slight. The short cylindrical connecting region between frond and float is somewhat less susceptible than

the basal portion of the flat frond. Even in these regions of high susceptibility in stipe and frond a susceptibility gradient appears. In HCl m/200 the change in color and loss of turgor show a gradation and require ½—1 hour to progress over the length of the growing region and usually several hours more to reach the base of the stipe and the tip of the frond. The presence of this gradient in the growing region undoubtedly means that growth is most rapid at the apical end of this region in the stipe and at its basal end in the frond.

With other agents used, KNC m/100, KOH m/100, ethyl alcohol 10%, the changes in color are less conspicuous, but with all agents the loss of turgor occurs. In all cases the susceptibility gradients in stipe and frond are the same. In this species then, as in the others examined, the regions of most rapid growth show the highest susceptibility and the susceptibility gradients represent gradients in physiological condition.

The only form examined in which a distinct gradient in susceptibility was not observed was *Callymenia phyllophora*. In the elliptical or nearly circular fronds of this species the susceptibility was almost uniform throughout, some irregular areas here and there showing a slightly higher susceptibility than other parts. Fronds ranging in length from 20—120 mm. gave the same results. The form of the frond in this species indicates clearly that growth cannot be solely or chiefly apical, but must occur more or less in all directions in the plane of the frond. The irregular areas of slightly higher susceptibility may perhaps indicate more or less definitely localized regions of more rapid growth, or they may be due to incidental or chance conditions. No very early stages in development were obtained, but it seems not improbable that such early stages might show a more definite gradient in growth and susceptibility, and that the gradient disappears relatively early in development as in the case in the leaves of many flowering plants

The pseudothallus of Navicula

The observations on the susceptibility of the pseudothallus of the diatom Navicula have a certain interest, as indicating the existence of conditions in this form similar to those in the thalli of algae with apical growth. This pseudothallus grows in the form of flattened stems of almost uniform width and thickness, with repeated, rather regularly arranged branches resulting from repeated bifurcation of the tips. The existence of a definite growth-form of this sort in this pseudothallus must evidently result from definite growth relations between the different diatoms composing it, moreover, such a growth-form is impossible except where the chief growing region is apical. If all diatoms composing the pseudothallus grew and divided with equal rapidity and without any regular arrangement,

the result would necessarily be a more or less nearly spherical mass. In this form, however, not only is there a definite growth-form of the axis as a whole, but the diatoms imbedded in a gelatinous substance, are arranged more or less regularly in longitudinal rows.

Although presumably no protoplasmic continuity between the individual diatoms exists, growth and division are evidently limited largely to the apical region of each branch, i. e., the diatoms of the apical region, or certain of them, continue to grow and divide, while growth and division must cease, at least to a large extent at a greater or less distance below the tips, as in the axes of true thalli with apical growth.

This being the case, it might be expected in the light of the results obtained with other algae that a gradient in suceptibility would appear in this Navicula pseudothallus, and this is actually the case. The extreme tip is the most susceptible region of each branch, and from the tip downward the susceptibility decreases over at least several millimeters. In the more basal, older portions of the pseudothalli irregularities appear, as in the true thalli of many other algae. With $KMnO_4$ a basipetal color gradient appears, as in thalli with apical growth.

Both as regards susceptibility and growth-form, then, the axes of the Navicula pseudothallus behave like physiological axes. In view of the facts, there seems to be no escape from the conclusion that some difference in physiological conditions must exist between the apical and other regions. As regards the nature of this difference, there are apparently two possibilities: either physiological correlation of some sort, similar in general effect to the influence of the growing tip on other parts in other plant axes, must exist between the apical region and other levels, or else growth and division are gradually inhibited by the formation of the gelatinous envelope, so that those diatoms which lie at the tip, where little of the gelatinous substance has been produced, are capable of the most rapid growth and division, and the rate of these processes decreases as the gelatinous substance increases. On the basis of the first alternative, the increase in gelatinous substance is probably a consequence of the decrease in rate of growth and division, while on the basis of the second, it probably determines the decrease.

It is impossible at present to determine which of these alternatives represents the facts, but certain points may be briefly noted. The second alternative, that decrease in rate of growth is determined by the increase in the gelatinous secretion, does not account for the regular bifurcation of the apical growing regions, since these regions consist, not of single but of many diatoms. At present it seems impossible to account for this process and its regularity of occurrence without some sort of physiological correlation between the component diatoms. Second, since both susceptibility and

the rate of growth and division decrease basipetally from the apical region, a difference in electric potential must unquestionably also exist between the apical and other levels. This difference in potential may constitute the basis for a transmission relation between the apical and other levels of the axis. And third, even though protoplasmic continuity from diatom to diatom presumably does not exist, there is continuity of the gelatinous envelope which is a colloid, and it is quite possible that transmission of some electrolytic change resulting from the difference of potential between the tip and other levels, may occur in it. R. S. Lillie's experiments (Lillie, 1917, 1918) with electrolytic gradients and electrolytic transmission in metals and his discussion of their significance for a conception of protoplasmic transmission may be referred to in this connection. The difference in potential along the axis may readily give rise to effects which constitute a factor in determining physiological condition of the diatoms at different levels, at least within a certain distance from the tip. At present it seems probable in the light of all the facts that some such physiological correlation exists in these pseudothalli and that their definiteness of growth-form is dependent upon it, in short, that they are to some extent physiologically as well as morphologically axes.

Conclusion

The facts presented above, together with many others recorded in earlier papers, leave no doubt that the difference in susceptibility observed at different levels of a single axis and in different axes of the same plant are in some way associated with differences in physiological condition of some sort. In axes of the usual kind, with the chief growing region at the apical end the gradient in susceptibility along the axis evidently corresponds to a gradient in growth activity, and in Nereocystis, in which the chief growing region of the stipe is apical and that of the frond basal, the correspondence between growth and suceptibility still holds. Moreover, in thalli with branching growth-form of definite type, the differences in susceptibility in different axes correspond to the relations of those axes to the general growth-form.

In animals it has been found that susceptibility to killing concentrations of various agents varies in general with the degree, intensity or rate of metabolic activity. There is good reason to believe that in animals the oxidations are fundamental reactions in the metabolic complex, and, so far as data are at hand, we find evidence that a general relation exists between susceptibility and rate of oxidation. Whether this relation will prove to be universal or not can be determined only by future investigation, but on the basis of our present knowledge, susceptibility appears to be in some way associated with the rate of fundamental reactions.

The relation between susceptibility and growth activity in the algae indicates that in these forms also susceptibility is in a general way a measure of certain aspects of physiological or metabolic condition. The process of photosynthesis in plants has no parallel in animal metabolism, but photosynthesis is dependent upon external conditions and varies in rate quite apart from the other metabolic activities of the plant. Apparently in the complex of these other activities, oxidations constitute for the plant, as for the animal, a fundamental factor. In general rapidly growing cells respire more rapidly than those growing slowly, and, since susceptibility shows a very definite relation to growth activity, we may expect to find a general relation between it and respiration or oxidation.

This, of course, does not imply that all agents used to determine susceptibility enter chemically into or directly influence the oxidative reactions. It means only that in general protoplasm which has a high rate of oxidation is in a condition that renders it more susceptible to the toxic action of at least many external agents. Different agents undoubtedly act upon protoplasm in different ways. The "vital" dye neutral red, for example, which enters readily all cells along an axis in most plants, though its rate of penetration decreases to some extent basipetally, does not kill the cell-surface by its entrance, but death results from its accumulation within the cell. To acids, alkalies and many other substances, on the other hand, the living plasma membrane in its normal condition is only slightly permeable, and these substances do not enter the cell, except to a slight extent, until they have killed its surface or produced in it irreversible changes which lead to death. Nevertheless, the differences in susceptibility in different regions of the plant are the same for neutral red as for acids, alkalies and many other substances.

It is evident, then, that these differences in susceptibility cannot depend solely upon differences in permeability of the living plasma membrane. Even with neutral red the differences in susceptibility are very much greater than the differences in permeability. The susceptibility of a cell, as determined by this method, really depends primarily upon the rapidity with which the surface, the plasma membrane, is killed or undergoes certain changes through the action of an external agent. The data on susceptibility do not give very definite information concerning the interior of the cell. It is probable that in neutral red physiological conditions inside the cell are greatly altered before the surface dies or undergoes irreversible changes, while in acids and alkalies the interior of the cell undergoes little or no change in condition until the surface is killed or irreversibly altered. In other words, neutral red and various other vital dyes kill from within the cell, while it is practically true that acids, alkalies and many other agents kill or irreversibly alter the surface before they can act on the interior.

The cyanides, at least in most cases, apparently kill by their action on respiration, whatever the exact nature of that action. In most organisms, if not in all, respiration is very greatly decreased before death occurs, but this decrease, if not carried too far, is completely reversible. Hyman (1919) has shown, for example, that in *Planaria* a reversible decrease of 80—90% in oxygen consumption may occur in certain concentrations of KNC, and I have found a marked decrease in CO_2 production in the same species (Child, 1919b). Very similar effects of cyanide on respiration have been obtained with various other animals and plants. It is difficult to understand how cynaide can produce an effect so great and at the same time completely reversible, unless the protoplasm of the forms used for experiment is highly permeable to it, but if such high permeability to cyanide exists, differences in permeability can scarcely be entirely responsible for the differences in susceptibility which cyanide shows with such clearness.

In at least most other agents in killing concentrations irreversible changes begin before the reversible decrease in respiration has proceeded as far as is the case in cyanides. Notwithstanding these differences in action between cyanides and other agents, the susceptibility relations are in general the same. Moreover, in all cases thus far known, the same susceptibility relations appear with extremes of temperature and with lack of oxygen.

And finally, the limiting surface of living protoplasm, the plasma membrane, is itself alive and therefore the seat of a certain amount of metabolic activity, though in all probability the amount is small as compared with the total metabolism of the cell. In fact, the semipermeability of the plasma membrane is associated with this living condition and disappears when the membrane is killed. It is to be expected, therefore, that permeability itself will vary, at least to some extent, with the rate of metabolic activity in the plasma membrane within physiological limits, but beyond these limits, where irreversible changes occcur, this relation undoubtedly ceases to exist.

Susceptibility, as determined in my experiments, is primarily concerned with, and is, at least to some degree, a measure of the physiological condition of this superficial region of the cell, and differences in susceptibility in different regions of a cell or in different cells of an individual must depend upon differences in this condition. The exact method of action of each particular agent is not yet known, but since the susceptibility relations are essentially the same for at least many different agents within a certain range of concentration or intensity, it is evident that all of them, whatever their particular point of attack on the protoplasm, produce the same general effect, i. e., the retardation or cessation of the fundamental activities of life, and that the rapidity with which these changes occcur shows in general a

certain relation to the rate of the fundamental vital processes. So far as oxidation is such a fundamental process a relation between susceptibility and oxidation rate must exist. While in general the condition of the cell surface undoubtedly varies with conditions inside the cell, it does not follow that all changes within the cell must produce changes at the surface and so changes in susceptibility. It is probable that certain functional processes in the interior of the cell, such, for example, as digestion in the entoplasm of a protozoan do not affect the surface to any marked degree, though they may increase the oxidation of the cell as a whole.

Susceptibility, then, is measured by the time required under given conditions to kill or destroy the physiological and morphological characteristics of the superficial region of the cell. So far as the condition of this region is related to conditions in the interior of the cell, susceptibility is a measure of the condition of the cell as a whole.

The similarity of susceptibility relations to different agents and the general relation between susceptibility and rate of metabolism or oxidation undoubtedly result from the fact that living protoplasm is not a mosaic of independent reactions and conditions, but a system of correlated and interdependent states and changes, and that life consists in the maintenance of this system within a certain range of variation or oscillation about a condition of dynamic equilibrium. But whenever any essential factor in the system is altered beyond a certain limit by conditions external to the system, the whole system is affected, and if the change in the particular factor is sufficient, maintenance of the system and of life becomes impossible. Granting that the various agents and conditions used in determining susceptibility act upon the protoplasmic system in different ways, i. e., primarily or chiefly upon different constituent factors of the system, their general effect in the concentrations and intensities used is essentially the same. Whether a particular agent affects primarily colloid aggregation, lipoid condition, ion content or distribution, enzyme activity, the oxidative reactions or any other essential feature of the living system, this action, if sufficient in degree, will retard or prevent the continued activity of the system and alter the protoplasmic structure associated with this activity. Moreover, we find, as might be expected, that a relation exists between the sensitiveness of the system to such action and the rate or intensity of its own activities. To grosser disturbances, i. e., killing concentrations or intensities, the more intensely active system is more susceptible, while, on the other hand, adjustment or acclimation to less extreme disturbances, i. e., lower concentrations or intensities, occurs more rapidly or more completely in the more active than in the less active system.

Summary

1. In the algae with apical growth a gradient in susceptibility exists along each axis, the susceptibility decreasing from the apical region basipetally.

2. In branching thalli, in which a distinction in growth-form between main axis and lateral branches exists, the apical region of the main axis shows the highest susceptibility, and in the lateral branches the susceptibility decreases from the most apical branches basipetally. In such forms, then, a susceptibility gradient exists, not only in each single axis, but in each system of main and subordinate axes, and usually in the thallus as a whole, though irregularities are likely to appear in the older portions.

3. In *Nereocystis luetkeana* the susceptibility is highest near the apical end of the stipe and decreases basipetally, while in the frond it is highest in the basal region and decreases toward the tip. Here, as in other forms, the regions of most rapid growth are most susceptible.

4. The pseudothallus of the diatom Navicula shows a distinct gradient in susceptibility corresponding to the growth-form, the apical region of each axis being most susceptible.

5. With neutral red and methylene blue a gradient in penetration corresponding in axial relations to the susceptibility gradient usually appears during at least the early stages of staining, but the differences in susceptibility are much greater than the apparent differences in permeability. Methylene blue in the concentrations used is apparently not toxic to some algae and passes out of the cells only somewhat less readily than it passes in. The passage outward, like the passage inward, occurs most rapidly in the apical regions with a basipetal decrease in rapidity.

6. While permeability may perhaps be a factor in determining susceptibility, it alone will not account for the observed facts. In order to be toxic a substance must affect some constituent or reaction of the protoplasm, and it may produce its effect first at the surface and enter the cell only as it alters or kills the surface, or it may enter the cell readily without killing or appreciably injuring the surface and produce its toxic effect by accumulating within the cell. The axial susceptibility relations are essentially the same in both cases.

7. The general relation between susceptibility to a wide range of agents and conditions and rate of metabolism or oxidation results from the fact that living protoplasm is a system, and whatever the point of attack of a toxic agent or injurious condition, the general effect is essentially the same as regards the system as a whole, if the action is sufficient in degree.

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Notes on Melibe leonina [Gould]

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The genus Melibe (Rang) together with Tethys (Linn.) constitute the family tethymelibidæ, which forms one of the numerous groups of family rank in the suborder nudibranchiata of the opisthobranchiate mollusks. The families most closely related to the tethymelibidæ are the eolidæ, lomanotidæ, and dotonidæ.

The type of the genus Melibe was discovered at the Cape of Good Hope and was described by Rang in 1829. Since that time 11 species have been added by various authors. In 1852 Gould described *Melibe leonina* from Puget Sound, founding for it the genus Chioræra, now merged with Melibe.

Bergh has been the most voluminous writer upon this branch of the mollusks. In a series of publications extending over the period from 1871 to 1907 he completely revolutionized the classification of the nudibranchiata, creating many new families and genera and describing numerous species. He united the families Tethidæ and Melibidæ of Alder and Hancock (1845, 1864) into one, and removed Tethys and Melibe from the comprehensive family tritoniadæ of Gould (1852), and Tryon (1883), and united them to constitute the present family tethymelibidæ. He also gave careful attention to the broad classification of the major groups, dividing the nudibranchs into two sections, the kladohepatica and the holohepatica porostomata, the tritoniadæ serving as a connecting link between the two series. In the kladohepatica he recognized 11 families in bringing the species into systematic correlation. Although the work of Bergh was primarily systematic he based his work on rather extensive morphological studies, and laid an excellent foundation for subsequent more intensive investigations in the groups with which he dealt. In all he added 6 species to the genus Melibe: *M. rangii, M. capucina, M. vexillifera, M. ocellata, M. bucephala and M. pellucida.* The last mentioned was erected on a specimen taken on the coast of Washington, near the mouth of the Columbia River. His descriptions indicate such a close similarity between *M. pellucida* and *M. leonina* that it may be questioned whether *M. pellucida* is entitled to specific rank; material from the type locality may be necessary to settle this point.

Other workers in this field were Alder and Hancock (1845 and 1864) who described *Melibe fimbriata;* Pease (1860), Angas (1864) and De

Filippi (1865-1868), each of whom has described a species. Sir Charles Eliot (1902) has contributed to our knowledge of the morphology of the genus through comparative studies of Melibe and Tethys and has given an account of the feeding habits of Melibe. No detailed study of *Melibe leonina* has been made. Our knowledge of the species rests largely on the brief description and figure published by Gould (1852).

The known distribution of Melibe is restricted to the Pacific and Indian Oceans. On the eastern side of the Pacific 2 species have been described: e. g., *M. leonina* (Gould) in 1852, from Port Townsend, Washington, and *M. pellucida* (Bergh) in 1904, from the coast of Washington near the mouth of the Columbia River. On the western side of the Pacific various species have been found by Bergh (1875, 1880, 1884, 1888, 1892, 1880-1892, 1902, 1904, 1907-1908) from the Japanese islands to Singapore. One species (*Melibe pilosa*) has been described by Pease (1860) from the Sandwich Islands; another (*Melibe australis*) by Angas (1864) from Australia; and still another (*Melibe papillosa*) by De Filippi (1865-1868) from Japan. *Melibe rosea* was described by Rang (1829), and *Melibe fimbriata* by Alder and Hancock (1864) and Eliot (1902) from the Indian Ocean. To the present time no species of this genus seems to have been found on the coasts of the Atlantic Ocean. There and in the Mediterranean Sea Tethys has its home.

The specimens of Melibe upon which Gould (1852) founded the species *M. leonina* were collected at Port Townsend, in the northern part of Puget Sound. When the Puget Sound Biological Station was established in 1904 in the San Juan Archipelago, only about 50 kilometers from Port Townsend, it was found that this animal was not uncommon in the vicinity of the Station, although, like many pelagic organisms, its abundance was subject to great fluctuations. In the summer of 1912 it was particularly abundant, great numbers appearing among the fronds of Nereocystis which drifted past the floating dock in front of the Station. At this time Professor H. L. Osterud of the University of Washington gathered and preserved a considerable number of specimens. The largest obtained at this time were 6 cm. long. The time of collection was the latter part of July. In the season of 1913 very few were seen. In 1914 the writer found several specimens of large size, 8 to 13 cm. long. These were taken from the floating leaves of the eel-grass (*Zostera marina*). In the summer of 1915 only 2 specimens were found. It appears that the genus Tethys has a similar spasmodic recurrence as has been noted by observers in the Mediterranean. The appearance of Melibe does not appear to be determined by any particular season, since Professor Osterud found specimens spawning when visiting the Biological Station early in March during the spring of 1916.

There are striking similarities between the members of the kladohepatica, not only between those of any given family, but also between members of different families within the section. The former is well illustrated by Tethys and Melibe. This similarity is not only morphological, but equally true in the manner of living, and in the general behavior as seen in the method of swimming. *M. leonina* may appear to crawl on the surface of the water with the back downward, or it may float with the back up, the hood having air under it, or the papillæ serving as floats. When it has its back down Melibe bends its anterior end alternatingly from side to side to an angle of 45 degrees. These movements are most rapid when the body is relaxed from its bend, making a complete sweep over to the one side, thus pulling the body forward. By this method Melibe moves slowly through the water. *Tethys leporina* (*s. fimbria* Linn.) (Bergh, 1877) moves through the water in a similar manner. In this case, however, it is the large veil which plays an important part, together with the right and left bendings of the body. *Scyllaea pelagica,* classified by Bergh (1871, 1892) in the family scyllæidæ, is described by Collingwood (1879) from the China Sea. Its method of swimming is much like that of Tethys and Melibe.

Collingwood says in part: "Considerable numbers of this pelagic species were found upon the *Sargassum bacciferum* floating in Lat. 25 N., Long. 37 W.; most species of weed having one or more specimens. The animals were in constant movement of contracting and writhing. In the water they swam freely, moving the head and tail from side to side alternatingly, so as nearly to touch one another; and when thus swimming were always, owing to the weight of the papillary prolongations and tentacles, back downward, and bore grotesque resemblance to a four-legged animal with long ears, such as a sky terrier."

Another similarity between Scyllæa and Melibe is their manner of lowering themselves from the surface to deeper water. Scyllæa may be found at the surface or in deeper water. Collingwood mentions it as assuming a certain aspect when it falls through the water to a considerable depth, where it is frequently found. Melibe possesses a similar habit. Some of the animals were placed in a large jar of seawater. One morning all save one were apparently dead. All were removed save one, the water changed, and from time to time freshened by oxygenation or renewal. After some days this also seemed dead. It was thought that the water had become stale. Melibe lay absolutely motionless on the bottom, its muscles apparently completely relaxed. It showed signs of life only after the water had been oxygenated for several minutes. After this the writer became accustomed to this apparent death, and needed only to oxygenate the water to cause the animal to become active again, whereupon it would crawl along

the bottom and sides of the jar, and after a while swim about. Apparently it relaxes to sink, or sinks when relaxed. The means by which the animal reaches the surface is not known.

The eel-grass offers an excellent feeding ground for Melibe. Here the water is not only calm but abounds in small crustaceous forms of many kinds. Zostera grows in large beds in the bays near the Puget Sound Biological Station at Friday Harbor, Washington. It offers a suitable assembling place for Melibe, where the animals may pair and lay their eggs. At low tide the eel-grass floats on the surface of the water, leaving areas of open water between. In these open spaces these animals collect and copulate. This was observed in the summer of 1914, when the writer first found these animals. The animals were united head to tail in copulation.

The excellent condition of the water offered an ample opportunity to study the manner of feeding. This corresponds rather closely to Eliot's description for *M. fimbriata* (1902). *M. leonina* is not so definite in its movements during its feeding as is *M. fimbriata,* yet a similar method is pursued. Both species have a large hood. In the case of *M. leonina* this is distended very widely (Fig. 1) when the animal is searching for food, and is periodically contracted into a knob (Fig. 2) when food is obtained. When the hood is open it is tossed sideways and held in a direct position for the capture of small horizontally swimming crustacea. Melibe is actively predaceous. Its stomach has been found so completely filled with minute crustacea, such as copepoda, amphipoda, and isopoda that it bulged out in almost a perfect sphere. However, ordinarily the stomach only partially distends, with its diameter a little longer than that of the proventriculus anterior to it, or the intestine immediately behind.

One morning a nidosome was found which had been deposited by the animal during the night. It was a funnel-shaped structure (Fig. 3) and adhered by its small end to the side of the jar.

When touched the animal gives off a very strong offensive odor. This may be a means of protection.

Summary

1. *Melibe leonina* is an actively predaceous animal; it practically gorges itself, feeding mainly on small crustaceous forms of various kinds.

2. It occurs close to the surface on Puget Sound from March to August.

3. Its defense is probably an offensive odor.

4. It lowers itself to deeper water probably by relaxation of the muscles.

5. It collects in groups in quiet places among seaweed, where copulation takes place.

6. It spawns as early as March and as late as July.

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PLATE 49

Fig. 1. *Melibe leonina* (Gould). Drawing from life, by Bert Elliot, of the University of Washington. Natural size.

PLATE 50

Fig. 2 (above). Photograph of preserved specimen. The hood is contracted into a knob as when the animal swallows. Natural size. [Photo by author.]

Fig. 3 (below). Photograph of egg-body (nidosome). The dark band at right angles to the folds of the nidosome is a piece of eel-grass to which the egg-body is attached; the white radiating dots are the egg- capsules, each containing 10—22 eggs. Natural size. [Photo by author.]

Fig. 2

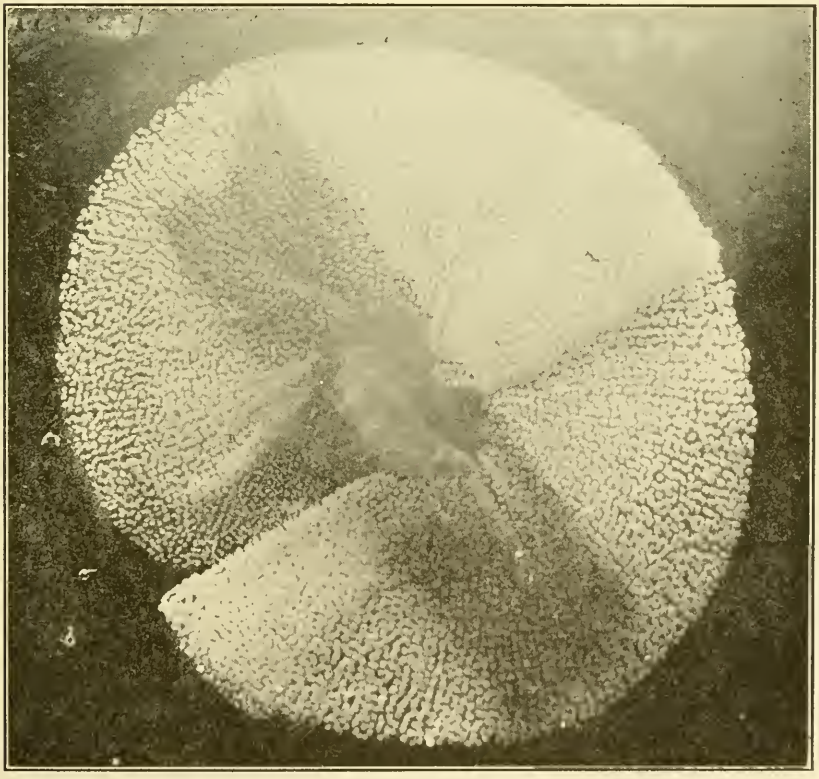

Fig. 3 PLATE 50

A List of Lichens from Southeastern Alaska

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The southeastern portion of Alaska has an exceedingly irregular coast line, most plentifully besprinkled with islands of all sizes and shapes. In the tide channels about the islands and fjords are vast forests of giant kelp. During the summer of 1913 the Bureau of Soils of the United States Department of Agriculture sent an expedition to investigate these kelp beds with a view to determining their availability as a source of potash. This party, headed by Dr. T. C. Frye of the University of Washington, studied not only the kelp forests but also the whole cryptogamic flora in general. Mr. A. S. Foster, well known as an enthusiastic collector of mosses and lichens, obtained most of the latter plants, tho Dr. Frye and Mr. Dean Waynick also collected many specimens of lichens.

About 462 species and varieties of lichens have hitherto been enumerated from Alaska, while the present list adds 20 species and subspecies, new to Alaska, making a total of 482. When one considers the enormous area and varied topography of Alaska this is a small number of lichens to be collected and shows that as a matter of fact the lichens of Alaska are but imperfectly known. The present collection of but 86 species and varieties contains over 18 per cent of species not previously collected, and also a number that have been collected only once or twice before, thus showing that every collection may be expected very greatly to increase our knowledge of Alaskan lichens.

It will be noted that the lichen flora of this region is, in general, about the same as that of all other northern regions having a cool, humid, foggy climate. The lichen flora of the islands visited by Dr. Frye's expedition may be considered as merely a northward extension of the lichen flora of Puget Sound and Vancouver Island, and as being intermediate between them and the arctic lichen flora of the interior of Alaska in geographical position. These facts and the ecological conditions of the region account for the poor representation of boreal species at this latitude.

Altho this region visited lies between latitude 54 and 58 degrees, it has a very mild climate for such a northern region. But in spite of this, the paucity of true arctic lichens in this list is to be also accounted for in part by the fact that all the lichens collected by Dr. Frye and his colleagues were obtained at or not very far above sea level.

The genus Cladonia, a characteristic group of northern latitudes, is amply represented, ten species, or nearly one-eighth of all the species

found being cladonias. The genus Parmelia and the genus Peltigera rank next in representation in this collection, six species of each having been found. As both genera comprise large and conspicuous foliaceous species they would naturally be among the first to attract attention.

The genera known as Lecanora and Lecidea comprise more species than any other lichen groups, but as the great majority are relatively quite inconspicuous bark and rock dwellers, it is perhaps only to be expected that but few species would be found in a reconnaissance of this character. Undoubtedly the region includes many more than the four species of Lecanora and two of Lecidea which were collected.

The number of endemic species in southeastern Alaska is very small and this is only what is to be expected. A lichen is not a species in the sense in which we speak of an oak, a maple, a Spirogyra, or an Agaricus. It is not a homegeneous unit, developed from an egg cell fertilized by a sperm cell from the same kind of an organism. There is therefore no inheritance in the ordinary sense, since there are no chromosomes to carry over the specific peculiarities of a given kind of lichen and accordingly there is no Mendelian principle involved, no dominant and recessive characters to be juggled about in the chromosomes.

A lichen is what one might call a physiological species, the result of the interaction of a fungus upon an enslaved alga, modified by the environment, and therefore in most respects not comparable to what is understood by the term "species" in most other groups of organisms. Their propagation is, in general, by soredia and by fragments detached and blown away. Soredia are minute masses of entangled algal cells and fungus threads, which form on the surface of many lichens. These powdery masses are widely distributed by the wind, and when they alight in favorable conditions develop into a thallus like the one from which they were formed. A large number of lichens have no other means of reproduction.

Where conditions are relatively uniform over great areas we find very few endemic species, because with these vegetative forms of reproduction it is difficult for considerable variation to arise under such conditions. It is only in regions of geographic isolation and with strongly diverse environmental factors, that we find a considerable number of endemic forms specifically distinct from those of continental distribution in the temperate regions of Europe and America.

As southeastern Alaska shares its climatic features with a very considerable area and is not greatly isolated, we may reckon upon the discovery of but few new species there, however much we may extend our knowledge of its lichen flora.

Following is a list of the species determined, together with the localities where they were collected:—

1.—*Verrucaria maura* Wahlenb.—Deweyville; Wrangell; St. John's Harbor, Zarembo Island; Howkan Bay; Heeeta Island.

2.—*Verrucaria muralis* Ach.—A variety of the species, with spores smaller than the type, 6-8 by 11 to 19 mikrons. Calder quarry, Prince of Wales Island.

3.—*Verrucaria fulva* Cummings—This species was found by the Harriman expedition at Port Wells only. Apparently common among the islands and to be looked for south at least as far as Vancouver Island. Our specimens are from Dall Island, Brownson Bay, and Nichols Bay.

4.—*Dermatocarpon miniatum* (L.) Mann var. *complicatum* (Sw.) Th. Fr.—Howkan Bay. New to Alaska.

5.—*Calicium curtum* Turn. and Borr.—Howkan Bay. New to Alaska.

6.—*Calicium hyperellum* Ach.—Zarembo Island. New to Alaska. Members of the Caliciaceae are so readily overlooked that is it probable several other species occur. They should be looked for especially on the trunks of rough-barked trees and on the dead limbs of trees.

7.—*Sphaerophorus globosus* (Huds.) Herre—Abundant; Kosciusko Island; Dall Island; Heeeta Island; Brownson Bay; Nichols Bay; Saltery Cove; Ratz Harbor.

8. *Arthonia cinero-fusca* Merrill—Spores 5-locular, larger at one end, 6.8 by 16.5 mikrons. Occuring on rocks touched by the ocean waves at St. John's Harbor, Zarembo Island. New to Alaska.

9.—*Arthonia punctiformis* Ach.—Our material is nearest to this species, the spores being 6-locular, 6.8 to 10 by 19 to 23.5 mikrons. On *Alnus,* at Augustine Bay, Dall Island.

10.—*Xylographa opegraphella* Nyl.—No spores were found but the material is very close to this; found on Zarembo Island.

11.—*Opegrapha saxicola* Ach.—On rocks in the bay at Deweyville, Prince of Wales Island; not previously reported from Alaska.

12.—*Graphis scripta* (L) Ach.—Common on bark of *Alnus;* Egg Harbor, Coronation Island; Shipley Bay; Kosciusko Island.

13.—*Thelotrema lepadinum* Ach.—This widespread lichen, found on *Alnus sinuata* at Kell Bay, Kuiu Island, seems not to have been reported from Alaska heretofore.

14.—*Lecidea flexuosa* (Fr.) Nyl.—Our specimens are nearest this species, which has not been collected in Alaska before. The material was found on slate rock at Wrangell; spores measure 3 to 4 by 9 to 11 mikrons.

15.—*Lecidea silacea* Ach var. *oxydata* Fr.—On rocks in the bay at Deweyville, Prince of Wales Island; new to Alaska.

16.—*Mycoblastus sanguinarius* (L.) Th. Fr.—Ratz Island; Augustine Bay.

17.—*Mycoblastus sanguinarius affinis* (Schaer.)—Port Alice, Heceta Island.

18.—*Mycoblastus sanguinarius alpina* Fr.—Thallus of this variety is yellow and it seems to be very common, tho not previously reported from Alaska. Shipley Bay; Kosciusko Island; Augustine Bay, Dall Island; Egg Harbor, Coronation Island; Kell Bay, Kuiu Island.

19.—*Bacidia* sp.—Spores 4-locular, 4.5 to 5 by 20 to 23 mikrons. Found on bark of dead cedars at Augustine Bay, Dall Island.

20.—*Pilophoron cereolus Hallii* Tuck.—St. John's Harbor, Zarembo Island.

21.—*Pilophoron cereolus acicularis* Tuck.—St. John's Harbor, Zarembo Island; Augustine Bay, Dall Island; Nichols Bay; Woewodsky Island.

22.—*Pilophoron cereolus fibula* Tuck.—Nichols Bay; this variety is new to Alaska.

23.—*Cladonia sylvatica* (L.) Hoffm.—Nichols Bay.

24.—*Cladonia alpestris* (L.) Rabenh.—Nichols Bay; near Wrangell.

25.—*Cladonia rangiferina* (L.) Web.—Kuiu Bay; Port San Antonio, Baker Island; near Wrangell.

26.—*Cladonia uncialis* (L.) Hoffm.—Nichols Bay.

27.—*Cladonia bellidiflora* (Ach.) Schaer.—Egg Harbor, Coronation Island; Kuiu Island; Ratz Harbor; Augustine Bay, Dall Island; Nichols Bay; Brownson Bay; St. John's Harbor, Zarembo Island; Prince of Wales Island.

28.—*Cladonia gracilis* (L.) Willd.—Prince of Wales Island; Egg Harbor, Coronation Island.

29.—*Cladonia squamosa* (Scop.) Hoffm.—Shipley Bay, Kosciusko Island.

30.—*Cladonia pyxidata* (L.) Hoffm.—Heceta Island.

31.—*Cladonia fimbriata* (L.) Hoffm.—Prince of Wales Island; Zarembo Island.

32.—*Cladonia furcata* (Huds.) Schrad.—Egg Harbor, Coronation Island; Brownson Bay; Heceta Island; Kuiu Island; Shipley Bay, Kosciusko Island.

33.—*Stereocaulon paschale* (L.) Ach.—Brownson Bay; Woewodsky Island; Wrangell.

34.—*Stereocaulon denudatum* Floerke—Augustine Bay, Dall Island.

35.—*Phylliscum demangeonii* (Mont. & Maog.) Nyl.—This very interesting and little observed plant is new to Alaska; the material was obtained on rocks at high tide mark, Zarembo Bay, Zarembo Island.

36.—*Collema pulposum* (Bernh.) Ach.—

37.—*Leptogium lacerum* (Sw.) S. F. Gray—Our material seems to be a variety of this species, which is new to Alaska. Port Alice, Heceta Island; Deweyville, Prince of Wales Island.

38.—*Leptogium lacerum pulvinatum* (Hoffm.) Nyl.—Growing on mosses over rocks, at Howkan. New to Alaska.

39.—*Leptogium palmatum* (Huds.) Mont.—This interesting European species which has only been found on the Pacific coast of North America, from Southern California northward, has not been reported from Alaska heretofore. The expedition obtained specimens from Egg Harbor on Coronation Island; on East Sound, in the Kashevarof group; and at Swanson Bay, B. C. At Howkan were found specimens of the species which were sterile and non-typical.

40.—*Parmeliella triptophylla* (Ach.) Müll. Arg.—I find no previous record of this lichen from Alaska, tho it seems to be abundant among the Alaskan Islands. It occurs on rotten wood at Deweyville, Prince of Wales Island; Port Alice, Heceta Island; Nichols Bay; and Coronation Island. On Kuiu Island it was found on the bark of alders. Material from Nichols Bay, growing on rocks and moss, yields spores 8 to 10 by 13.6 to 20 mikrons, the thecium deep blue with Iodine.

41.—*Parmeliella lepidiota* (Sommerf.)—Occurring on bark of alders at Augustine Bay, Dall Island; and Aats Bay, Coronation Island. New to Alaska.

42.—*Pannaria brunnea* (Sw.) Mass.—Augustine Bay, Dall Island.

43.—*Lobaria pulmonaria* (L.) Hoffm.—Heceta Island; Dall Island; Hidden Inlet; Ratz Harbor.

44.—*Lobaria oregana* (Tuck.) Herre—Prince of Wales Island; Saltery Cove; Augustine Bay, Dall Island; Heceta Island.

45.—*Sticta crocata* (L.) Ach.—A single scanty but typical specimen was found at Egg Harbor, Coronation Island. Recorded by Miss Cummings from Yakutat and undoubtedly overlooked by collectors. Probably occurring thruout the islands and mainland of southeast Alaska.

46.—*Sticta anthraspis* Ach.—Egg Harbor, Coronation Island.

47.—*Peltigera aphthosa* (L.) Hoffm.—St. John's Harbor, Zarembo Island; Port San Antonio, Baker Island; Wrangell.

48.—*Peltigera canina* (L.) Hoffm.—Wrangell; Port San Antonio, Baker Island.

49.—*Peltigera canina membranacea* (Ach.) Nyl.—Heceta Island.

50.—*Peltigera rufescens* (Neck.) Hoffm.—Saltery Cove.

51.—*Peltigera polydactyla* (Neck.) Hoffm.—Our material obtained on Prince of Wales Island is abundantly fertile.

52.—*Peltigera horizontalis* (L.) Hoffm.—Port San Antonio, Baker Island.

53.—*Nephroma arcticum* (L.) Fr.—Egg Harbor, Coronation Island.

54.—*Nephroma laevigatum* (Ach.)—Egg Harbor, Coronation Island.

55.—*Pertusaria multipuncta* Nyl.—Brownson Bay.

56.—*Pertusaria multipuncta ophthalmiza.* Nyl.—This subspecies found at Shipley Bay on Kosciusko Island is new to Alaska.

57.—*Pertusaria pocillaria* Cummings—This species is described by Miss Cummings from material obtained by the Harriman Expedition at Farragut Bay. It seems to be common in southeastern Alaska, and probably occurs southward into the Queen Charlotte Islands and perhaps the northern part of Vancouver Island. Our material is from Ratz Harbor; from Port Alice, Heceta Island; and Egg Harbor, Coronation Island.

58.—*Pertusaria* sp.—Sterile material from Saltery Cove could not be determined with certainty, other than it is different from all the other specimens collected.

59.—*Lecanora saxicola* (Poll.) Ach.—Saltery Cove.

60.—*Lecanora gelida* (L.) Ach.—Wrangell Island.

61.—*Lecanora subfusca* (L.) Ach.—Zarembo Island.

62.—*Lecanora subfusca campestris* Schaerer—St. John's Harbor, Zarembo Island; Shipley Bay, Kosciusko Island.

63.—*Ochrolechia tartarea* (L.) Mass.—Port San Antonio, Baker Island; Heceta Island; Shipley Bay, Kosciusko Island.

64.—*Icmadophila ericetorum* (L.) A. Zahlbr.—Nichols Bay.

65.—*Icmadophila aeruginosa* (Scop.) Mass.—Kasook Inlet; Augustine Bay, Dall Island; Port San Antonio, Baker Island.

66.—*Candelariella cerinella* (Falk.) A. Zahlbr.—On sandstone. Heceta Island.

67.—*Parmelia perforata* (Wulf.) Ach.—Port San Antonio, Baker Island; Howkan.

68.—*Parmelia saxatilis* (L.) Ach.—Fertile specimens were found at Port San Antonio, Baker Island; other material was obtained at Nichols Bay, Saltery Cove, and on Kosciusko Island; found growing on boulders at Kell Bay, Kuiu Island.

69.—*Parmelia saxatilis isidiata*—Kosciusko Island; on slate rock at Wrangell, the latter specimens being fertile.

70.—*Parmelia fuliginosa* (E.Fr.) Nyl.—This species not previously recognized from Alaska, was found at Shipley Bay, Kosciusko Island.

71.—*Parmelia glabra* Schaer.—This species, also new to Alaska, was likewise found at Shipley Bay.

72.—*Parmelia enteromorpha* Ach.—This excessively abundant lichen was obtained at Augustine Bay, Dall Island; Port San Antonio, Baker

Island; at all points on Dall Island where the collectors landed; on Kosciusko Island; at Tlevak Narrows. An evernioid form with linear lobes was collected at Brownson Bay.

73.—*Cetraria aculeata* (Schreb.) Fr.—Nichols Bay.

74.—*Cetraria lacunosa* Ach.—Brownson Bay; Kell Bay, Kuiu Island; Augustine Bay, Dall Island.

75.—*Cetraria glauca* (L.)—Egg Harbor, Coronation Island; Shipley Bay, Kosciusko Island; Port San Antonio, Baker Island.

76.—*Cetraria tuckermani* Herre—Brownson Bay; Heceta Island. Apparently new to Alaska.

77.—*Alectoria jubata* (L.) Ach.—Wrangell.

78.—*Alectoria sarmentosa* Ach.—Augustine Bay, Dall Island; Nichols Bay; Saltery Cove; Brownson Bay; Prince of Wales Island; Coronation Island.

79.—*Ramalina farinacea* (L.) Ach.—This species, tho common in southeastern Alaska seems to have been reported but once previously, having been collected by Dr. Bean among the Shumagin Islands. Our specimens come from Shipley Bay, Kosciusko Island; Howkan; and Egg Harbor, Coronation Island.

80.—*Usnea florida hirta* (L.) Michx.—Augustine Bay, Dall Island.

81.—*Usnea barbata* (L.) Web.—Port San Antonio, Baker Island.

82.—*Usnea plicata* (L.) Web.—Port San Antonio, Baker Island; Wrangell Island; Shipley Bay, Kosciusko Island.

83.—*Usnea longissima* Ach.—Shipley Bay, Kosciusko Island.

84.—*Xanthoria polycarpus* (Ehrh.)—Growing intermingled with *Physcia caesia,* on Heceta Island.

85.—*Buellia albo-atra* (Hoffm.) Th. Fr.—On slate, Wrangell.

86.—*Physcia caesia* (Hoffm.) Nyl.—Heceta Island; Zarembo Island; Wrangell.

Hydrogen Ion Concentration and Other Factors Affecting the Distribution of Fucus

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Introduction

During the summer of 1918 the writer worked on the effect of light and desiccation on the distribution of *Fucus evanescens* Agardh. In places, however, little or no Fucus was found where there was sufficient light and where desiccation to any considerable extent did not occur. Fucus frequently did not occur in bays which had light as well as suitable places for attachment. The writer has never found growing Fucus in tide-pools, although some were completely surrounded by it. Fucus does not grow where Ulva is found in any considerable quantity. Both Fucus and Ulva utilize the CO_2 in the sea-water during the day in the synthesis of their carbohydrates, thus causing the water to become more alkaline. During the night CO_2 is given off, thus causing the water to become more acid. Animals also add to the acidity of the water. Tests showed the hydrogen ion concentration to be much higher where Ulva grew than where Fucus grew.

Historical

Little work has been done on the factors causing the distribution of Fucus. Davis (1911) says the depth to which certain algae may descend depends upon the penetration of light. The factor that determines the lowest limits of algal life is not the depth of the water but the absence of light. The green algae require the most light, the red the least and the browns are intermediate. The same writer also considers that the influence of temperature must be of fundamental importance in the distribution of algae where the seasonal extremes are as great as those of the summer and winter at Woods Hole. There Fucus is found in its best vegetative condition during winter and spring, fruiting most abundantly during the latter season. It is represented during the summer by dwarfish growths, frequently lighter in color than the winter condition in which the growth and fruiting is more uniform. Most of the winter growth matures during the spring, hence the display of Fucus during the summer is comparatively poor.

Gail (1918) found that light and desiccation are controlling factors in the distribution of Fucus.

Little work has been done dealing with the effect of hydrogen ion concentration on plants, and no literature was found dealing with its effect on Fucus. Gillespie (1918) found the growth of the potato scab *(Actinomycoses chromogenus)* in media at the exponent 5.2 was slower and generally less vigorous than at less acid exponents. Sometimes the strain succeeded in growing well in a medium which had initially an exponent of 5.2 or even 4.8, but the growth was accompanied by a marked decrease of acidity, and the manner of the growth gave reason to doubt whether even in these cases more than a poor growth can occur in such exponents.

Cohen and Clark (1919) find in several species of Bacillus that there is a broad zone of pH within which the rates of growth are quite uniform for these short periods during which the increase of viable cells approaches the logarithmic rate. On borders of these zones of pH slight change in the pH produces marked effect upon reproduction.

Itano and Neill (1919) report that *Bacillus subtilis* germinates at 25° and 37° C if the hydrogen ion concentration of the broth is kept between pH 5 and pH 10 but not higher or lower pH values.

Shelford (1918) found that death among young herring occurred after an exposure of 8 hours when the pH was brought down to 6.825 or just on the acid side of true neutrality. Lillie, Loeb, Medes and Moore have gotten similar results on sperms and newly fertilized eggs.

Methods and materials

Experiments were started in June, 1919, at the Puget Sound Biological Station to determine if the hydrogen ion concentration had any bearing on the distribution of Fucus.

The salts used were potassium acid phosphate (KH_2PO_4), boric acid (H_3BO_3), potassium chloride (KCl) and sodium hydroxide (NaOH). The first three salts were recrystalized from 3 to 5 times. In all essentials the methods used were those of Clark and Lubs (1917).

The sodium hydroxide was prepared from metallic sodium. A piece larger than would be required was placed in a paraffined bottle which contained sufficient conductivity water to cover the sodium. The mouth was closed with a paraffined stopper. The solution was poured off and discarded when the outer layer had dissolved and approximately the desired amount of the unoxidized sodium remained. At once, and as rapidly as possible, unused conductivity water was poured on the sodium in the paraffined bottle and the mouth was closed with a paraffined stopper. After 24 hours, when the sodium had thoroly interacted with the water, the desired normality of the solution was obtained by adding a sufficient amount

of conductivity water and titrating with benzoic acid 99.90% pure fused, Bureau of Standards. All other solutions, including the buffers, were made according to the methods of Clark and Lubs (1917). These solutions were also kept in paraffined bottles or resistance glass.

The conductivity water was prepared by the distillation of barium hydroxide ($Ba(OH)_2$) sat. sol. 100cc, barium chloride ($BaCl_2$) sat. sol. 75 cc, alkaline potassium permanganate sol.* 50 cc, and enough distilled water to make 2 liters. The first and last 600 cc of distillate were discarded. The conductivity water and buffers were tested with the calomel cell.

The indicators used were phenolsulphonphthalein and o-cresolsulphonphthalein. A hydrogen set put up by Hynson, Westcott and Dunning of Baltimore was also used, and proved very helpful as a check.

pH Determinations

Tests were made on alternate days for the hydrogen ion concentration during the last half of June, thru the entire month of July, and thru the greater part of August. They were made in the following locations:

I. Places where Fucus was abundant.
   1. Sunny exposure on Brown Island.
   2. Southeast half of Turn Rock.
   3. Southeast exposure on San Juan Island opposite Madrona Point.
   4. On beaches having good rock for attachment of Fucus, much light and little or no Ulva.

II. Places where little or no Fucus grew.
   5. On beaches having good attachment, good light and an abundant growth of Ulva.
   6. In tide-pools containing no Fucus but with an abundant growth of it about them.
   7. Out in the Sound away from visible vegetation.
   8. On beaches having poor attachment for Fucus, much light and little or no Ulva.
   9. Locations having little or no direct sun light.

* NaOH 200g., $KMnO_4$ 8g., distilled in H_2O made up to 1000 cc.

Table 1. *Average monthly p H values of seawater June 15 to August 15, 1919.*

	June		July		August		Av. for Per.	
Time of Testing	a.m. 5-6	p.m. 1-4	a.m. 5-6	p.m. 1-4	a.m. 5-6	p.m. 1-4	a.m. 5-6	p.m. 1-4
Location				*Much Fucus*				
1 Southeast exposure	7.8	8.37	7.65	8.15	7.73	8.35	7.72	8.31
2 " "	7.81	8.3	7.8	8.26	7.8	8.15	7.80	8.23
3 " "	7.8	8.175	7.77	8.23	7.75	8.25	7.77	8.21
4 Beach, good attachment	7.6	8.0	7.72	8.27	7.53	8.2	7.62	8.16
				Little or no Fucus				
5 Beach, Ulva present	7.71	8.60	7.56	8.73	7.80	8.80	7.69	8.71
6 Tide pools	7.41	8.45	7.23	8.62	7.66	8.70	7.43	8.59
7 Out in Sound	7.90	8.00	8.00	8.16	8.14	8.25	8.01	8.15
8 Gravel beach; no Ulva	7.90	8.00	8.10	8.20	8.00	8.15	8.00	8.12
9 Little direct sunlight	7.91	8.13	7.84	8.15	8.03	7.97	7.92	8.07

Table 1 shows the different pH values of the seawater and the locations are referred to by numbers which correspond to those above. The pH value of the locations having much Fucus does not vary much *either* side of 8.0 in the afternoons. The average difference between the forenoon and the afternoon is 0.50. In case of tidepools and of beaches having much Ulva, but neither having Fucus, the pH values of the water in the afternoons is considerably above that of 8.00. The average difference between the forenoon and afternoon is 1.09, which is much greater than the average difference where much Fucus is found. This would seem to indicate that the pH values of the water were not favorable since all other conditions were good.

The last three locations, namely (7) out in the Sound away from visible vegetation, (8) on beaches having no Ulva and (9) on exposures receiving little or no direct sunlight, have pH values well within the limits for a good growth of Fucus. They have an average difference between the morning and afternoon of only 0.035. There must be other factors which prohibit the growth of Fucus in these locations, and these factors will be considered in a later part of this paper.

Effects of Ulva on Fucus

To study the effect that Ulva might produce on Fucus, 2-4-lobed plants, and more mature ones but not fruiting, with their natural attachments, were selected. These were placed in the Sound, where much Ulva was growing, others in Fucus beds. All plants were apparently of equal vigor. A stout cord was tied to each rock containing the Fucus plants which were placed among the Ulva. To the other end of each cord a piece of wood was tied. The cord was of sufficient length to permit the wood to float at high tide. Thus the Fucus could readily be found. The rocks containing the Fucus which was placed in the Fucus beds were suffi-

ciently heavy to hold them in place, and they were less difficult to find than among the Ulva. There were six rocks bearing the 2-4-lobed Fucus plants and the same number bearing the more mature plants among the Ulva. The same number of each kind were placed in the Fucus beds. All were placed about 1.5 meters vertically above the —1 tide line.

After three weeks both the young and mature Fucus plants placed among the Ulva had become much darker in color, and by the end of 4.5 weeks they had a decidedly reddish tinge. This is an abnormal color. At the end of 11 weeks the reddish tinge was still present except for a space of 3.5 mm around the tip ends and margin of the thallus where they were very dark thruout. A microscopic examination of sections thru the thallus showed a very evident gradient of susceptibility. The cells at the margin were dark brown in color and the walls were in a state of collapse. A little farther in they were still intact but the protoplasm was very dark. In cells still farther in, the protoplasm was more normal in color and normal chloroplasts could be detected. The plants had the same number of lobes that they had in the beginning of the experiment. Measurements showed that on the average they had not increased in size.

Both the young and the mature plants placed in the Fucus beds had normal color at the end of 11 weeks. The young plants placed in the Fucus bed had grown to twice the size of the young plants placed among the Ulva. A microscopic examination showed the cells to be normal.

The average pH value of the water about the Ulva at about 6:00 A. M. was 7.85, and at about 2:00 P. M. was 8.72. The average pH value of the water about Fucus at about 6:00 A. M. was 7.88, and at about 2:00 P. M. was 8.21. The average temperature of the water among the Ulva was 13° C at about 2:00 P. M. The average lower extreme was 11° C. The light was of the same intensity since they were the same distance above a —1 tide and were on the same exposure. All conditions seemed to be the same except that of the pH value of the water. The difference in growth and color in this experiment points toward the difference in the pH values of the water in the two locations, as a means by which the presence of Ulva may limit the distribution of Fucus.

Effect of pH and temperature on young plants

Experiments were started to study the effect on Fucus plants of pH values on the acid side of true neutrality and on up thru the higher alkaline values.

Open 4-liter glass jars were used, and three liters of water were used in each jar. The water was taken from the Sound, away from visible vegetation. It had an average pH value of 8.05. Commencing with pH value of 6.6 each jar was labeled respectively 6.8, 7.0, 7.2, etc., thru 8.8.

The correct pH value from 6.6 to 8.0 was secured by the addition of HCl. From 8.2 thru 8.8 the correct pH value was secured by the addition of sufficient amounts of water from about Ulva. When the tide was high it was necessary to add NaOH in place of water taken from about Ulva. In the addition of both the HCl and NaOH a medicine dropper was used. The indicator used for testing the pH values was phenolsulphonphthalein. The hydrogen ion set was used wholly for the comparison of colors. In those cases in which the pH values ranged from 6.6 to 8.0 it was necessary to make the pH corrections of the water nearly every hour of the day. The number of changes necessary depended largely upon the intensity of the sunlight. The correction of the pH values above 8.0 was necessary two or three times per day depending also upon the intensity of the sunlight. These changes kept the pH values of the water reasonably accurate. The seawater was changed completely in each jar once each day.

The temperature of the water in two of the sets of the experiment varied from 11° C to 24° C. These were the temperatures secured on the float where the jars remained during the time of the experiment. The temperature of the water in the third set varied from 10.5° to 13° C. This temperature was obtained by suspending in the Sound trays with bottoms of wire netting. The trays were fastened by cords between two logs on a float. The jars were placed in the trays. The trays were regulated in such a manner that the surface of the water in the jars had the same level as the surface of the water of the Sound. The lower temperature, 10.5° C, was the average temperature of the water in the jars at night. The higher temperature, 13° C, was the usual temperature at about 1:00 P. M., which was about 1° C higher than the temperature of the Sound at that time. Two-lobed young Fucus plants with natural attachments were used. Three such sets were continued over a period of nearly 11 weeks. All conditions were the same except that of temperature.

The results of the two sets having the temperatures range from 11° C to 24° C will be considered first. The Fucus plants in the seawater having a pH value of 6.6 and 6.8 lived about 9 days. The thalli of the plants commenced to curl on the second day. They showed signs of whiteness about the margins on the third day. On the fifth day the larger portion of the entire thallus looked whitish and soft. A microscopic examination of the cells on the seventh day showed those near the tips and margins to be completely broken down. The cells a little farther in were very much plasmolyzed. Still farther in, a few of the cells were apparently normal. No cells were normal on the ninth day. Here also the gradient of susceptibility is well shown. These plants were kept two weeks longer in the seawater having their respective pH values. At the end of this time

practically all of the cells were broken down and the plants were becoming frayed. The effect on the Fucus plants in seawater having pH values of 7.0 and 7.2 was similar, but three or four weeks were required to bring about the same results.

Those plants in seawater having pH values of 7.4, 7.6 and 7.8 showed little or no effect except that the growth was somewhat inhibited. There appeared to be a physiological adjustment to some extent, at least temporarily. Since Fucus is rarely found in seawater having a pH value as low as 7.4, and this for only a short period in the early morning, it would seem that it might be a matter only of time until the plants in seawater having this pH value and possibly the plants in seawater having a pH value of 7.6, would show results similar to those in the lower pH values.

The plants in seawater having a pH value of 8.0 to 8.2 had increased on the average 1 cm in length, and about 80 per cent of them were 4-lobed. In one jar containing seawater having the same pH value, the Fucus plants had not grown as much. These had increased in length only about 7 mm but the same percent of them were 4-lobed. There was no cause apparent for this difference since the conditions were the same and they had received the same treatment. There may have been some difference in the vigor of the plants but they appeared the same at the beginning of the experiment. At the end of 11 weeks the experimental plants were compared with those on the shore from which they were taken. The growth was practically the same. Those in the jars having seawater with a pH value of 8.0 to 8.2 were slightly lighter in color. The cause was undoubtedly due in a large degree to the difference in temperature, as will be explained later.

The Fucus plants in seawater having a pH value of 8.4 became abnormally darker after about 4.5 weeks and remained darker thruout the entire time. They showed an average increase in length of 3 mm at the end of 11 weeks. Two of the 30 plants in the jars containing seawater having a pH value of 8.4 had become 4-lobed.

The Fucus plants in seawater having pH values of 8.6 and 8.8 became very dark at the end of the first week. They also appeared more leathery and soon took on a reddish tinge. A microscopic examination after 8 weeks showed all the cells to be dead, except those in the interior part of the thallus. The protoplasm was brownish in color and many of the cell walls near the outer margin were collapsing. No growth had taken place at the end of 11 weeks and very few cells appeared normal.

The results from the set having the temperature range from 10.5° to 13°C will now be considered. In jars containing seawater with pH values of 6.6, 6.8 and 7.0 the results were practically the same as in the two previous sets except in degree and in that a longer period of time was required. No effects were noticeable until on the 8th day on the Fucus

in seawater having a pH value of 6.6, and none until at the end of three weeks on those in 6.8 and 7.0.

No effects could be seen in 7.2, 7.4 and 7.6 except that growth appeared somewhat inhibited. In 7.8 there was an average increase in length of 5.5 mm, and some showed the beginning of four lobes.

The plants in seawater having pH values of 8.0 to 8.2 had on the average the same growth as the plants in the same pH values but with the temperatures ranging from 11° to 24°C. The color, however, was more nearly the same as the color of the Fucus plants of the same age growing on the shore. This wide range of pH values and higher temperature must account to considerable extent for the lighter color of the Fucus plants in the previous set having the same pH values.

The Fucus plants in the seawater having pH values of 8.4, 8.6 and 8.8 were affected in practically the same manner as in the previous set with the higher and wider range of temperature. The period of time necessary to show the same results was from 4 to 6 days longer in this narrow range and lower temperature.

Percent of germination of oospores

Experiments were also undertaken with a view to determining effects both of temperature and of pH values of the seawater on the acid side of true neutrality and on up through the higher alkaline values, upon the germination of oospores and upon the subsequent growth of the sporelings produced. The oospores were obtained in the manner previously described (Gail, 1918). They were germinated on microscopic slides placed in glass jars containing seawater having the different pH values. Four different ranges of temperature, 10.5°-13°C, 11°-17°C, 11°-24°C and 11°-30° were used. The temperature of 10.5°-13°C was secured by suspending trays with bottoms of wire netting in the Sound as was previously described. The temperature of 11°-17°C was secured by placing the jars containing the different pH values of seawater in porcelain pans containing seawater, and setting these on a float on the Sound. The pans were about 60 cm high. The water in the pans was changed as often as was necessary to keep the temperature at 17°C or below. The temperature of the seawater having the different pH values usually went down to 11°C at night on the float. The temperature of 11°-24°C was the temperature produced by the atmosphere on the float some distance from the bank. The last temperature, 11°-30°C, was that produced by the atmosphere on the float but near the shore where it was protected from the wind. Each set was run in duplicate.

The per cent of germination will be considered first. An examination of table 2 will show the following:

TABLE 2. *Percentage of germination in different pH values and temperatures of water.*

pH	10.5°-13°C	11°-17°C	11°-24°C	11°-30°C
8.8	30	12	13	0
8.6	75	60	40	15
8.4	90	90	67	20
8.0-8.2	95	92	85	20
7.8	80	71	75	13
7.6	75	82	49	10
7.4	60	60	41	3
7.2	40	28	15	0
7.0	40	31	3	0
6.8	36	10	5	0
6.6	32	5	0	0

1. The higher percent of germination occurs in seawater having pH values above 7.4 and below 8.6 in all temperatures considered. The maximum germination occurs in seawater having pH values between 8.0 and 8.2.

2. The per cent of germination usually diminishes above a temperature of 17°C.

3. A microscopic examination of the oospores in seawater having pH values above 8.2 with a temperature above 24°C showed that the cell wall and plasma membrane were ruptured and that the protoplasm was exuding.

PERMEABILITY OF OOSPORES

Experiments were now made to determine whether permeability is related to inhibition of germination of oospores and to the death of oospores in different pH values of seawater. Oospores were secured in the usual manner. These were stained one to three hours in a weak neutral red solution. Watch glasses were washed in seawater and rinsed thoroly with seawater having the pH value that was to be used in that particular watch glass. The watch glass was then labeled with the correct pH value. Watch glasses for each pH value used were treated in the same manner. A large number of stained oospores were now placed in each watch glass by means of a medicine dropper. The medicine dropper was also used to place seawater having correct pH values on the stained oospores. The water on the oospores was changed four times. This was done to make certain that the correct pH value was in each glass, as the oospores were stained in seawater having a pH value of 8.2.

During the first attempts the oospores were examined every 15 minutes under a compound microscope to observe any change. As no

changes could be detected, the time for the examination was extended to about one hour. Decided changes in color usually occurred after about 12 hours. Each oospore is covered with a gelatinous substance which probably accounts for the long period of time required before any definite changes resulted. In no case did a change in color take place in all oospores.

Eighty per cent of the oospores in seawater having pH values of 6.6 and 6.8 changed from red to darker red or purple. No change of color occurred in oospores which were in seawater having pH values between 6.8 and 8.2. This indicates that substances potentially acid or alkaline do not permeate the plasma membrane in sufficient quantities to change the color produced by staining the oospores with neutral red. Oospores in seawater having pH values below 7.6 were not killed but were inhibited when the temperature was below 24°C. This was shown by the fact that when the pH values were allowed to rise, germination and some growth took place. When the temperature of the water was above 24°C the oospores were killed in 3 to 24 hours, depending upon the height of the temperature. Better germination occurs in seawater having pH values between 7.4 and 8.4. The maximum germination is at about 8.0 or 8.2 and gradually decreases on either side. This is in accord with the observations that the plasma membrane was not permeated by OH or H ions in any considerable quantity in these pH values as is manifest by no change in color. In seawater having pH values of 8.4, 8.6 and 8.8 about 70 per cent of the oospores changed from red to yellow. Usually there was a considerable space between the cell wall and the protoplast. The OH ion had probably produced a sufficient disturbance of the colloidal equilibrium of the plasma membrane to bring about plasmolysis. Harvey (1911) reports injury to be possible when the concentration of the base was 0.025N.

Plate 51 shows a graphic representation of the germination during the first 7 days at different temperatures and the various pH values of the seawater.

Growth of sporelings

Oospores were germinated in the usual manner and the sporelings were grown for four weeks. The same conditions and manner of treatment were continued that were used in the study of the per cent of germination of oospores. The percentage of living sporelings is based on the number of oospores that had germinated by the seventh day. Table 3 shows the percentage of living sporelings at the end of four weeks together with the pH values of the water and the variations in the

temperature. With a few exceptions there is a gradual decrease in the number of living sporelings as the temperature becomes higher than 17°C. The percentage of living sporelings is very small when the temperature reaches 30°C. The maximum number is nearly always found in water having pH values of 8.0 to 8.2. For a graphic representation see plate 51.

TABLE 3. *Percent of living sporelings at the end of 4 weeks, in different temperatures and pH values*

pH	10.5°C-13°C	11°C-17°C	11°C-24°C	11°C-30°C
8.8	0	0	0	0
8.6	29	30	22	0
8.4	56	83	70	45
8.0-8.2	96	98	81	5
7.8	86	90	82	3
7.6	85	91	81	4
7.4	52	40	26	0
7.2	45	28	7	0
7.0	45	29	0	0
6.8	20	8	2	0
6.6	19	3	0	0

The size of the sporelings growing in seawater having the various pH values and a temperature ranging from 11°-17°C were recorded at frequent intervals. Both the length and the width of the sporelings were measured. The increase in width is very small during this period of growth and will be considered later in this article. Each measurement recorded represents the average length and width of 12 typical sporelings. Very little growth occurred in seawater having a pH value of 6.6. The sporelings were still alive at the end of four weeks, as they increased in length from 0.104 mm to 0.16 mm when the pH value of the sea water was raised from 6.6 to 7.4.

The growth of sporelings in seawater having pH values of 7.0 and 7.2 was almost completely inhibited after the eighth day.

The growth in seawater having pH values of 7.4, 7.6 and 7.8 was very much the same. Growth was inhibited to some extent after about two weeks.

No inhibition of growth is apparent in seawater having pH values of 8.0-8.2. Numerous sets of experiments showed the same results in all temperatures tried below 24°C. The sporelings increased in length from 0.072 mm to 0.192 mm in 72 hours. This was an increase of .12 mm. By the eighth day the length was 0.404 mm. Growth continued thruout the period.

The sporelings in seawater having a pH value of 8.4 increased from 0.072 mm to 0.224 mm in the first 72 hours. This was an increase of 0.152 mm, which was a greater increase than occurred in seawater having a pH value of 8.0-8.2. By the eighth day the length was 0.30 mm, and by the thirteenth day it was 0.332 mm, an increase of only 0.032 mm in five days, while the sporelings in the seawater having pH values of 8.0-8.2 were 0.40 mm long in the same time. Growth seems to be inhibited after about 72 hours in seawater having a higher pH value than 8.2.

The sporelings growing in seawater having a pH value of 8.6 were living at the end of four weeks. At this time they were 0.24 mm in length, an increase of 0.178. The growth was quite rapid for the first eight days, when it was practically inhibited, and many appeared dead by the eighteenth day. A considerable number of the sporelings became loosened from the slide during the remainder of the four weeks.

The sporelings growing in seawater having a pH value of 8.8 grew very slowly and life was considered extinct after the seventh day. No growth took place after this time and the sporelings were dark gray instead of brown in color. Many of the sporelings soon became loosened from the slide. The length of the sporeling on the seventh day was 0.08 mm. As the oospore is about 0.072 mm in diameter, the sporeling had increased 0.008 mm in length in seven days. For a graphic representation of this increase in length see plate 52.

The effect of different pH values and temperatures on the size of sporelings as measured by width and length at the end of four weeks is summarized in table 4, from which the following is evident:

Table 4. *Size of sporelings in different p H values and different temperatures of water at the end of 4 weeks.*

pH	10.5°C-13°C		11°C-17°C		11°C-24°C		11°C-30°C	
	width	length	width	length	width	length	width	length
	0_u	0_u	0_u	0_u	0_u	0_u	0_u	0_u
8.8	90	320	90	214	69	123.5	0	0
8.6	90	384	90	396	78	217.5	78	116
8.4	96	640	112	680	89	288	80	168
8.0-8.2	88	496	92	336	84	288	76	128
7.8	90	502	90	256	69	208	76	96
7.6	72	388	90	272	75	276	0	0
7.4	76.8	416	90	208	72	144	76	102
7.2	76	320	78	176	78	88	0	0
7.0	76	240	76	128*	78	81	0	0
6.8	76	208	76	160	0	0		
6.6								

* Dead on 24th day.

1. The maximum growth is found in seawater having a temperature not higher than 17°C.

2. The largest plants as a rule are found in seawater having a pH value of 8.0-8.2, in any of the temperatures considered.

3. The growth in width of the sporeling is small in comparison with the growth in length. The greatest growth in width is found where the temperature of the water is not higher than 17°C, and where the pH value of the water is 8.0-8.2. Plate 51 shows a graphic representation of the size of the sporelings as affected by the different temperatures and the various pH values. It will be observed that in this particular set the sporelings made little or no growth where the temperature ranged from 11°-17°C and the water had a pH value of 6.8. The sets were all run in duplicate and there was a more normal growth in the corresponding set of the same pH.

Study of beaches

In the case of beaches having neither Ulva nor Fucus and only smooth rolling stones for attachment, some other factor or factors than that of the pH value of the water must be responsible since the average pH value of the water at such places was 8.07, which is well within the limits for an abundant growth of Fucus. Experiments previously conducted by the writer (Gail, 1918) threw some light on the situation. It was then believed that desiccation was the limiting factor. Considering the evidence presented in this paper, it is now considered that temperature is also an important factor.

As during the previous summer, oospores were planted on smooth flat stones which were firmly attached to the beach, a 10 per cent germination resulted on two different occasions when the temperatures did not go above 19° or 20°C. At another time scarcely a 1 per cent germination resulted, when the temperature remained at 26°C for nearly 4 hours. In one case all of the sporelings disappeared in five days. In another trial they disappeared in eight days. In the last trial, during which it was more cloudy than usual, most of the sporelings remained on the stones until the thirteenth day. The temperature in the first case went up to 24°, 24.5° and 26°C, during the first three days. When the sporelings remained on the stones eight days the temperature rose to 26°C on two different days, but only for about an hour on each occasion. In the last case the temperature did not go higher than 20°C until the last two days, when it rose to 25°C and 28°C, respectively. The temperature of 28°C was maintained for over two hours.

In order to determine whether desiccation or temperature was the limiting factor experiments were started as follows. Oospores were

planted on glass slides and placed in three different glass jars each containing two liters of seawater having a pH value of 8.2. The temperature of one jar did not go higher than 13°C. In a second jar it did not go higher than 17°C. In a third jar it did not go above 27°C. Each jar contained two slides and each slide contained over 75 oospores. A 95 per cent germination took place in those jars in which the temperature varied from 11° to 17°C. About a 3 per cent germination resulted in those jars in which the temperature went as high as 27°C, when this high temperature was maintained 2 hours or more. In these experiments the desiccation factor was eliminated and the effect of temperature was clearly demonstrated. Undoubtedly desiccation on the beach is harmful to the germination of oospores and the growth of sporelings but the high temperatures often reached may be the determining factor if they continue any considerable length of time.

Study of tidepools

A study of tidepools was also made in order to find why Fucus does not grow in them. Chambers (1912) claims that young plants of *Prionitis lyallii* are always found starting around the rim of tidepools where the CO_2 would supposedly be abundant, but never on the bottom, where both CO_2 and oxygen would in all probability be absent or much diminished. He believes the whole problem would resolve itself into a question of aeration. The writer has made numerous tests in tidepools and found that the average pH value of the seawater in them between 1:00 P. M. and 4:00 P. M. was 8.59, and that it was often 8.8. This is partly brought about by the seawater draining from the surrounding Fucus beds into the tidepools when the tide is going out. As the seawater makes its way down into the tidepools, it is continually taking up oxygen and is well aerated. The microscopic algae and *Prionitis lyallii* also make the water more alkaline in the manufacture of their carbohydrates. During the night CO_2 is liberated largely and oxygen is used in respiration. This CO_2 unites with H_2O, forming H_2CO_3, which makes the seawater in the tidepools more acid. The average pH value was 7.43 a little before sunrise. The writer has never found a good growth of Fucus in seawater having as high pH values as occur in tidepools during the day nor in seawater having as low pH values as occur in tidepools in the early morning.

The average temperature of the tidepools between 1:00 P. M. and 1:00 P. M. was 24.7°. This high temperature has already been shown to be harmful to Fucus. When oospores were planted on shells and placed in the tidepools there was less than a 5 per cent germination. Those that did germinate soon became loosened from the shells. The

only conditions of the tidepools measured by the writer that were different from those of the surrounding Fucus beds were temperature and the pH values of the seawater in the tidepools. In the light of this investigation, these two factors are believed to prevent the growth of Fucus in tidepools.

Depths at which Fucus grows below surface of water

It was shown by experiment (Gail, 1918) that the oospores, sporelings, young and mature plants of Fucus died, or that decomposition took place, when suspended in the seawater of the Sound at a depth greater than 1 meter. The average pH value of the seawater taken the following summer at the same location and at a depth of nearly 1 meter was 8.0. This is well within the limits for a good growth of Fucus. The pH value of the seawater, however, does decrease with the depth. This would also indicate a low oxygen content. At the close of the season of 1918, light was believed to be a controlling factor, and is still so regarded. This is in accord with Davis (1911) who says "The depth to which certain algae may descend depends upon the penetration of light." The writer now considers that the pH value and the low oxygen content of the seawater at the greater depths may also be important factors. Lacking facilities for measuring the pH value and the oxygen content at any great depth, no accurate measurements could be made.

Summary

1. The growth of sporelings as well as larger plants of Fucus is almost completely inhibited in seawater having a higher pH value than 8.6, and is very much inhibited in seawater having a higher pH value than 8.4.

2. Sporelings as well as larger plants of Fucus will not live in seawater having a pH value of 8.6 when the temperature is higher than 24°C.

3. Sporelings as well as larger plants of Fucus are very much inhibited in growth when the pH value of the water is below 7.2. Neither will live in seawater having a pH value of 7.0 when the temperature is above 24°C.

4. Fucus is not found on beaches, even tho there be good attachment, where there is much growth of Ulva, since Ulva causes the seawater to have too high pH values.

5. The results of the experiments on permeability of the oospores indicate that the plasma membrane is sufficiently permeable to OH and H ions in seawater having pH values above 8.4 and below 6.8 to reduce the percent of germination and to inhibit the growth of sporelings.

6. Fucus is not found on smooth gravel on beaches even where Ulva is not present, since the high temperatures and extreme desiccation decrease the germination and prevent the growth of sporelings.

7. The oospores of Fucus in seawater having a pH value of 7.0 do not germinate if the the temperature is as high as 30°C for three hours or longer. Germination is retarded at lower temperatures in seawater having pH values below 7.2.

8. Fucus is not found in tidepools because the temperature of the water is too high and because the extremes of the pH values of the water are too far apart.

9. Reduced light is a controlling factor in determining the lower limit of Fucus. The probable low pH value and low oxygen content of the seawater at any considerable depth may also be important factors.

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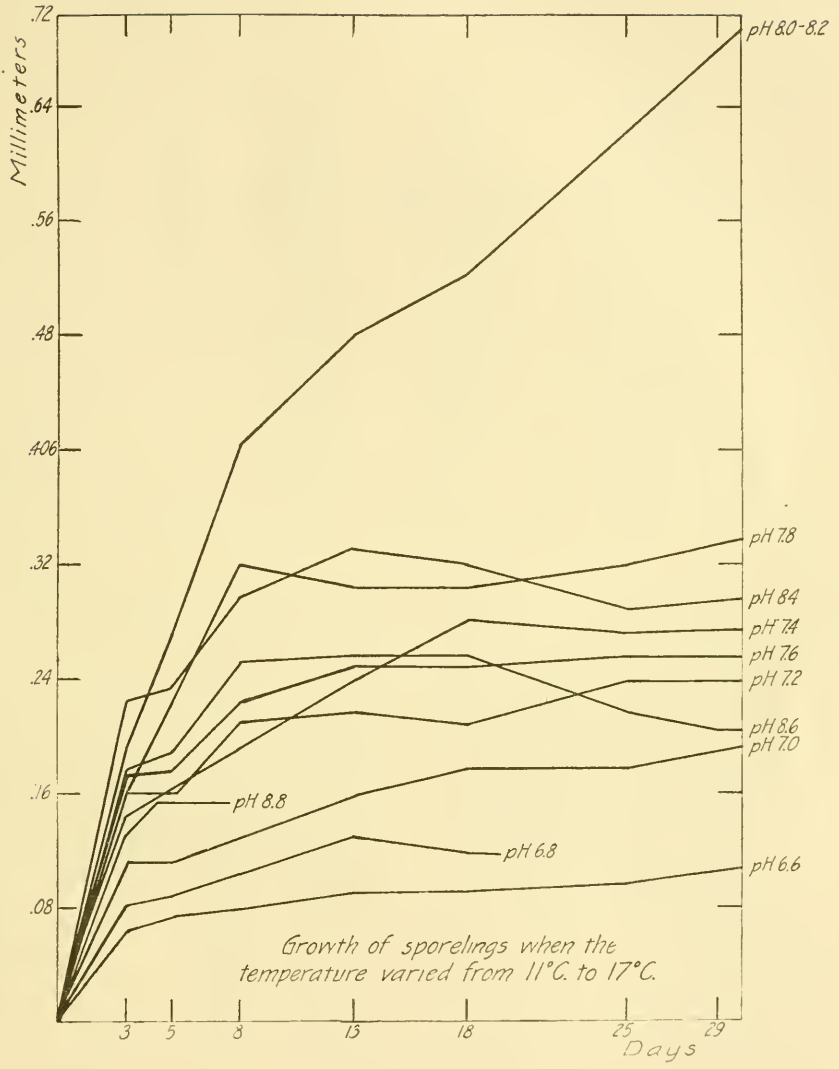
.72
.64
.56
.48
.406
.32
.24
.16
.08
Millimeters
pH 8.0-8.2
pH 7.8
pH 8.4
pH 7.4
pH 7.6
pH 7.2
pH 8.6
pH 7.0
pH 8.8
pH 6.8
pH 6.6
Growth of sporelings when the temperature varied from 11°C. to 17°C.
3
5
8
13
18
25
29
Days

PLATE 51

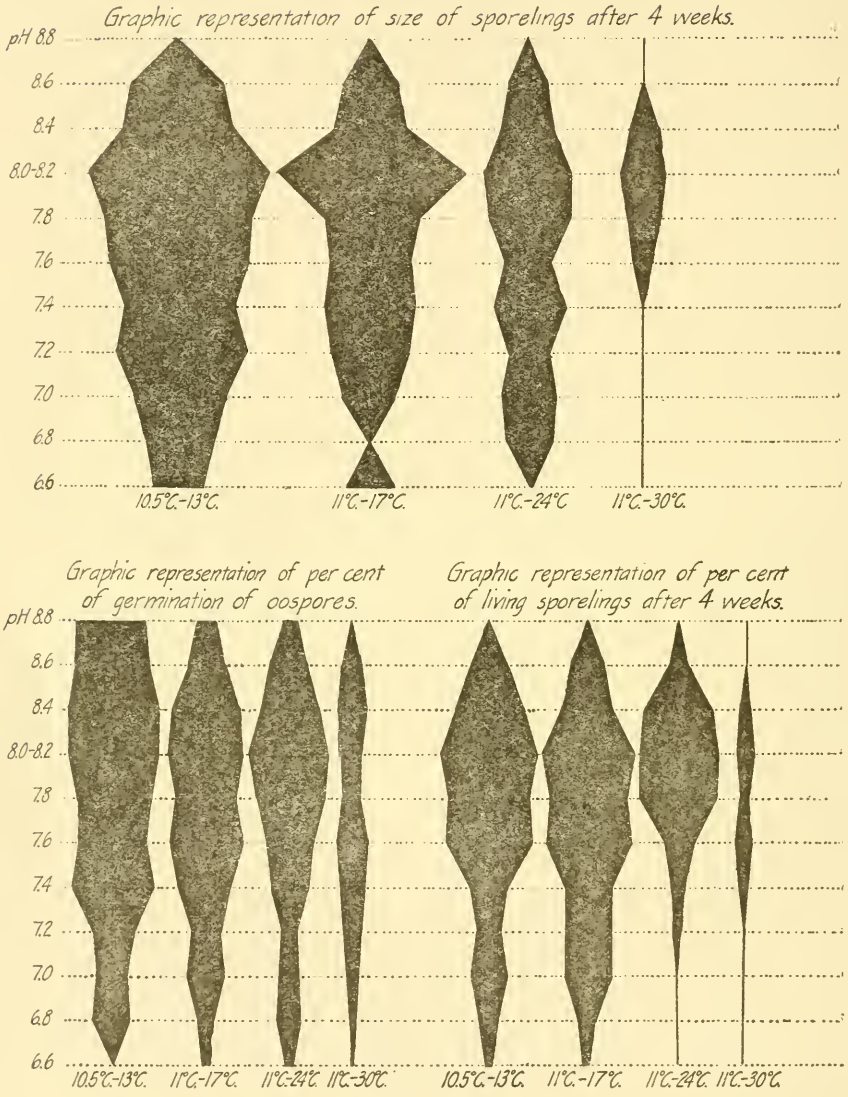
Graphic representation of size of sporelings after 4 weeks.
pH 8.8
8.6
8.4
8.0-8.2
7.8
7.6
7.4
7.2
7.0
6.8
6.6
10.5°C.-13°C.
11°C.-17°C.
11°C.-24°C
11°C.-30°C.
Graphic representation of per cent of germination of oospores.
Graphic representation of per cent of living sporelings after 4 weeks.
pH 8.8
8.6
8.4
8.0-8.2
7.8
7.6
7.4
7.2
7.0
6.8
6.6
10.5°C.-13°C.
11°C.-17°C.
11°C.-24°C.
11°C.-30°C.
10.5°C.-13°C.
11°C.-17°C.
11°C.-24°C.
11°C.-30°C.

PLATE 52

Variation in the Number of Ribs in Costaria costata

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Postels and Ruprecht (1840) describe the genus Costaria as "5-3 costata," and give a plate showing a 5-ribbed specimen. J. Agardh (1848) agrees with this. Most of the literature on the genus succeeding these records no other variation in the number of ribs. Whether 3-5 (the figures are given 5-3) mean that there are fronds with 3, 4 and 5 ribs respectively, or with 3 and 5 ribs only, is not distinctly specified. Some descriptions state that "the ribs . . . are five in number" (Setchell 1893), and mention no variation at all. Setchell (1911) says the blade has "several (4-5) longitudinal ribs." This is the only specific reference found to a 4-ribbed Costaria, and 3 ribs are not included. Muenscher (1917) describes Costaria as having "5, or rarely 3, longitudinal ribs." The species referred to in every case mentioned is *Costaria costata* (Turn.) Saunders [*Costaria turneri* (P. & R.)]

During the summer of 1919 in the waters in the vicinity of the Puget Sound Biological Station, many fronds of *C. costata* were gathered which show great variation in the number of ribs. All specimens were gathered and preserved for the Director of the Station by Miss Leona Sundquist of the University of Washington, and handed over to the writer to report upon. Most of them were secured off the shores of Blakeley and Turn Islands, perhaps because these localities were visited most often. The frequency of finding these variants indicates that they are not extremely uncommon. No account of *C. costata* with more than 5 ribs seems to have been published, nor any variation in the number of ribs within the length of a single blade. For these reasons it is thought that perhaps the observations on the unusual features recorded in this paper may be of interest.

It has been established that the growing point of Costaria is near the base of the blade (Fallis 1916). Small plants are generally the only ones which have all their parts, since the early growth of larger fronds is invariably worn away . In all cases here discussed the tips of the fronds are missing, and in one instance the holdfast end is also torn away.

No 3-ribbed fronds were found. The number or ribs ranged from 4 to 9. Not all the ribs necessarily extend the whole length of the frond. In fact, of the fronds which are not typically 5-ribbed, there are

as many which have a variable number of ribs in a single frond as those which have a constant number. It is conjectured that if no part of the frond wore away the complexity of venation might be still further augmented.

The typieal frond has a midrib with two ribs on each side. The corresponding ones on the sides are approximately the same distance from the midrib. In the variants a rather common feature is the absence of a midrib. Thus we have the 4-ribbed plant (Figs. 1 and 2). In no case was a 4-ribbed one found which had a rib in the middle. Of the 6-ribbed fronds several groupings of ribs were found. One group seems to be the result of the separation of one of the outside ribs into two. thus giving two on one side of the midrib and three on the other (Fig. 6). Another plant has a midrib with one on one side and four on the other, apparently due to the failure of the rib on one side to grow, and to the splitting of the 2 ribs on the other side into 2 each (Fig. 8).

A third abnormal form with 6 ribs is characterized by the complete absence of the midrib, and by the presence of two groups of 3 ribs each growing symmetrically at equal distances from the middle of the blade (Fig. 7). The 3 ribs of each group are close together in this. The first and third of each group are not as far apart as the two side ribs normally would be, and the one of each set nearest the center of the frond is stronger than the other two. At the junction of the stipe and blade the two outer ribs of each group almost coalesce, giving the appearance of four ribs arising from the stipe tissue. A fourth variation with 6 ribs has a midrib with two normal ribs on one side of it and a group of three on the other (Fig. 5).

All the fronds having more than 6 ribs show variation in the number thruout the length of the blades. This lack of constancy is also present in the 4 and 5-ribbed forms, and may appear in any part of the frond length. This shows that new ribs may appear or old ones be suppressed at any point in the growth of the plant.

Variation in the midrib may affect the number of ribs in the following three ways: (a) By its disappearance in the late growth of the plant (Fig. 5); (b) By its appearance in a plant in which it was not present (Fig. 3); (c) By its disappearance for a time and its reappearance farther down (Fig. 4). Specimens were found showing all three of these variations in 4-5-ribbed combinations. The dropping of the midrib was found in a 5-6 combination. At no point in a frond was a group of ribs found in the middle; thus there is no indication of the separation of the midrib into several. Of course, in the stipe all the ribs arise very close together and one might consider this the region in which the lateral ribs arise from the midrib.

The changes in the side ribs are more irregular than those in the midrib. A discrimination may be made between the variations in the number of distinct ribs, and the apparent splitting or separation of one rib into two or more which remain comparatively close together, forming what may be called a rib-group.

The side ribs show the same variations as the middle one, and in addition may form rib-groups. The individual ribs in these groups exhibit the same variations as the side ribs. One frond shows a variation from 4 to 7 depending upon the region of the blade at which the ribs are counted (Fig. 8). The most complex venation found is in a fragment of a very large plant in which there are from 7 to 11 ribs (Fig. 9). The midrib and one outer side-rib are constant thruout, but the others form groups. Two of these ribs in a group may coalesce and then separate, after which one or the other may disappear completely. In one group is a small, not very clearly defined rib which extends only 6 cm. and does not reappear again or have any connection with any other rib.

In one frond an injury near a rib resulted in the almost complete disappearance of the injured rib (Fig. 2). Whether some of the variations, either of suppression or proliferation, may be the result of injury is not known. No explanation for the cause of the variations is attempted.

PLATE 53

DIAGRAMS

Fig. 1. A 4-ribbed specimen with no variation in the number of ribs thruout.

Fig. 2. An injured frond which shows almost complete disappearance of the injured rib.

Fig. 3. Showing variations of the midrib.

Fig. 4. Showing variations of the midrib.

Fig. 5. A 6-ribbed type, showing variations of the midrib.

Fig. 6. A 6-ribbed type.

Fig. 7. A 6-ribbed type.

Fig. 8. A 6-ribbed type, showing variations in the side ribs .

Fig. 9. Most complex system of ribs found.

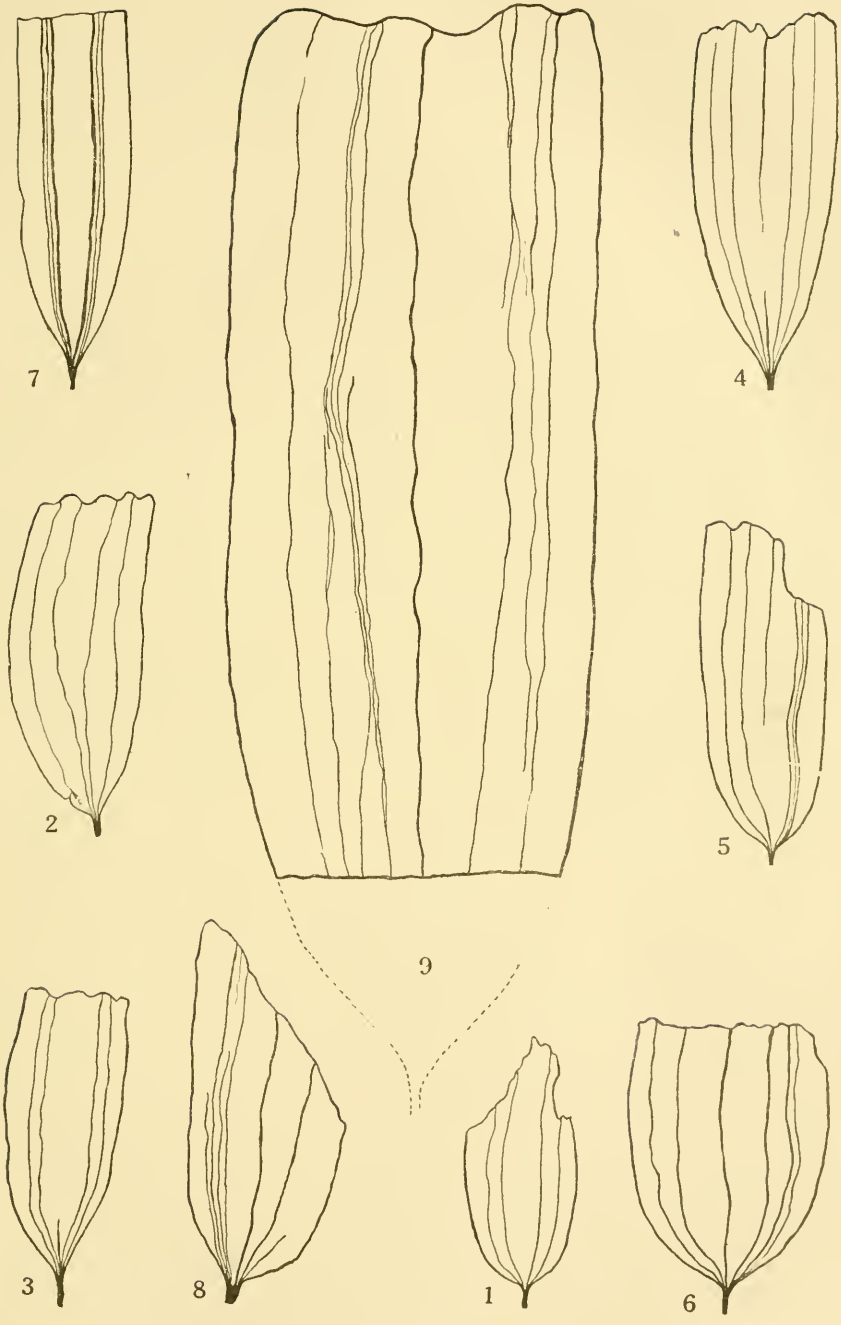

PLATE *53*

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Taxonomy and Morphology of the Ligulate Species of the Genus Desmarestia*

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Contents

1. Introduction

In a preliminary paper on the north Pacific coast species of Desmarestia, the writer (Pease, 1917) discussed briefly the principal characters of the genus, and described five species which she had collected in the San Juan Islands. The paper proposed two or three changes in classification, eliminated one species, and inserted *D. tabacoides* Okamura, which had previously been reported only from Japan.

The present paper deals with the taxonomy and morphology of the ligulate species of the genus. Reproducing material of these species has not been collected in the San Juan Islands, and the writer is not yet ready to discuss the functions of the various tissues.

* A thesis submitted to the Faculty of the Graduate School of the University of Minnesota, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1919.

2. Taxonomy

a. General

The genus Desmarestia was established by Lamouroux in 1813, in his masterly "Essai sur les Genres de la Famille des Thalassiophytes non articulées", and as outlined by him consisted of four species, *D. aculeata* (L.), *D. viridis* (Muell.), *D. ligulata* (Lightf.) and *D. herbacea* (Turn.). Agardh (1824), the celebrated Swedish algologist, adopting a different, but no less scholarly system of classification, grouped the four species which Lamouroux had placed by themselves, with several others under the genus Sporochnus. Agardh sub-divided his genus Sporochnus into two groups according to external appearance, the filiform and the plane, putting Lamouroux's *D. aculeata* and *D. viridis* in the filiform group, and *D. ligulata* and *D. herbacea* as the only members of the plane group. Postels and Ruprecht (1840) classified the filiform species as Desmarestia and the plane species as Desmia, the latter a genus name proposed by Lyngbye (1819) to take the place of Lamouroux's Desmarestia, but severely criticised by Greville (1830). Subsequent writers have seen fit to retain Lamouroux's genus Desmarestia with Agardh's sub-divisions.

Naturally with increased facilities for exploration and collection, and for study, numerous additions have been made to both groups of the genus. The present paper, dealing with the ligulate species which occur on the north Pacific coast of America, includes four species: *D. ligulata* (Lightf.) Lamour., *D. herbacea* (Turn.) Lamour., *D. latissima* Setch. and Gard. in litt. n. sp., and *D. foliacea* n. sp.

b. Desmarestia ligulata (Lightfoot) Lamouroux

(Plate 54, Figs. 1, 2; Plate 62; Figs. 1-7; Plate 63.)

Fucus ligulatus	Lightfoot	Flora Scotica 946.	1777
	Smith	English Botany, t. 1636	1790
	Goodenough	Linn. Trans. 3: 123.	1797
Fucus herbaceus	Hudson	Flora Anglica p. 582.	1798
Fucus ligulatus	Lightfoot	Nereis Britannica. (App.).	1801
Fucus herbaceus	Esper	Icones Fucorum 7:118	1808
	Turner	Hist. Fuc. 2:74. pl. 98	1809
Laminaria ligulata	Hooker	Flora Scotia, Part 2. 99.	1821
Sporochnus ligulatus	Agardh	Sp. Alg. 1:158.	1821
		Syst. Alg. 261	1824
	Greville	Flora Edinb. 287.	1824
	Sprengel	Syst. Veg. 4:330.	1827

Desmia ligulata	Lyngbye	Hyd. Dan. 33. pl. 7	1819
	Postels and Ruprecht	Illust. Alg. 13.	1840
Desmarestia ligulata	Lamouroux	Ess. 25.	1813
	Greville	Alg. Brit. 37 pl. 5.	1830
	Kützing	Phyc. Gen. 343.	1843
	Agardh, J.G.	Sp. Gen. Ord. Alg. 1.2.1. 165.	1843
	Harvey	Phyc. Brit. pl. 115.	1846
	Hauck et Richter	Phyc, Univ, 420.	1886
	De Toni	Syl. Alg. 3:460.	1895

Desmarestia ligulata (Lightf.) Lamour. was first described by Lightfoot (1777) in the Flora Scotica as follows: "Fronde plana, avenia, subtriplicato-pinnata: ramis ramulisque distischis; foliis lineari-lanceolatis, spinoso-dentatis." Turner (1809) describes the type species as "frond cartilaginous, flat, almost nerveless, doubly pinnate; segments linear-lanceolate, serrated at their margins with spinous teeth." He also describes two forms: "β angustior has its frond trebly pinnated, and nowhere above two lines wide; the teeth of its ramuli are so small and obsolete as to be scarcely observable without a glass, whence the whole plant has a naked appearance.' "Y dilatatus, which is a native of more southern seas, is distinguished from the specimens of our coasts by the width of its branches, extending to four lines or more: its ramuli are nearly elliptical, and remarkably attenuate at their base, so that they seem to be supported upon very short petioli."

These three forms have not been kept separate, the difference in growth habit being accounted for, no doubt, by difference in age or vigor of individual specimens.

Turner's description, which is the most complete of those of the early writers, calls attention to the irregular form of the holdfast; the almost cylindrical base of the stipe; the narrow, flattened thallus, with branches of various lengths, "the lowest generally longest, the upper ones regularly shortest, but long and short mostly mixed together, all opposite and undivided, but pinnated with others still smaller, arranged in the same manner, and these occasionally with a third series....." These branches are "attenuated at their base and apices" and "separated by intervals of two or three lines." This species was known to all the early European collectors, but was first collected on American shores on the coast of British Columbia by Lyall, and identified by Harvey (1862). Harvey, however, considered that this and the following species were identical, remarking that "some of the specimens are of ordinary breadth; others are of the widest variety constituting the *D. herbacea* of authors." Setchell and Gardner (1903) quote Harvey, and remark that very few, if any, of the

plants which they have examined correspond to the slender form from European waters.

This narrow form, corresponding to the descriptions of the European type, has been collected for years by members of the Puget Sound Biological Station, on the rocky shores off Kanaka Bay, on the seaward side of San Juan Island. The writer has examined specimens in the Station herbarium and in private collections, and in the summer of 1918 was fortunate enough to secure an abundance of excellent material. The plants were growing on the most exposed point of a steep rocky headland, where strong tidal currents continually sweep along shore, and the heavy surf or ground swell from the open Strait of Juan de Fuca continually breaks on the rocks. Collections were made by hand, with the greatest difficulty, at extreme low tide, as the plants were anchored to the perpendicular faces of great rocky ledges where they were continually submerged, even during the lowest tides.

Early English writers describe the species as varying from two to six feet in length, about 60 cm. to 2 m., but the writer found no specimens exceeding 60 cm. However, the main axis and all the longer branches were, without exception, frayed at the ends, due no doubt to the beating of the plants against the rocks by waves and currents.

The holdfast is not smoothly conical, as in other species examined, but roughly lobed, not only around the circumference, but also over the upper surface, and is much larger than in other species. From about the center of this rough, irregular holdfast arises a single frond with stipe almost cylindrical at the base, but widening immediately, or within 2 or 3 cm. at the most, into a flattened thallus. The main axis reaches an extreme width, in the fresh plant, of 6-8 mm., and is closely and regularly pinnated, at distances varying from 2-8 mm., with opposite distichous branches, which vary extremely in length. Short branches alternate with long without definite sequence, although in general the larger branches are toward the base. Usually both branches of a pair reach about the same stage of development, barring accident, indicating some correlation in growth. The shortest branches are simple, from a few millimeters to one or two centimeters in length, dentate along their margins and comparatively few. From these simple structures up to the longest branches, which may be 30 to 50 centimeters long, and several times pinnate, all degrees of development are found. Branches of all orders narrow down to a slender cylindrical base, and taper to a long point at the extremity. The margins of all the pinnules are sharply and finely dentate, the teeth bearing, early in the spring, tufts of slender branching filaments which are shed later in the season. Toward the base of the frond the midrib is very prominent. In fresh or formalin material it stands out as a distinct

thickening throughout almost the entire length of the main axis and the larger branches and is visible to the unaided eye as a definite ridge on the surface of branches of the third and fourth orders which are only a centimeter long. In herbarium material it is much less evident.

Reproduction has not been observed, but probably takes place as Kuckuck (1894) and Rosenvinge (1894) have reported for *D. aculeata*, and Skottsberg (1907) for *D. firma*.

c. *Desmarestia herbacea* (Turner) Lamouroux
(Plate 54, Fig. 3; Plate 60, Figs. 1-4.)

Fucus herbaceus	Turner	Hist. Fuc. 2:78. pl. 99.	1809
	Mertens	Linnaea. 4:62.	1829
Desmarestia herbacea	Lamouroux	Ess. 25.	1813
	Greville	Alg. Brit. syn. 40	1830
	Kützing	Phyc. Gen. 343	1843
	Grünow	Novara 51	1867
	Skottsberg	Antarkt. Meeresalg. 23.	1907
	Pease	P. S. M. S. Publ. 1:388.	1917
Sporochnus herbaceus	Agardh	Syst. Alg. 261.	1824
	Sprengel	Syst. Veg. 4:329	1827
Desmia herbacea	Postels and Ruprecht	Illust. Alg. 13.	1840
	Reinsch	Flora. n.r. 46:189.	1888
Desmarestia ligulata var. herbacea	Agardh,J. G.	Sp.Gen.Ord.Alg. 1.2.1. 169.	1848-51
	De Toni	Syl. Alg. 3:460.	1895
	Set. & Gard.	U. Cal. Pub. Bot. 1:247.	1903
	C., H. & Set.	P.B.-A. Fasc.D. 79 a. and b.	1905

This beautiful species, brought from the northwest coast of North America by Menzies, was first published by Turner (1809) under the name *Fucus herbaceus,* with the following description: "frond membranacea, plana, obsolete costa, bipinnata; segmentis oppositis, ellipticis, base attenuatis, apice obtusis; margine spinoso dentatis."

The writer can formulate no better description of the growth habit of the plant than given by Turner, which is quoted in full. "Root, a small callous disk. Frond flat, two feet or more long, rising with a single individual stem, at its base nearly cylindrical, and as thick as a crow's quill, but almost immediately becoming flat, and gradually widening to the height of a few inches, where it acquires a width of half an inch, or three quarters of an inch, after which it continues linear till, on approaching the extremity, it is again slightly narrowed and terminates in a rounded apex;

the margins are throughout the whole length serrated with small spiniform, rather remote teeth; the stem, from root to summit, is pinnated with opposite distichous branches, of the same substance as itself, between horizontal and patent, separated by intervals of about half an inch, a foot or a foot and a half long, and the middle ones apparently longest, their greatest width nearly an inch, attenuated at their bases into very short subcylindrical petioli, rounded at their apices, toothed at their margins, and in their turns pinnated with a series of others, similar to them in every particular, except their small size; throughout the whole frond runs a midrib thick and rather wide in the stem, but in the branches thin and faint, so as scarcely to be visible, unless the plant is held to the light, and appearing only like a dark line."

He compares this new species with *D. ligulata,* setting forth very clearly his reasons for considering it a distinct species. Postels and Ruprecht (1840) also discuss the specific differences. J. G. Agardh, (1848), however, placed *D. herbacea* as a variety under *D. ligulata* and was followed by other systematists, including De Toni (1895) and Setchell (1903). Harvey (1858 and 1862) went so far as to consider *D. herbacea* merely a broad form of *D. ligulata.* Grünow (1867) again raised the question of specific difference, supported by Skottsberg (1907); and the writer (Pease 1917), in a preliminary paper, published *D. herbacea* as a distinct species. In this discussion, however, all of the forms too broad to be included with the typical *D. ligulata* were considered as belonging to the species *D. herbacea* (Turn.) Lamour. In the present paper the writer wishes to correct that statement, limiting the species *D. herbacea* to those forms which correspond exactly with Turner's original description, and setting apart the larger, broader forms in a group by themselves.

As thus limited, the specimens distributed by Collins, Holden and Setchell (1905), P.B.-A. 79 a. and b., under *Desmarestia ligulata* f. *herbacea,* would belong here.

During the summer of 1918 an abundance of material was collected, which corresponds remarkably with Turner's description and plate. These plants were collected in the same locality and at the same time as the specimens of *D. ligulata.* No intermediate forms were found. The two species were absolutely distinct. A glance at the mass of fronds as they lay floating in the water was sufficient to identify the plant. In color, in width of frond, and in texture, there was no possible room for doubt. The same outstanding differences are manifest in formalin material and herbarium specimens. Anyone who has seen the two plants together could not possibly doubt their identity as distinct species. The two plants are of about the same size. The writer has not collected a specimen of either species which had attained a meter in length. *D. ligulata* is darker in

color, with a greenish tone; has a narrow thallus, not exceeding 6-8 mm. in the widest parts; and has a firmer, heavier texture, with the midrib much more prominent in the older basal portion of the plant. *D. herbacea* is lighter in color, a yellowish brown, with a comparatively broad thallus, 10 to 30 mm. wide; and a much thinner, more delicate texture.

d. Desmarestia latissima Setchell and Gardner in Litt. n. sp.

(Plates 55, 56, 57; Plate 58, Figs. 1-4; Plate 60, Figs. 5-9; Plate 62, Figs. 8-10.)

Fronde magna, foliacea, latissima, inferne sub-coriacea et evidenter costata; pinnis distantibus, margine dentis distantibus.

Plant body very large, length up to 8 m.; frond membranaceous, subcoriaceous at base, foliaceous, very wide, diam. 4-100 cm.; with an evident midrib and lateral veins; occasionally bipinnate; pinnae and pinnules widely separated, either blunt or pointed, with margins coarsely spinulose-serrate; veins branching from the midrib, giving off secondary branches which break up into fine veinlets and form a network in the broad thin tissue of the lamina, visible to the eye in both fresh and dry material as faint branching lines.

The species name is credited to Drs. W. A. Setchell and N. L. Gardner of the University of California, who were kind enough to furnish the writer with a brief outline of their provisional arrangement of the Pacific coast species of the genus Desmarestia. Type specimens are deposited in the Alga Herbarium of the University of Minnesota.

The writer believes that this species should be set off from *D. herbacea* on account of its extreme size and the heavier, coarser texture of the thallus; also because it has fewer branches, more widely separated, and only in rare instances do the lateral branches bear branches of the second order.

The species, as designated, includes a wide range of forms, and further study may make it necessary to subdivide still more or at least to segregate one or two varieties, but up to the present time a sufficient number of examples have not been examined and compared so that definite limits can be set, and it is not the writer's intention to split up the genus unnecessarily.

In a series of 25 young plants collected by the writer the length of the main axis varies from 1 to 50 cm. and the width from 0.2 to 13 cm. While in general an increase in width accompanies one in length, the two are not proportional. In these young plants the number of pairs of lateral branches varies from 2 to 14, without any apparent relation to the length or width of the main axis from which they arise. These lateral branches vary in length from 0.5 to 22.5 cm., and in width from 0.2 to 8 cm.

In a series of 58 mature specimens the variation in length of main axis is from 23 cm. to 8 m., and in width from 4 to 40.5 cm. Increase in length seems to have no relation to increase in width. These mature plants bear from 3 to 24 pairs of lateral branches, the number of pairs seemingly having no relation to the length or width of the main axis. The lateral branches vary in length from 21 cm. to 2.5 m., and in width from 3.5 to 23 cm. Table 1 gives some idea of the variation among mature plants. It is evident that it would be exceedingly difficult to find a point where a division could be made on the basis of width of frond.

The specimens of *Desmarestia ligulata* (Lightf.) Lam. var *herbacea* (Turn.) J. Ag., distributed by Tilden (1898), belong here. She includes part of a young plant of the narrow form, and also one of medium width.

Table 1. *Desmarestia latissima, mature, with holdfasts, tabulated according to length of main axis.*

	MAIN AXIS		LATERAL BRANCHES		
	length cm.	width cm.	No. of pairs	length cm.	cm. width
1.	55.+	10.	6	69.	15.
2.	78.+	5.5	21	54.+	13.
3.	100.+	10.	12	24.	5.
4.	127.+	6.	18	58.	8.
5.	137.+	13.	17	48.	9.5
6.	165.+	5.5	—	—	—
7.	182.+	6.5	11	24.	3.5
8.	188.+	12.	12	117.+	16.
9.	195.+	10.	12	50.	8.
10.	200.+	16.	3	14.	5.5
11.	206.+	10.	14	50.5	7.7
12.	228.+	14.	10	154.	15.
13.	248.+	29.	11	255.	14.5
14.	295.+	9.5	13	77.	7.3
15.	303.+	10.	17	63.	9.
16.	319+	11.	14	41.	6.
17.	344.+	10.5	15	91.+	9.5
18.	383.+	17.	12	121.	12.
19.	448.+	13.5	16	95.	11.
20	800.+	17.	—	136.	18.

Superficially, this species is distinguished from *D. herbacea,* which it most resembles, by its extreme size. Specimens of typical *D. herbacea* have not been collected in the San Juan Islands with fronds longer than one meter, nor wider than 3 cm., and all the specimens collected were of about the same size and general appearance. Specimens of *D. latissima* with fronds up to 8 meters in length and 40 cm. in width were collected by the writer during the summer of 1918; several specimens were found in the summer of 1916 from 40 to 60 cm. in width, and during that same summer Muenscher (1917) collected one specimen 1 m. wide. Setchell and Gardner (1903) mention plants of *D. ligulata* f. *herbacea* "cast ashore at Esquimalt, B. C., in fragments several meters long and full 30 cm. wide." Rigg (1912) says it "sometimes reaches a length of 8 to 10 feet and a width of from 12 to 16 inches (about 3 m. long and 30 to 40 cm. wide—*Author*), although it is usually much smaller." The figures given apply to the main axis of the plant. This axis bears opposite lateral branches which may attain a length of 2.5 m. and a width of over 20 cm.

The lateral branches begin to appear quite close to the holdfast, are at first small and rather crowded, but soon become farther apart and much longer. Well developed branches are usually separated by from one to three pairs of spines, which are merely abortive branches. Practically all of the lateral branches are borne within one meter of the holdfast, plants 3 and 4 meters long having no more branches than those from a meter to a meter and a half in length (table 1). The same thing is true also of young plants, those with a main axis measuring less than 10 cm. bearing as many branches as plants four times as long. Also the main axis usually attains its maximum width within one meter of the holdfast. The occurrence of wider intervals between the upper branches is due to two factors: (1). A larger number of abortive branches between the long branches; (2). Greater elongation of the thallus of the main axis between the branches of the midrib, which form the midribs of the lateral branches.

Abortive branches vary from short sharp spines to flattened outgrowths 3 to 4 cm. long and several mm. wide, which give no external evidence of venation, and do not bear spines along their margins. Normal branches, especially on the narrower forms, are sometimes no larger, but have a definite midrib with visible lateral branches which run out to spines along the margins.

An occasional plant was found in which one or two of the lower pairs of lateral branches were themselves branched, but this is not common and seems to take place when the main axis has been broken off rather close to the holdfast, a phenomenon which reminds one forcibly of the growth habits of higher plants.

In common with other ligulate species, a distinct midrib runs the length of the main axis and gives off at regular intervals opposite branches which extend to the edge of the frond and end in the spine-like teeth, or passing through the attenuate bases of the lateral branches, become the midribs of these structures, giving off in turn pairs of opposite branches which extend to the margins. The midrib is much thickened in the basal portion of the thallus. It also forms a ridge that can be felt with the finger in the basal portion of the long branches.

The one outstanding difference between this species and *D. herbacea,* aside from the extreme difference in size, lies in the system of fine veins which ramify through the laminae of the main axis and the lateral branches. These veins do not originate from the midrib, but are given off as secondary outgrowths from its pinnate, lateral branches. They are given off singly, at irregular intervals, both above and below, branch repeatedly, and form a loose, irregular network in the lamina. The whole effect reminds one strongly of the venation of a long narrow pinnately-veined leaf. Kützing (1858) indicates this method of venation in his plate of *D. herbacea* f. *latior.*

Young plants ranging in length from 10 mm. to 50 cm. seem to show that relative width of frond is determined from the beginning. Some are relatively wide, with long wide branches, the branches equaling or exceeding the main axis, while others are relatively narrow, with short, narrow branches. These represent the extremes of the type however. In both young and mature plants a fairly complete series has been secured, and there seems to be no break in the progressive increase in width of the thallus which would form a basis for dividing the species into two, or even for segregating a form or variety. A factor which complicates the situation is that entire plants have not been found. Even in the smallest specimens secured, the tipe of the long strap-like axis is worn away, so that the real length of the plant in proportion to its width can not be ascertained.

e. Desmarestia foliacea n. sp.

(Plate 58, Figs., 5-10; Plate 61, Figs. 1-5.)

Fronde latissima, ligulata, simplici, tenui stipitata, tenuissime membranacea, margine irregulariter sinuata dentataque, dentibus distantibus, costa media tenuissima et ramosa opposite percursa, ramis se ramulosis, omnibus patentissimis, subtilissimis.

Plant body comparatively small, up to 1 m. long; frond thin, membranaceous, wide, strap-like, diameter to 15 cm.; unbranched; margins irregularly sinuate with occasional obscure teeth; midrib evident, with

opposite branches which break up into fine veinlets. The species name is given on account of the resemblance of the simple frond to a long narrow pinnately veined leaf.

Type specimens are deposited in the Alga Herbarium of the University of Minnesota.

In a preliminary paper the writer (Pease, 1917) described and figured a series of plants collected during the summer of 1916, which she referred to *D. tabacoides,* first described by Okamura (1908) from material collected in southern Japan. Okamura describes his new species thus: "Fronds very large, leaf-like, shortly stipitate, with broadly oval, very usually obliquely lobed, simple, midribbed, and coriaceous lamina. The midrib is slightly prominent below, but gradually becoming fainter upwards, with opposite veins which dissolve, even from the base, into numerous fine veinlets. . . . . The outline of lamina is of large oval shape. . . . . with the length of 30-70 cm. and breadth of 20-50 cm." The description is accompanied by an excellent plate. Since publishing the article referred to, the writer has had the privilege, through the kindness of Dr. W. A. Setchell of the University of California, of examining an authentic herbarium specimen of Okamura's plant. She detects the following differences: 1. The oblique lobing which Okamura mentions in his description. Plants collected on the American coast have sinuate margins with obscure teeth rather widely separated, while the Japanese specimens were "very usually obliquely lobed." 2. The opposite veins, "which dissolve even from the base, into numerous fine veinlets." These are close together, and all their branches come off at very acute angles, corresponding to the "erect branching" of some of the filiform species, and take the same general direction toward the margin of the frond; while in American material the secondary branches are given off more nearly at right angles and have a more definitely reticulate appearance. The distance between the pairs of opposite veins seems to vary with the size, and hence the age, of the specimen. 3. The stipe seems to be much more slender in American specimens. 4. The frond, which in Japanese material is of a large oval shape and reaches a breadth of 20-50 cm., is long and strap-like in American specimens, and not over 15 cm. wide, so far as known.

The description of *D. pinnatinervia* given by De Toni (1895) applied so well to both Japanese and American specimens that the earlier literature was consulted. *D. pinnatinervia* was first described by Montagne (1842) with the comment, "a single specimen collected in 1823 on the coast of Spain, in the harbor of San Sebastian and for a long time considered as a Laminaria." He describes the new species as follows: "Frond stipitata tenuissime membranacea margine denticulis distantibus instructa nervo

pertenui longitudinali aliisque transversis oppositis parallelis percursa." The characteristics which finally decided him to remove the plant from the genus Laminaria and consider it as the type specimen of a new species of Desmarestia were the presence of: 1, a definite stipe; 2, a median vein which extends throughout the length of the frond and gives off opposite lateral branches. The colored plate which accompanies the description shows the same general growth habit as the Japanese and American specimens, i. e., a broad, flat frond, unbranched, with a median vein which gives off opposite lateral branches, the latter branching again. It does not, however, give the details of the venation, only one secondary branch from each lateral vein being shown.

Agardh (1848), who according to Montagne, had examined the original specimen, lists it under "Species inquirendae" with the remark, "Videtur pars inferior frondis latioris Desmarestiae ligulatae." His description, "Fronde latissima plana costate simplice, margine edentato serrata, in stipitem attenuata," varies somewhat from Mantagne's and may have been either from the specimen itself or from Mantagne's plate.

Kützing (1849) must have written his description from the plate, as the phrase "costis apice bifurcatis" would indicate, the plate showing only one secondary branch from each lateral vein and no finer subdivisions. De Toni (1895) quotes Kützing's description, adding Agardh's comment concerning its resemblance to "*D. ligulata* var. *firma*."

Johnstone and Croall (1859) close their discussion of *D. ligulata* by saying, "Under the present species we would place the form *D. pinnatinervia*, our impression being that they are identical; although at first sight the great breath of the frond, and the very narrow and distinct pinnated nerve, would indicate a distinct species." Although claiming to have specimens collected in the north of Ireland, they give no plate, and the species is not mentioned by other writers in lists of British marine algae.

It would seem then, that all authentic published accounts of this species are based on Montagne's and Agardh's opinions of a single specimen, Kützing apparently having written his description from Montagne's plate and De Toni having quoted Kützing and Agardh.

Until specimens of the three can be placed side by side for comparison, the most that can be said is that the Japanese, the American, and the Spanish plants resemble each other very closely, and if not three very closely related species, are three forms of the same species.

The ligulate species of the genus Desmarestia should be further subdivided, then, into two groups, one to include the branched forms, the other the simple forms. It was this unbranched condition of the frond which caused Montagne to hesitate for nearly 20 years before finally classifying

his plant as a Desmarestia, and then he did so with the comment: "Si c' est un Desmarestia, comme tout le fait croire, on peut considerer la fronde entière comme formée par la soudure des pinnules opposées que representent les nervures."

A few specimens of the proposed new species, *D. foliacea,* were secured during the summer of 1916, and referred at the time to *D. tabacoides* Okamura. In the summer of 1918, while collecting over the same ground, an abundance of material was secured in all stages, from plants 2 or 3 cm. long up to those about a meter in length.

In very young plants, the lateral veins can be traced unbranched to the margin of the frond, and a few of these young plants have been collected which still show the protruding stubs where the assimilating hairs have broken off. In older specimens, however, the margins are sinuate, with obscure teeth rather widely separated, and the lateral veins which branch out from the midrib do not appear to extend to the edge of the lamina, as in other ligulate species, but break up into a fine branching network. The lateral veins stand almost at right angles with the midrib, and their branches, in turn, are almost at right angles. These secondary branches subdivide again and again, the finer branching being followed easily with a hand lens. This visible network of fine veinlets in the lamina relates the species closely to *D. latissima.* In fact, except for its extreme thinness, a plant of *D. foliacea* bears a remarkably close resemblance to a lateral branch of *D. latissima.*

The writer has not seen reproducing material. Okamura (1908), in his discussion of *D. tabacoides,* describes and figures plurilocular sporangia intermingled with sterile hairs, in sori forming irregularly roundish patches on both surfaces of the lamina. To the writer these bear a close resemblance to a species of Phycocelis which she has found growing on the fronds of several species of Desmarestia (Plate 60, Figs. 1, 2, 3). In the light of work done by Rosenvinge (1894), Kuckuck (1894) and Skottsberg (1907), on reproduction in various species of Desmarestia, it is highly probable that Okamura's interpretation is not correct.

f. Key to Species

In accordance with the ideas expressed in the preceding pages, the author wishes to present the following scheme of classification, which affects only the ligulate species of the genus Desmarestia which appear in the Puget Sound region, with the exception of *D. pinnatinervia* Mont. and *D. tabacoides* Okamura, which are introduced for the purpose of comparison.

A. Frond much flattened, blade-like; midrib evident (ligulate species).
  B. Frond unbranched.
    C. Stipe short (4-5 mm.), heavy; secondary veins not pronounced ..... *D. pinnatinervia* Mont.
    CC. Stipe long (10-20 mm.), heavy; secondary veins pronounced, appressed ..... *D. tabacoides* Okam.
    CCC. Stipe long (8-16 mm.), slender; secondary veins pronounced, at about 90 degrees ..... *D. foliacea* n. sp.
  BB. Frond branched.
    D. Branches of first and occasionally second order; plant body large, with main axis up to 1 m. wide, 8 m. long. *D. latissima* Setch. and Gard. in litt. n. sp.
    DD. Branches of third and fourth orders; plant body small.
      E. Fronds up to 8 mm. wide. *D. ligulata* (Lightf.) Lamour.
      EE. Fronds 10-30 mm. wide. *D. herbacea* (Turn.) Lamour.

AA. Frond terete or compressed, not flattened; midrib not evident. (Filiform species).

3. Morphology

a. Historical

There are two types of thallus in the genus, represented by *Desmarestia aculeata* and *D. ligulata*. In the *D. aculeata*, or filiform type, the main axis and all its branches, to the last order, are slender and terete, or only slightly compressed, with the axial filament and its surrounding tissues, the so-called midrib, not at all evident from the surface. In the *D. ligulata*, or ligulate type, the main axis and all its branches are much flattened, with a very evident midrib. The latter type, the ligulate group, represented on the Pacific coast according to the opinion of the writer by four species, will be considered in the following discussion.

The general growth habit of all species studied is the same, and has already been worked out by various writers. The plan of the present writer has been to gather together from many scattered sources and to summarize the work already done and then to add the results of her own observations.

The plant is attached to its substratum, which may be a rock, a shell, or the holdfast or stipe of another alga, by means of a simple disk-shaped or flattened-conical holdfast. From the center of the holdfast arises a single round or slightly compressed axis which begins to widen out within 1 or 2 cm. into the ligulate thallus. The main axis may remain unbranched, as in *D. foliacea*, or may bear branches to the fourth order, as in *D. ligulata*. Lateral branches are opposite, distichous, patent to erect, with

serrate margins, and begin to appear not more than 2 or 3 cm. above the holdfast.

The mature parts of the frond are differentiated into several tissue systems. The main axis, together with all its branches of all orders, is traversed by a single row of cells, the axial filament or rachis, surrounded by an inner assimilation tissue of small cells, the whole forming the so-called "midrib." Surrounding this, and forming the main substance of the broader parts of the thallus, is a ground tissue consisting of very large cells, among which ramify slender filaments containing scattered chromatophores. The whole plant body is covered by a cortical layer from one to three cells thick.

The entire young plant and the younger parts of old plants are clothed during the active growing season in early spring, with opposite, distichous rows of monosiphonous branching filaments, which give the plant a most beautiful feathery appearance. The cells of the filaments are crowded with chromatophores and according to Söderström (1889) can be considered as assimilatory organs which have an important bearing on the rapid development of the young twigs.

According to Janczwski (1875), lateral and terminal filaments are the same in structure and can be distinguished only by their position. They are either simple or branched and the main filament and its branches develop basipetally, the outermost cells being the oldest and in his opinion the most vigorous. On account of the fact that one finds younger, shorter branches among the older ones, Söderström reasons that the cells of the rachis divide transversely and the new cells thus formed also send out branches. The branches originate as opposite outgrowths from the upper part of cells of the rachis. These become cut off from the parent cell by a cross wall, elongate, divide transversely, and increase in length by repeated transverse divisions of the basal cell formed by their first transverse division.

Toward the end of the growing season the hairs break off just above the growing region and are shed bodily. Their bases remain as small thorn-like projections along the sides of the thallus twigs, gradually become covered by the growth of the cortical cells, and persist in the form of serrations or small thorn-like "short branches."

The growing region, as in many other Phaeophyceae, is intercalary, lying between the thallus and the hair-like filaments, and giving off cells to each. According to Jönsson (1901) the first two or three cell divisions in the growing region are necessarily apical, but by subsequent divisions of the inner cells the growing region becomes intercalary. The earliest stages of development have never been observed, but the development of the various tissues can be followed in the growing tips of the fronds.

The newest part of the thallus is a single row of articulate cells, lying just below the growing region, which bears opposite distichous branches at frequent intervals. According to Söderström, each original cell of the monosiphonous thallus below the growing region bears opposite branches, which must therefore stand very close together. This is not the case for long, however, as observation will show that at a short distance below the growing region the segments bearing branches are separated by a distance the length of three cells, showing that the original segment cell, through successive divisions, has given rise to four cells. Söderström says that no further division of the segment cells takes place, only an elongation to keep up with the growth of the thallus, as repeated observation shows branches arising from every fourth cell.

According to observations made by Reinke (1880) and confirmed by Söderström, the cortical cells arise as outgrowths from the axils of opposite lateral branches of the monosiphonous thallus at some distance below the growing region. By repeated transverse divisions these outgrowths, instead of developing as hair branches, form filaments which are applied to the axial cell. These filaments, encircling the axial cell, meet and fuse, and by repeated divisions form a layer of small cells surrounding the original single cell row of the thallus. To the writer it would seem that the idea expressed by Jönsson is more nearly correct, namely, that from the bases of opposite lateral hairs cells are cut off which, by repeated divisions, form a girdle about the central cell. Repeated examination of the growing tips of three different species, both in cross section and from the surface, gave no indication of the development of filaments which were applied to the axial cell.

When the axial cell has become completely enclosed, the cells of the investing layer, or cortex, divide tangentially, forming a surrounding tissue two cells thick. The inner layer, immediately surrounding the central axis, is the beginning of the ground tissue, and from it, later, through secondary growth, develops the "inner assimilation tissue." The outer layer remains permanently meristematic, cutting off by repeated tangential divisions cells which are added to the ground tissue, and dividing radially to accommodate the increasing size of the thallus. According to Jönsson the cells of the ground tissue never increase their number by division, the increase is always by addition from the outer meristematic layer. The cells of the ground tissue become very greatly enlarged in length and diameter, are smaller at the extremities, with their ends lapping past each other, and in older parts of the frond the walls are considerably thickened.

According to Harvey (1858), the manner of growth in *D. ligulata,* which is typical of the ligulate forms, is precisely similar to that in the filiform species, "except that the new cellular integument to the primary

filament is not developed equally on all sides, but extends chiefly laterally, so as to form first a two-edged and then a flat or even leaf-like stem. In this process of lateral extension, or widening of the stem, the lower portions of the pinnae of the primary filament, being enclosed within the cellular wings of the flattened branch, become the lateral nerves of the frond."

The outer cells remain small and are crowded with small lens-shaped chromatophores, thus forming the principal assimilating region of the plant. The cells immediately within the outer cortical layer are somewhat larger, the chromatophores are less crowded, and the protoplast becomes peripheral, having within it a single large vacuole in which the nucleus is suspended by slender strands of cytoplasm. The cells rapidly increase in size toward the center and the chromatophores rapidly decrease in number and finally disappear, though the large cells of the ground tissue remain nucleated after all trace of chromatophores has vanished. Thus it can be seen that in the primary tissues only the three or four outermost layers of cells of the thallus are engaged to any appreciable degree in photosynthetic work.

At the end of the period of elongation, then, which occurs early in spring, the newly formed thallus consists of three primary tissue regions: (1) The axial row of cells formed from the primary growing region, which bears regular opposite distichous branches; (2) The cortical region, consisting of two or three layers of small isodiametric cells densely filled with chromatophores. The outermost layer remains permanently meristematic, cutting off cells laterally to accommodate the increasing width and length of the thallus, and dividing parallel to the surface to add to its thickness. The inner cortical cells enlarge to form (3) the ground tissue, composed of greatly enlarged cells with bluntly tapered ends in which the protoplasm is distributed as a very thin peripheral layer within which a few scattered chromatophores may be imbedded.

At about the close of the growing period, which is marked externally by the shedding of the filamentous hairs and the complete cortication of the thallus, the formation of secondary tissues begins. This includes the "inner assimilation tissue" and the "conducting hyphae" which ramify through the intercellular spaces of the ground tissue. The tissue of small cells which completely surrounds the original thallus cells of the axial row, called by Söderström the inner assimilation system, is described by him as consisting of several layers of extremely small, thin-walled, endochrome-containing cells which originate by repeated oblique divisions in all directions of the cells of the ground tissue which lie next to the central cell. Söderström is at a loss to explain the presence of chromatophores deep within the thallus. But Wille (1895), under whose direc-

tion he carried on this work, explains in a later article "the peculiar appearance of an inner assimilation tissue," assuming that lively respiration goes on in the large protoplasm-filled cells of the central cylinder, and that the CO_2 formed in this way is taken up immediately in sunlight by the inner assimilation system, while the outer assimilation system makes use of the CO_2 absorbed from the surrounding water. This arrangement he considers necessary because of a lack of either stomata or intercellular spaces, as in higher plants, to facilitate gas exchange.

Söderström also described and figured cross sections of the thallus from near the holdfast, where "certain cells of the ground tissue" had also become surrounded by a system of very small thin-walled cells which had the same appearance as those of the inner assimilation system. Jönsson, by means of diagrammatic longitudinal sections, proves that the central cell of that group does not belong to the ground tissue, but is formed by secondary branching of the lateral branches of the central axis; these secondary branches, arising from the lower side of the lateral branches, grow downward toward the base of the plant between the cells of the ground tissue, and become surrounded, like the cells from which they originate, by a tissue of small assimilation cells.

Jönsson calls Söderström's inner assimilation tissue a "secondary assimilation tissue" and describes a "tertiary assimilation tissue" in the older parts of the thallus. This is formed by outgrowths from the secondary assimilation system which pierce the walls of the central cylinder cells and form within the cell cavities a dense mass of hypha-like growths. These divide by cross walls to form small, closely packed cells which contain many chromatophores and therefore aid in assimilation. If Wille's assumption as to the necessity for the secondary assimilation system be correct, one wonders how the formation of this tertiary system can be explained on the basis of function?

The conducting hyphae, as described by Söderström, also originate from the cells of the ground tissue. They seldom arise in young parts of the thallus, but develop gradually, with the growth of the plant. In older parts they are found penetrating everywhere between the cells of the ground tissue, so that the latter may become widely separated. The hypha first appears as a sac-like outgrowth from the side of a cell of the ground tissue, is cut off from the parent cell by a cross wall, and elongates by terminal growth. Cross walls are formed as growth progresses. It forces its way among the cells of the ground tissue, twisting and turning, but keeping its course steadily downward toward the base of the plant. These hyphae branch repeatedly and twist and turn about themselves and about the cells of the ground tissue until it is impossible to follow a single hypha for any distance through the tangled mass. Since their growth is steadily

downward, the lower parts of the thallus are made up almost entirely of this tangled mass of hyphal threads, the original cells of the ground tissue having been pushed widely apart by them. They continue down through the base of the stipe and into the holdfast, where they spread radially, and form the main bulk of the disk. In the basal portions of the plant, where they constitute most of the substance, their walls are much thickened, according to Söderström, and their function is almost entirely mechanical.

b. Methods

The foregoing summary is intended to present in a concise form what is already known of the structure and development of the thallus in the genus Desmarestia. Practically all morphological work has been done with *D. aculeata* as the basis for study. Since this was the first member of the genus to be recognized and described by the early systematists, is the most widely distributed species, and seems to be at least fairly common throughout its entire range; it is not surprising that it has received more attention than other species of the genus, and that its structure as worked out by Kützing (1843), Janczewski (1875), Reinke (1880), and especially by Söderström (1889) and Jönsson (1901), should be considered as typical. But since the type of thallus in the two groups of the genus is so entirely different it is natural to suppose that there would be at least minor differences in the formation and development of the various tissues, if not in their structure. The writer has studied the structure of *D. aculeata* (L.) Lamour., *D. viridis* (Muell.) Lamour. and *D. media* (Ag.) Grev. among the filiform species; and *D. ligulata* (Lightf.) Lamour., *D. herbacea* (Turn.) Lamour., *D. latissima* Setchell and Gardner in lit., n. sp., and *D. foliacea* n. sp. among the ligulate species. The work done on the filiform species was for the purpose of verification and comparison, and will not be discussed in the present paper. Microphotographs of cross and longitudinal sections through the mature part of the thallus of *D. aculeata* are given (Plate 61, Figs. 6 and 7) so that the size, shape, and distribution of cells may be compared with those of the ligulate species. Reproducing material has not been collected in Puget Sound waters, and the writer will not attempt to discuss reproduction.

All material used was collected in the San Juan Islands in July 1916 and July 1918, and preserved in 2% formalin in sea-water. As wanted for study since, the material has been removed from formalin, cut into pieces of suitable size, dehydrated by running up slowly through a close series of alcohols, and imbedded in paraffin, using both xylol and cedar oil as solvents. Sections were cut from 10 to 15 micra in thickness, and stained with Bismarck brown. Most of the material was stained after sectioning, but toward the end of the work a few slides were made by

staining in bulk and afterwards sectioning and mounting. The results were very satisfactory. The material is easy to handle; any good method carefully followed brings the desired results. Drawings were made with the camera lucida or with projection apparatus.

Chlorzinciodide was used to determine the composition of the cell walls. Formalin material was rinsed in tap water and the concentrated reagent was applied directly, to flat pieces of the entire thallus, to pieces stripped of the cortical layers, and to sections. The cell walls of both primary and secondary tissues gave the characteristic violet coloration of cellulose. Both Söderström (1889) and Jönsson (1901) recognize the marked resemblance of the cells of the central cylinder to the sieve tubes of higher plants, but neither is willling to concede that they are true sieve tubes, though Jönsson figures and describes the pores in the cross walls and the protoplasmic connections extending through them. Both chlorzinciodide and Bismarck brown distinctly show the presence of callose covering the sieve plate in older parts of the frond, and in younger stages the progressive development of the callose covering is clearly shown. The callose is first formed as a ring at the outer edge, on both sides of the sieve plate, and gradually extends inward across the plate, finally forming a complete covering, through which, in some cases, the pores can be traced. The original cell row of the thallus, then, is a row of sieve tubes, as are all of its branches and their subdivisions. The system of veins which forms a delicate though clearly visible network throughout the fronds of the extremely broad species is thus a system of sieve tubes.

Desmarestia ligulata, the type species of the ligulate group, has been used as the basis for study. A detailed description of the formation and development of the thallus and its various tissues will be given, and the three remaining ligulate species, *D. herbacea, D. latissima,* and *D. foliacea,* will be compared with it.

c. Desmarestia ligulata (Lightfoot) Lamouroux

(Plate 54, Figs. 1, 2; Plate 62, Figs. 1-7; Plate 63.)

Although collected in midsummer, some of the material still bore assimilating hairs, so that all stages in the development of the thallus could be observed,—the structure of the hairs themselves, the intercalary growing region, the beginning of corticaion, the development of the axial filament; in fact, the formation and development of primary and secondary tissues from the tip of the youngest thallus branch to the base of the holdfast.

Söderström (1889) says of the hairs of *D. aculeata,* "Their cells contain much chlorophyll, they are closely and regularly branched, and up to 3 cm. long." In *D. ligulata* the hairs are very much shorter, be-

ing visible to the naked eye only by the filmy appearance which they give to the edges of the ultimate pinnules. They are set much more closely together and hence do not appear to be in tufts, as Oltmanns (1904) illustrates them for *D. aculeata* (Plate 59, Fig. 1). Many of the hairs, especially those arising near the base of the pinnule, are short and simple, and of less diameter than the more vigorous branched hairs toward and at the distal end. The main axis of the hair, formed by cells cut off form the upper side of the intercalary growing point, may consist of from 10 to 50 cells. The hair is spindle-shaped. The youngest cells, next the growing point, are about 100 micra wide and 15 or 20 micra long. The largest cells, about its middle, are 125 to 200 micra in diameter and 175 to 225 micra long. The tip ends in a small conical cell about 37 micra wide at the base and 45 to 70 micra long. The individual cells are slightly distended in the middle, and thus barrel-shaped.

In branched hairs, the branches arise as outgrowths on the sides of the cells of the main axis, which are soon cut off by cross walls. A cell thus cut off divides again and the branch elongates by repeated division, this always occurring in the innermost cell, so that the hair branch develops basipetally. These branches, like the axis from which they grow, are spindle-shaped, with a conical end cell. Although the lateral branches of the central axis below the growing point always arise in pairs, and both members of the pair persist, so that branching is always opposite, the lateral branches above the growing point quite often arise singly, and several single branch hairs in succession may be produced, either on the same or on opposite sides of the axis (Plate 62, Fig. 1). In fully grown hairs, both proximal and distal ends of the axis, which are respectively the youngest and oldest regions, are devoid of branches, while in the intermediate region branches are closely crowded. In this crowded region every cell, or every second or third cell, bears a pair of branches, or occasionally only a single branch. Usually, from 3 to 10 cells of the axis bear branches, and the first 10 to 15 cells at the distal end of the axis are devoid of branches. The branches themselves consist of from 5 to 15 cells each and do not branch again, as they sometimes do in *D. aculeata*.

The writer has examined the assimilating hairs in *D. aculeata* and *D. viridis* as well as in *D. ligulata*, and finds that the size and shape of the cells and also the arrangement of the branches is different in each species. It would be interesting to examine the assimilating hairs of other species. It may be possible that this could be made one of the distinguishing characters in separating species.

At the end of the growing season all the assimilating hairs are shed, a definite method of abscission being quite apparent. Entire pinnules were stained in Bismarck brown, cleared in cedar oil, and mounted in balsam. The tissues are so transparent that one can focus through the cortical layers

of the midrib, trace its lateral branches out to the edge of the cortex, and note their continuation, through the short cells of the intercalary growing point, into the axis of the assimilating hairs. Just at the edge of the cortex, the basal cell of the assimilating hair and the outer cell of the midrib branch round away from each other at their separating wall, making the axis thinner at this point and therefore weaker. The hair breaks off at this weak point and the cortical cells, by lateral divisions, soon cover the broken stub, leaving a blunt projection on the edge of the pinnule (Plate 62, Figs. 2 and 3).

Renewal of growth has not been observed in *D. ligulata*, but in *D. media* (Ag.) Grev., collected in the same region in April 1917, the first cell of the assimilating hair was just protruding through the cortex at the tips of many of the ultimate branches.

At the same time that the assimilating hairs are being developed the central axial row of cells of the midrib, the "original thallus" of Janczewski (1875), is being increased in length by the addition of cells from the lower side of the intercalary growing point. These cells, almost as soon as formed, send out projections on either side which are cut off by cross walls and develop into lateral branches. Branches of the axial row above the growing point may or may not be in pairs, and do not develop from every cell; but in the permanent axial row below the growing point, every cell that is cut off bears two opposite branches. The axial cell elongates below leaving the branches on its upper part, and divides by a cross partition, the branches being on the upper cell. Each of these cells elongates and again divides. Thus the pair of branches will be found on the uppermost of the series of four cells formed by two successive divisions of the original cell laid down at the growing point. Söderström (1889) states that in *D. aculeata* no further divisions take place in the cells of the axial row, every fourth axial cell throughout the thallus bearing branches. Each axial cell, therefore, must become greatly elongated to keep pace with the elongation of other tissues of the thallus. In pinnules of *D. ligulata*, well cleared and not too deeply stained, the writer has been able to trace the axial row for 3 or 4 millimeters. Branches arise from every second, third or fourth cell, but no wider intervals have been observed. The same holds true for longitudinal sections of older parts of the thallus. Each original cell does not necessarily subdivide to form four cells. For instance, in the basal region of a branch of the first order, branches have been found on every second axial cell, indicating that only one division has taken place. In other sections, every third axial cell bears branches (Plate 59, Fig. 2), indicating that the original cell and one of the daughter cells has divided. When every fourth cell bears branches, it is evident that both daughter cells of the original axial cell have again divided. No greater intervals between lateral branches have been found in any part of the thallus, hence

cell division in the axial row probably takes place in *D. ligulata* in the same way that Söderström has already demonstrated for *D. aculeata.*

Since the original wall which cut off the branch cell was not perpendicular to the transverse wall of the axial cell, but slightly oblique to it, the branch, at its beginning, was not exactly at a right angle but inclined slightly forward toward the growing point. The lateral walls of both axial cells and lateral cells in the young stages are not cylindrical but barrel-shaped. As a result, the supper side of the basal cell of the branch is pressed against the base of the axial cell next above, and the two walls become adherent. Subsequent changes in the shape and position of the cells due to the development of surrounding tissues so modify their appearance at the point of juncture that unless the early stages were traced it is impossible to tell from which axial cell the branch originates (Plate 62, Figs. 2 and 3, and plate 59, Figs. 2, 3 and 6).

Cortication begins immediately below the growing point, and according to the observation of the writer does not proceed as indicated by Reinke (1880), who claims that filaments develop which become applied to the axial cell, encircle it, meet and fuse, and by repeated division form a layer of small cells lying close to each other about the original cell of the thallus. The cortical layer originates, as described by Janczewski (1875) and Reinke (1880), as a small cell cut off on the lower side of the basal cell of a lateral branch of the main axis. This new cell does not divide again immediately, but another small cell is cut off from the side of the same basal cell. The first cell to be cut off then divides, either below or on one side. Since the same process is proceeding on the opposite side of the hair branch, a cluster of small cells is thus formed about its base. Meanwhile, the opposite branch hair of the pair is also developing a cluster of small cells about its base. At this time there is then a cluster of small cells on opposite sides of the axial cell, and this is the beginning of the flattened thallus. The axial cells at this point have a length only about half their diameter, and each cell bears a pair of branches, hence the branches are very close together, and the cluster of small cortical cells about the base of one lies in contact with the branch below it. These cortical cells now divide laterally without any definite sequence, and gradually spread toward each other until the axial cell and the basal cells of its paired branches are completely covered (Plate 63).

Since elongation of the axial row is taking place at the same time, several stages of this process of cortication can be observed on a single filament. From the point at which the first small cell is cut off to the point at which complete cortication is attained, from two to six axial cells may intervene, in all intermediate stages of cortication.

By the time complete cortication is reached, the basal cells of the

branches of the axial filament, from which the first cortical cells were cut off, have elongated and the adjoining cells have divided. The growing region of the branch hair is thus moved outward, so that branches below the growing point of the main axis have intercalary growth, while branches above the growing point, as previously explained, develop basipetally (Plate 62, Figs. 1 and 2). The dividing cortical cells extend outward along the bases of the lateral branches, as well as around the axial cells, so that when the axial cells have one layer of cortical cells covering their lateral walls between the paired branches, there is a flat extension of cortical cells surrounding and between the bases of the successive lateral branches which is two or three cells broad. Division of cortical cells in the plane of the lateral branches proceeds quite rapidly, the cells of the axial filament elongate and divide, and there is thus formed a flat thallus consisting of a filament of large cells bearing distichous branches, the whole covered by a single cortical layer of very small cells. The lateral branches increase in length by addition of cells at the intercalary growing point, and the cortical growth spreads outward, keeping the branches covered almost to their growing points.

At a distance 4 to 6 axial cells behind the point where complete cortication is effected, the cortical cells have begun to divide parallel to the surface of the thallus, thus forming the beginning of the ground tissue. A few cells farther back, another parallel division has taken place, the inner cell of the first division having greatly enlarged meanwhile, accompanied by the rapid division of the meristematic outer cortical layer in a plane perpendicular to the flat surface of the thallus, to keep pace with its rapid increase in length and width. In a small pinnule well stained and cleared one can focus through the successive layers of cells and make out their size, shape and arrangement very distinctly. At this point all the cells of the thallus still contain chromatophores, the small cortical cells of the surface layer being densely crowded with them, while the rapidly enlarging cells of the ground tissue still have a few scattered through the layer of peripheral protoplasm. They are also present in the axial cells.

As Jönsson (1901) says, there is no definite point at which primary growth stops and secondary growth begins. The material used in this study still bore assimilating hairs, though most of them had been shed. Cortication was still in progress at the tips of some of the pinnules, while the formation of the secondary assimilation system had already begun farther back toward their bases.

In focusing down through the superficial walls, the inner assimilation system first appears as a series of long slender filaments lying alongside the large cells of the axial filament. In cross section the axial filament appears to be surrounded, or partly surrounded, by small cells (Plate 62, Fig. 6). In longitudinal section, the filaments can be seen originating

in the large cells of the ground tissue. When the filaments first begin to elongate, cross walls are rather infrequent, the cells of the filaments having a length of from 2 to 5 times their diameter (Plate 62, Figs. 3, 4, 5). Intercalary cross walls soon appear, the filaments become more numerous and begin to twist and turn about the central axis and about each other as they force their way along the path of least resistance toward the base of the pinnule. In this way a mass of small cells is formed about the axial cells (Plate 59, Figs, 2-5). The filaments making up this assimilating tissue elongate not by elongation of the individual cells, which remain small and pratically isodiametric, but by apical growth, the end of the filament elongating and forming cross walls.

The "conducting hyphae" of Sŏderstrŏm originate in the same way as the inner assimilation tissue, as outgrowths from the large cells of the ground tissue. Sŏderstrŏm says that the cells of the ground tissue never divide, but any increase in their number is by addition from the cortical meristem. The writer has found all degrees, from the elongation of the entire cell with formation of cross walls, forming a chain of several large cells which gradually narrows to a small filament, down to small slender outgrowths from the end or side of the ground tissue cell. The hyphae vary exceedingly in diameter, and branch repeatedly, forming a tangled network which binds the cells of the ground tissue firmly together, making the lamina a tough pliable sheet.

Inner assimilation tissue and conducting hyphae have the same origin and both develop in the same way. The only structural difference seems to be that in the first hyphal filaments formed, which surround the axial cell row, intercalary cross walls are formed which divide the filament into small isodiametric cells, while in the hyphal filaments formed later, and farther from the axial cell row, intercalary cross walls do not appear, the filaments being made up of long cylindrical cells. Moreover, the major part of the conducting hyphae are developed immediately surrounding the inner assimilation tissue. In *D. aculeata,* according to Sŏderstrŏm, "the inner assimilation tissue consists of five or six layers of very small, thin-walled, endochrome containing cells," while in *D. ligulata* there are only one or two layers of small iso-diametric cells containing chromatophores, which are surrounded in turn by a zone of conducting hyphae of varying width, depending upon the age of the portion of thallus examined, and the region of the plant body from which it is taken (Plate 59, Figs. 2-6).

In the mature thallus the cells at the outer edge of the lamina are younger, hence are smaller and have produced less of the hyphal growth. The cells surrounding the axial row and its covering of small assimilating cells produce more of the hyphal growth than do the cells of the flat lamina; thus causing a thickening of the thallus, and producing the evi-

dent midrib which characterizes all the ligulate species. This would account also for the increased thickness of the midrib toward the base of the thallus (Plate 59, Fig. 4).

In the short cylindrical stipe and the slender petiole-like bases of the branches, very few ground tissue cells were laid down originally, and the number is not increased from year to year. The greater part of the tissue in these regions is made up of hyphal filaments which follow down the main axis of the thallus or of its lateral branches.

Jönsson (1901) was the first worker who correctly interpreted the origin and structure of the secondary inner assimilation systems which appear in the basal portions of the thallus of old *D. aculeata* plants. He shows, by means of diagrammatic longitudinal sections, that the axial cell row originates as a branch, growing down from the lower side of one of the basal cells of a lateral branch of the main axial strand. This branch elongates, growing downward among the cells of the ground tissue, and becomes covered with layers of small cells containing chromatophores, in the same way as does the main axial strand. This same secondary development occurs in all the ligulate species of the genus, but is carried out on a much more extensive scale.

It was brought out earlier in the discussion that while the lateral branches of that portion of the axial filament above the intercalary growing point develop basipetally, the permanent branches below the growing point increase in length by means of an intercalary growing point which is formed after cortication begins, and which remains always at the edge of the flat thallus. Just as the original cells of the main axis may by repeated division form 2, 3, or 4 cells, so the cells of the permanent branches also increase in number by repeated divisions. This is especially noticeable at the base of the branches, where division takes place so rapidly that a series of short cells is produced. It will be remembered that originally the lateral branches are inclined at an angle a little less than 90° from the main axis. During this period of elongation and rapid cell division in the lateral branch, the new cross walls formed by the dividing basal cells are laid down parallel with the main axis, so that the basal portion of the branch, as these cells elongate, stands squarely at right angles with the main axis (Plate 59, Figs. 2, 3, 6 and Plate 62, Fig. 3).

It is from the lower side of one of these basal cells that a lateral branch grows out which ultimately becomes the axis of a secondary inner assimilation system. This secondary branch may appear at any point along the lower side of the cell, though it is usually at about the middle, giving the branched cell the appearance of a T-tube. The branch elongates by apical growth and cross walls are laid down back of the growing tip. As the cells thus formed mature, they take on the appearance of typical sieve

tubes, their cross walls give the callose reaction, and protoplasmic connections through the sieve plates can be demonstrated. As the branch develops it becomes invested with a layer of assimilating cells in its older parts, and as it grows downward through the ground tissue of the lamina it may give off branches which in turn acquire an investment of assimilating cells (Plate 59, Figs. 2, 3, 6, and Plate 62, Fig. 7).

It is obvious that these secondary assimilation systems must originate in younger parts of the thallus, before the cells which bear them have become closely invested with assimilating cells and several layers of closely interwoven conducting hyphae. The entire mature thallus is penetrated by these branching systems of sieve tubes surrounded by their assimilating cells, and all in connection, through the lateral branches of the midribs of the pinnae, with the larger branches, and so with the main axis of the compound thallus.

Söderström developed the idea of two secondary tissues originating in the cells of the ground tissue. The foregoing discussion shows that the axial cells of the "original thallus" also give rise to a secondary tissue in exactly the same manner. There are thus two systems of secondary tissues, one developing from the original thallus, and the other from the ground tissue which surrounds it.

The holdfast in *D. aculeata,* according to Söderström, is made up in the central part mainly of a mass of interwoven and outspreading hyphae, which function more as mechanical support than for conducting, while the tissue of the peripheral parts is made up of rectangular cells, lying in regular parallel rows. In *D. ligulata* the central tissue of the holdfast is a mass of filamentous hyphae branching and running in all directions between the few cells of the original ground tissue, which have become widely separated by this secondary growth. These hyphae originate not only in the ground tissue among which they lie, but descend along the midrib of the stipe from the ground tissue cells of the lower parts of the frond. Through all this central tissue ramify numerous branching sieve tubes surrounded by assimilating cells, the sieve tubes originating as secondary outgrowths from the lowest branches of the main axis, which naturally forms the central structure of the holdfast, as it does of all other parts of the frond.

In the cortical region of the holdfast, the new cells which are cut off from the inner side of the outermost layer do not enlarge to form ground tissue, but remain of practically the same diameter as the mother cell, although they may elongate somewhat perpendicular to the plane of division. Since several of these cells may be cut off during one season's growth, the resulting tissue has the appearance of a mass of closely packed, roughly parallel filaments.

There is a sharp line of demarcation between the cortical growth of two seasons, which gives the appearance in section of annual rings (Plate 59, Fig. 7). This is due to the fact that the cells first formed in the spring elongate much more than those formed later in the season, while those cells which form the outer cortical layer of the resting season are small and isodiametric, and develop heavier walls.

d. Desmarestia herbacea (Turner) Lamouroux

(Plate 54, Fig. 3; Plate 60, Fig. 1-5).

Neither young plants nor plants bearing assimilating hairs were collected, hence it has been impossible to determine the process of development of the various tissues. However the tissues of the mature plant in the resting condition resemble so closely those of *D. ligulata* that it is natural to suppose that they are formed in the same way.

The increased width of the thallus of *D. herbacea* over that of *D. ligulata* is due to the fact that all primary growth, in older parts of the plant, takes place at the edge of the thallus. The cortical cells in this region divide parallel to the surface of the thallus, and the inner cells develop into ground tissue, while the superficial cells divide rapidly at right angles to the surface to accommodate the increasing width. In the older region of the lamina, nearer the midrib, the only growth is secondary, and consists in further development of the conducting hyphae and sieve tubes.

There are five definite tissue regions in the thin flat lamina of the thallus, the two cortical layers, each underlaid by the large cells of the ground tissue, and between the two ground tissue regions, forming the central layer, a weft of interwoven conducting hyphae, among which ramify the branching sieve tubes (Plate 60, Figs. 1, 2, 3).

It is in the increased development of the sieve tubes of the secondary inner assimilation tissue that *D. herbacea* differs most materially in structure from *D. ligulata*. The secondary branches of the lateral veins, which originate as in *D. ligulata*, continue their growth among the conducting hyphae of the central layer of the lamina, branching and rebranching, without the investment of small inner assimilation cells with which older parts of the system are provided, but themselves containing many chromatophores, as do all young cells of the primary thallus. They are easily distinguished from the conducting hyphae, many of which are of about the same diameter and also contain chromatophores, since in sections lightly stained in Bismarck brown, the ring of callose which is forming about the edge of the sieve plate takes the stain much more deeply than do the cellulose walls (Plate 60, Fig. 1).

e. Desmarestia latissima Setchell and Gardner in Litt. n. sp.

(Plates 55, 56, 57; Plate 58, Figs. 1-4; Plate 60, Figs. 5-9; Plate 62, Figs. 8-10.)

Several very young plants from one to five centimeters long were collected, one of which still bore a few assimilating hairs. Not enough hairs were observed to make general statements possible. Those studied were small and sparsely branched; their main axis spindle-shaped and less than 20 cells long; their branches either single or in pairs, tapering at the ends, and composed of from 5 to 8 cells. Several pinnules terminated in unbranched hairs.

As in *D. ligulata* every cell of the permanent central axis, laid down below the intercalary growing point, bears a pair of lateral branches. By subsequent repeated divisions of the original axial cells, these paired branches become separated by a series of up to 8 cells, whereas, in *D. ligulata* not more than 4 axial cells were observed between successive pairs of branches. The cells of the axial row could be counted very easily by focusing down through the superficial cells of entire young plants which had been lightly stained in Bismarck brown.

The young tips of the branches were in the same stages of development as the tips of the pinnules in *D. ligulata*. Cortication proceeds in the same way. At the tips of those branches whose axial cells did not produce hair branches, a pair of lateral cells was cut off, as in the beginning of branch hairs, but these single cells cut off small cells below, then at the side, and cortication proceeded in the usual way, by repeated division of these cells.

In these same plants the beginnings of the inner assimilation tissues were visible as slender filaments made up of cylindrical cells, arising in the cells of the ground tissues and closely applied to the cells of the central axis. Secondary growth from the lateral branches of the central axis was also well under way, the secondary branches themselves branching and ramifying among the cells of the ground tissue.

All these cells in these young plants were extremely small, and lateral branches were very close together. Young plants up to 50 cm. in length bore no greater number of branches, hence a tremendous elongation of the cells of the thallus between the branches must take place.

The structure of the mature thallus does not differ from that of *D. herbacea* except in degree. The lamina consists of the same five tissue regions, developed to a much greater extent, as the enormous width of

the thallus in some individuals testifies (Plate 60, figs. 5-8). The secondary growths of sieve tubes, which in *D. herbacae* ramify among the conducting hyphae, in this species become invested with inner assimilation cells in their older parts (Plate 60, fig. 5), and are visible on the surface of the lamina as the network of veins between the lateral branches of the midrib. Toward the outer edge of the lamina, in the younger tissue, they are still uninvested, and are not visible from the surface.

The cells of the ground tissue are so firmly bound together by the long branching filaments of the conducting hyphae that in formalin material the cortical layers, which become loosened and form large blisters, can be stripped from both surfaces and an entire lateral branch will preserve its original shape and texture, and the veining can be traced as easily as in a skeleton leaf. In plate 62, figs. 8 and 9, is shown the method of branching of the conducting hyphae, while fig. 10 shows how firmly the cells of the ground tissue are bound together by the interwoven meshes of the hyphae.

In studying sections of the stipe and holdfast of a young specimen, a single longitudinal section plainly showed the axial cell row of the midrib or "original thallus" extending through the base of the stipe to the bottom of the holdfast (plate 60, fig. 9); and the covering of assimilating cells, gradually becoming less and finally disappearing at the base of the holdfast. This would seem to indicate that the plant originates as a simple monosiphonous filament which later develops branches and becomes corticated. This is probably true of all members of the genus, as it is the condition found at the thallus tips of all species at the beginning of the active growing season.

f. Desmarestia foliacea n. sp.

(Plate 58, Figs. 5-10; Plate 61, Figs. 1-5)

Except for its extreme thinness and delicacy of texture, this species closely resembles a single lateral branch of *D. latissima*. In structure the two are identical. Plate 61, fig. 1 shows a flat section through a younger portion of the lamina, with the large cells of the ground tissue, the interwoven conducting hyphae, and the secondary growth of sieve tubes, not yet surrounded by assimilating cells. The sieve tubes are easily distinguished from the hyphae by the heavy callose rings formed on the cross walls. In fig. 2, from an older part of the lamina, the sieve tubes are surrounded by a layer of small assimilating cells.

In fig. 3, a cross section through the edge of the lamina, the primary growing region of the plant is shown, that is, the region in which new ground tissues are added to older parts of the thallus, the result being an increase in the width of the thallus. The increase in thickness of older

parts of the thallus, aside from increase in size of the original ground tissue cells, is brought about, as stated before, by secondary growth, that is, by continued development of conducting hyphae.

A longitudinal section through the upper part of the stipe of a mature plant is shown in fig. 4. Contrast the length of the portion of an axial cell shown with the length of the axial cells in fig. 5, a longitudinal section through the base of the stipe and upper part of the holdfast in a young plant. Note also in fig. 5 the mass of conducting hyphae surrounding the central axis. In this young plant an inner assimilation tissue had not yet developed, though sections of the same region in mature plants show an abundant development of assimilating cells.

In discussing the structure of his *D. tabacoides*, Okamura (1908) says, "The axis is not composed of a single longitudinal row of cylindrical cells, but of many short filamentous cells, densely packed and firmly coalesced in a very irregular manner." This would correspond to the "tertiary assimilation tissue" which Jönnson (1901) describes in *D. aculeata*, formed by outgrowths from the secondary assimilation tissue, or "inner assimilation" of "Söderström" (1889), which pierce the walls of the axial cells and form dense masses in the cell cavities. The writer has found nothing resembling a development of this sort in any of the ligulate species examined.

g. Conclusions

In the four ligulate species of the genus Desmarestia under discussion, the difference in width and thickness, in venation, and in texture of the thallus is due, not to fundamental differences in structure, but to differences in the relative amounts of primary and secondary tissues, and to differences in the size of the cells of corresponding tissues and the thickness of their walls. A comparison of the figures in plates 59-61 will make this very evident.

In *D. ligulata* there is comparatively little lateral extension of the lamina and an abundant development of conducting hyphae, making a thick and narrow, but very tough and pliable thallus. In *D. herbacea* there is greater lateral extension but less development of hyphal tissue, and hence a broader but much more delicate lamina. In *D. latissima* the lamina is enormously extended, there is comparatively little hyphal tissue, except in the basal portions, but the individual cells are extremely large and thick-walled. There is also an extreme development of secondary branches of the inner assimilation tissue. In *D. foliacea* the extreme thinness of the lamina is due to the fact that it is the minimum number of cells in thickness, and the cells are very thin walled, especially in younger plants, so that when dry there is very little substance.

4. Summary

In the taxonomic portion of the paper, the writer has discussed two well established ligulate species of the genus Desmarestia, *D. ligulata* (Lightf.) Lamour., and *D. herbacea* (Turn.) Lamour.; has presented a species recently separated from the latter, *D. latissima* Setch. and Gard. in litt., n. sp.; and has proposed a new species, *D. foliacea* n. sp.

In the discussion of the morphology of these ligulate species, all the available literature has been summarized and the following new points have been presented:

1. The transitory assimilating hairs during the period of elongation in early spring differ in several species examined, and further examination in other species may lead to the conclusion that difference in assimilating hairs can be made to constitute a species difference.

2. The primary axis of the assimilating hair is not regularly branched, but branches may occur singly or in pairs, and are separated by a varying number of axial cells.

3. At the end of the growing season the assimilating hairs are cut off by a definite method of abscission.

4. The cortical layer does not originate as filaments growing out from the basal cells of branch hairs which become applied to the axial cells, but as two groups of cells cut off from the basal cell of opposite paired branches, which divide without definite order and produce a single layer of cells covering the axial cell and the bases of its lateral branches, and so form a flat thallus.

5. There are two systems of secondary tissues: (a). The "inner assimilation system" and "conducting hyphae" which originate from the cells of the ground tissue. (b). The secondary outgrowths from the lateral branches of the axial filament or "original thallus," which in very broad species form a network of veins in the lamina, visible from the surface.

6. The cells of the axial filament and all its branches, both primary and secondary, develop into true sieve tubes.

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PLATE 54

Figs. 1 and 2. *Desmarestia ligulata* (Lightfoot) Lamouroux. × 0.26.

Fig. 3. *Desmarestia herbacea* (Turner) Lamouroux. × 0.26.

PLATE 54

PLATE 55

Figs. 1-4. *Desmarestia latissima* Setchell and Gardner showing bases of plants bearing lateral branches of various widths. X 0.20.

PLATE 55

PLATE 56

Figs. 1-4. *Desmarestia latissima* Setchell and Gardner showing various widths of main axis. × 0.20.

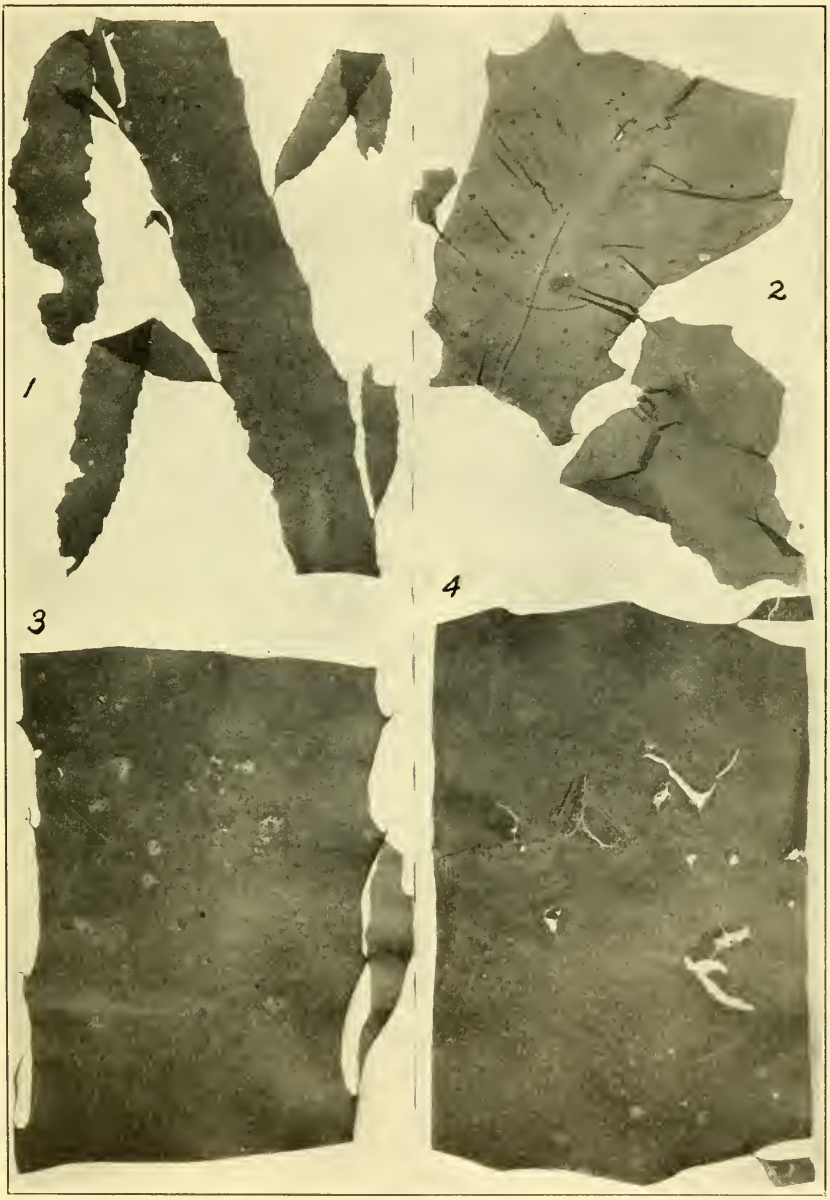

PLATE 56

PLATE 57

Desmarestia latissima Setchell and Gardner. Plants in condition as secured with a trawl. White bar on surface of thallus is 152 mm. (6-inch) ruler. × 0.04.

Fig. 1. Fragment of a plant. Main axis 40.5 cm. wide.

Fig. 2. A small plant attached to the base of the stipe of an older one.

PLATE 57

PLATE 58

Figs. 1-4. *Desmarestia latissima* Setchell and Gardner. Young plants. X 0.20.

Figs. 5-10. *Desmarestia foliacea*. Plants of various sizes and ages. X 0.20.

PLATE 58

PLATE 59

Desmarestia ligulata (Lightfoot) Lamouroux.

Fig. 1. Tip of branch, showing crowded arrangement of assimilating hairs. X 88.

Fig. 2. Longitudinal section through midrib showing sieve tubes of central axis, surrounded by inner assimilation tissue and conducting hyphae; three pairs of lateral veins, originating from every third cell of central axis; middle pair of lateral veins with secondary branches. X 88.

Fig. 3. Same as fig. 2. X 88.

Fig. 4. Cross section of main axis, showing arrangement of tissues. X 88.

Fig. 5. Cross section of branch of first order, showing central axis with pair of lateral branches. X 188.

Fig. 6. Longitudinal section of branch of second order, showing branching in secondary branches of veins. X 88.

Fig. 7. Section of holdfast, showing two years' growth. X 88.

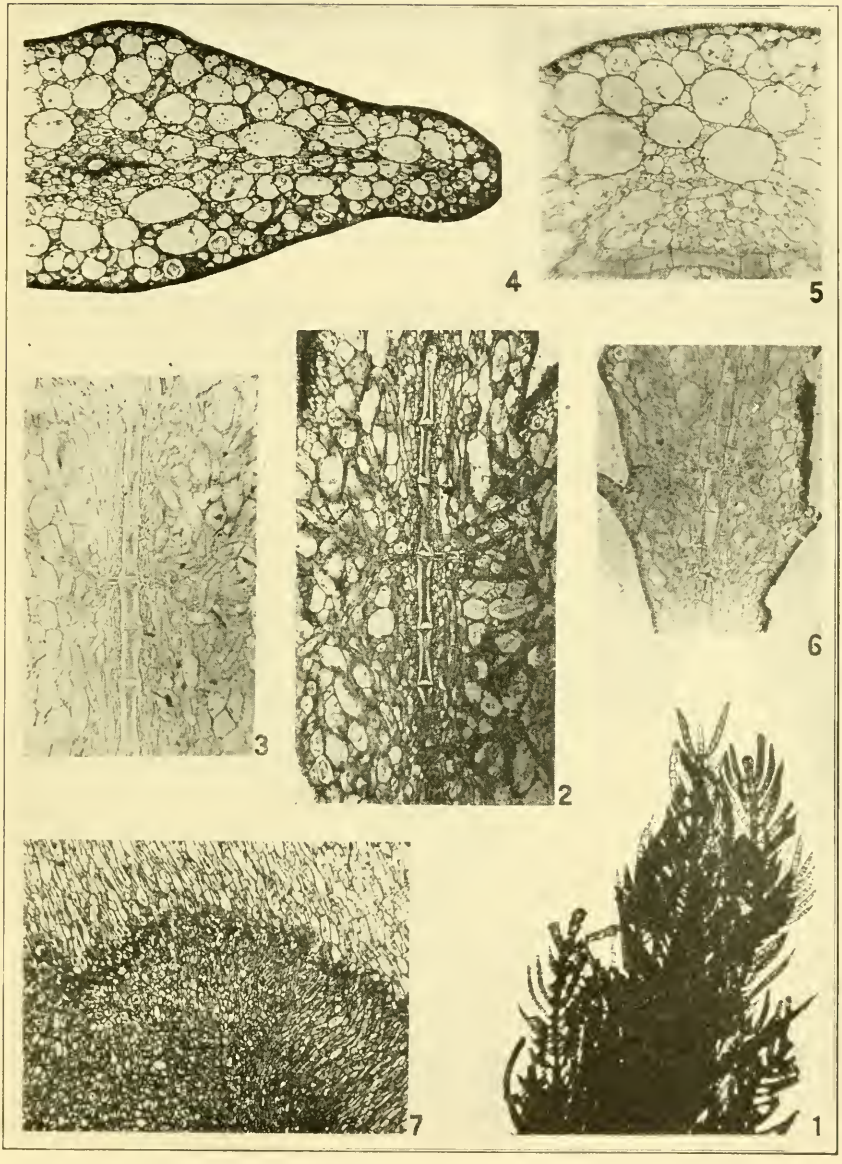

PLATE 59

PLATE 60

Figs. 1-4. *Desmarestia herbacea* (Turner) Lamouroux.

Fig. 1. Longitudinal section of young tissue, sieve tubes of central axis with beginning of inner assimilating tissue, and branch veins without assimilating tissue; callose at sieve plates. X 88.

Fig. 2. Cross section of lateral branch, through midrib and through lamina. X 88.

Fig. 3. Cross section of lamina of lateral branch. Phycocelis sp. epiphytic on surface. X 188.

Fig. 4. Cross section of thallus, near stipe, with Phycocelis sp. X 188.

Figs. 5-9. *Desmarestia latissima* Setchell and Gardner.

Fig. 5. Cross section of lateral branch, showing sections of small seondary veins surrounded by assimilating cells, large cells of ground tissue and small cortical cells. X 188.

Fig. 6. Longitudinal section of lateral branch, through midrib, perpendicular to flat surface of frond. X 88.

Fig. 7. Cross section of lateral branch. X 88.

Fig. 8. Longitudinal section of midrib, base of main axis. X 188.

Fig. 9. Longitudinal section of holdfast, showing central axis reaching almost to base. X 50.

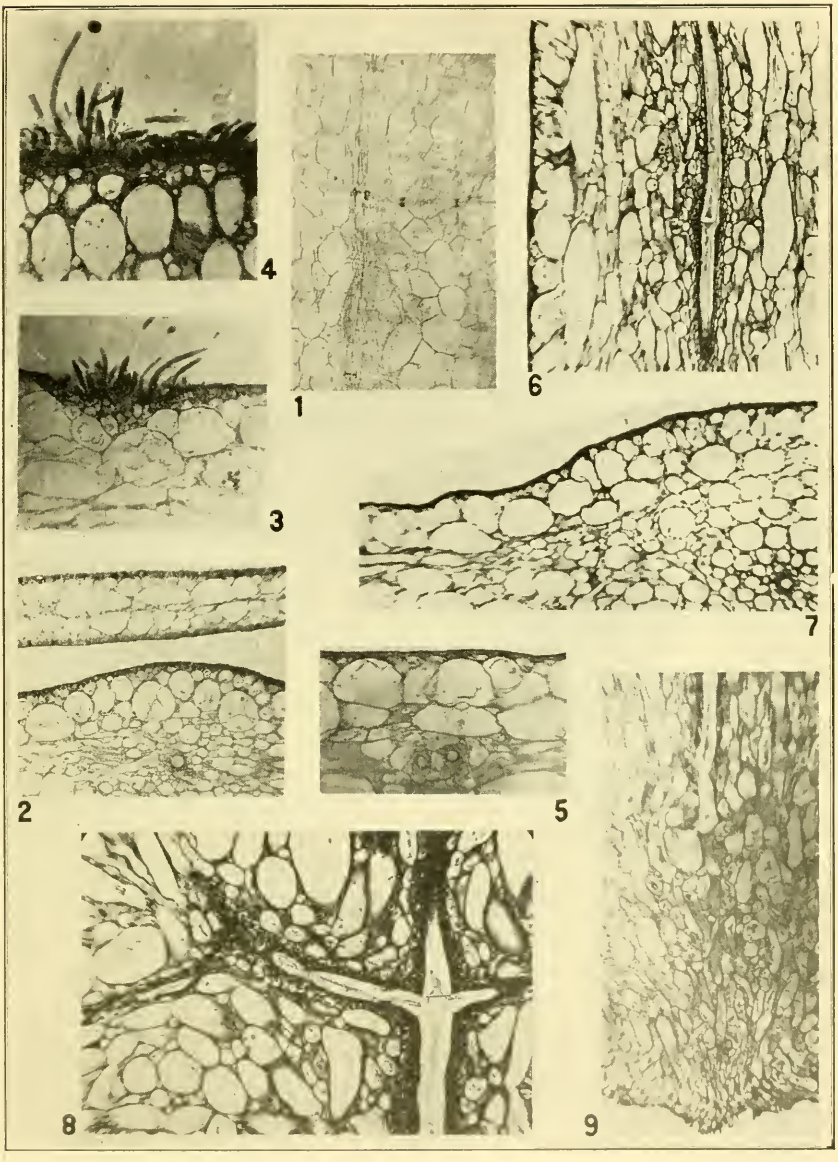

PLATE 60

PLATE 61

Figs. 1-5. *Desmarestia foliacea.*

Fig. 1. Longitudinal section, showing young secondary veins without investment of assimilating cells, distinguished from conducting hyphae by callose sieve plates. X 88.

Fig. 2. The same as Fig. 1 but of older tissue, showing inner assimilating tissue surrounding secondary veins. X 88.

Fig. 3. Cross section of edge of frond, showing arrangement of tissues and growing region at extreme edge. X 188.

Fig. 4. Base of stipe and central region of holdfast, showing central axis extending into holdfast. X 88.

Fig. 5. Longitudinal section of base of frond, showing central axis with pair of lateral branches surrounded by inner assimilation tissue and conducting hyphae. X 88.

Figs. 6, 7. *Desmarestia aculeata* (Linnaeus) Lamouroux. Longitudinal and cross sections of main axis, showing arrangement of tissues and size of cells as compared with cells of ligulate species. X 88.

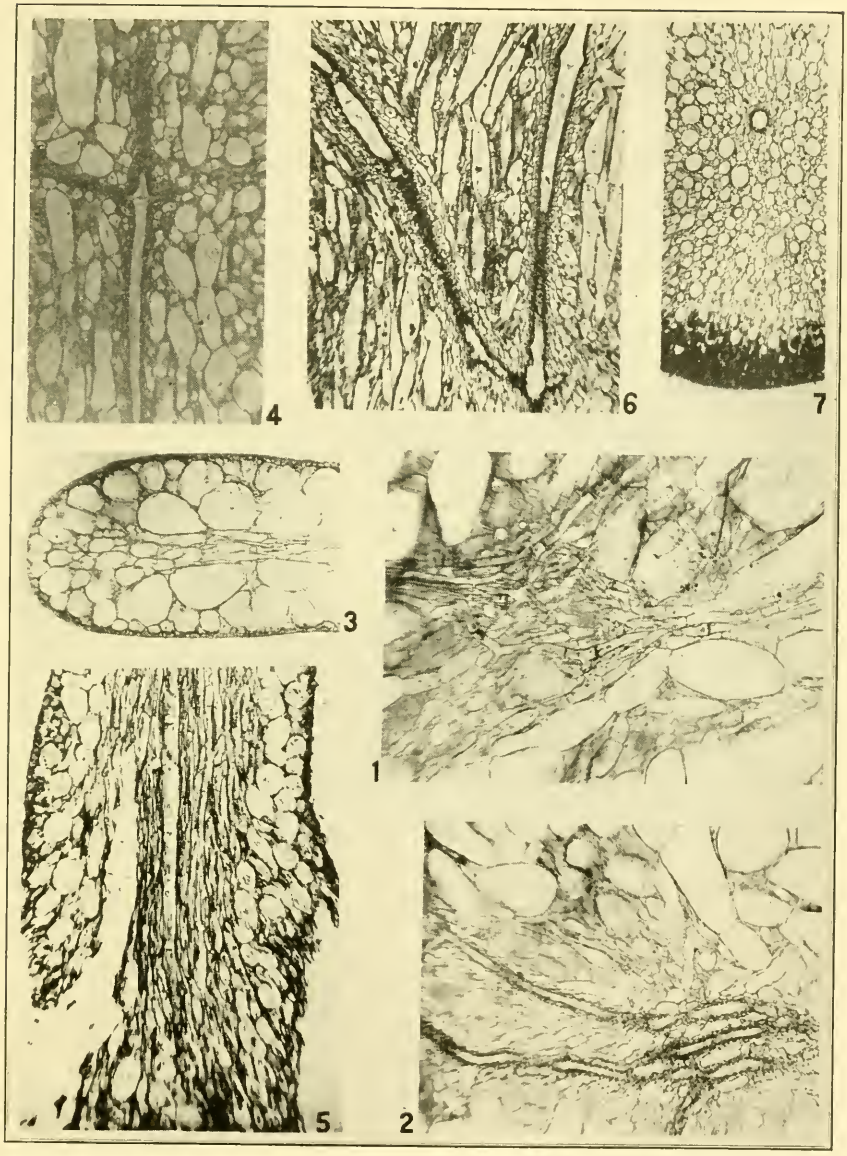

PLATE 61

PLATE 62

Figs. 1-7. *Desmarestia ligulata* (Lightfoot) Lamouroux.

Fig. 1. Original row of cells of thallus: a, intercalary growing region; b, terminal hair bearing lateral branches irregularly arranged; e, permanent axis with opposite lateral branches. X 275.

Fig. 2. Longitudinal section of young lateral branch: aa', central axis; bb', edge of thallus; c, intercalary growing region of lateral branches; d, point of abscission. X 170.

Fig. 3. Longitudinal section of young lateral branch: a, central axis; b, lateral branch; c, cortical layer; d, ground tissue; e, beginning of development of inner assimilation tissue. X 170.

Fig. 4. Cross section of young lateral branch, such as in Fig. 3. X 170.

Fig. 5. Longitudinal section of young branch perpendicular to flat surface of frond. X 170.

Fig. 6. Cross section of young branch, showing inner assimilation tissue surrounding central axis, and distribution of chromatophores. X 165.

Fig. 7. Branching sieve tube from secondary outgrowth from lateral branch of central axis. X 170.

Figs. 8-10. *Desmarestia latissima* Setchell and Gardner.

Figs. 8-9. Method of branching of conducting hyphae. X 275.

Fig. 10. Network of conducting hyphae with chromatophores, between two regions of large ground tissue cells. X 170.

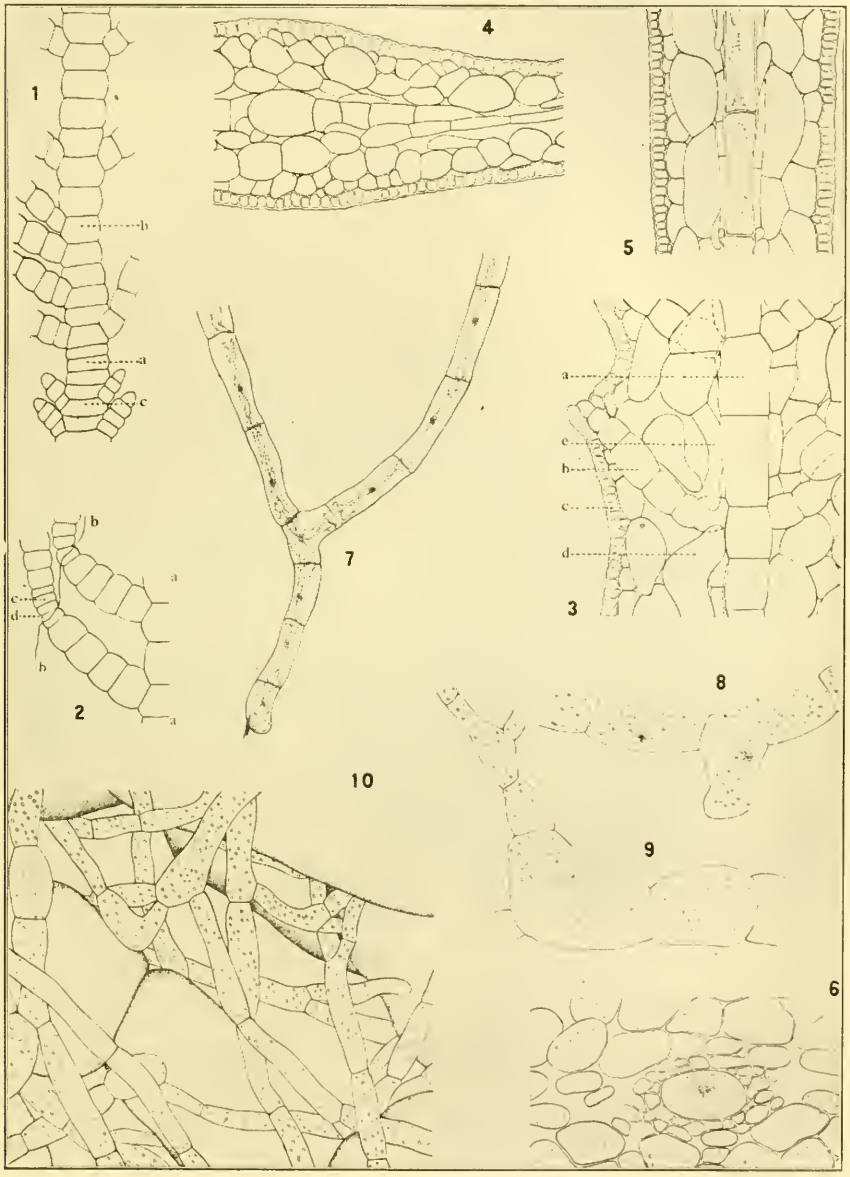

PLATE 62

PLATE 63

Origin of cortical layer in *Desmarestia ligulata* (Lightfoot) Lamouroux, partly diagrammatic. a. Intercalary growing region; b, Base of terminal hair; c, Bases of lateral hair branches; d, Basal growing region of branches; e, Permanent axis of thallus; f. Bases of lateral branches of permanent axis; g, Intercalary growing region of branch; h, First cell cut off in formation of cortex; i, Second cell cut off in formation of cortex; j, Cortication complete; k, Cells of original thallus covered by cortex.

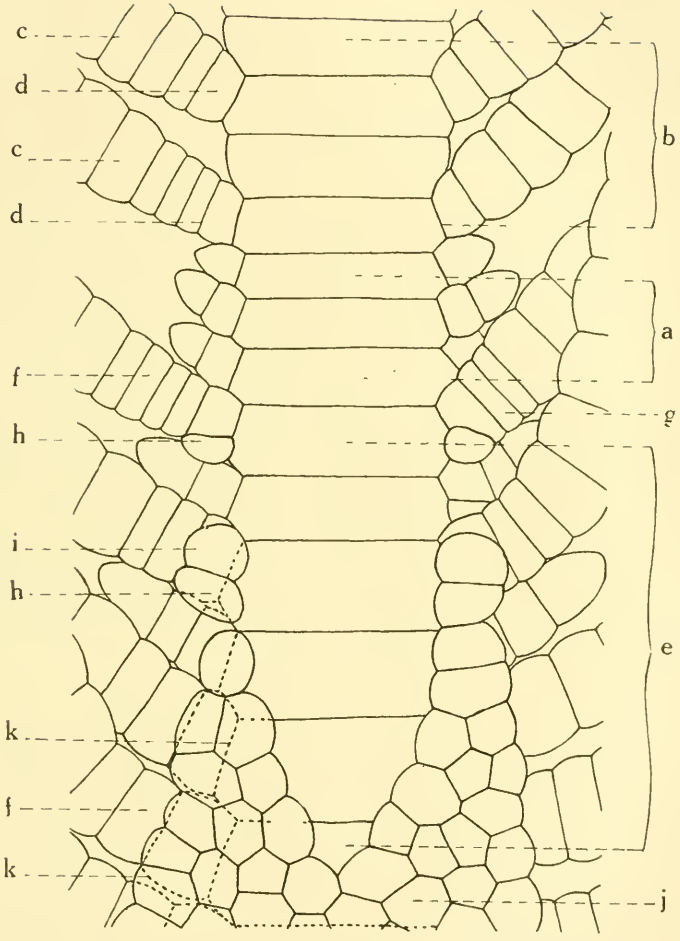

PLATE 63

The Variation of the Condition of Sea-water, Especially the Hydrogen-ion Concentration, and Its Relation to Marine Organisms*

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Contents

1. Introduction and methods

During the summers of 1918 and 1919 work was undertaken at the Puget Sound Biological Station to determine the condition of the sea-water in the vicinity of the station. Special attention was paid to the hydrogen-ion concentration in relation to marine organisms.

The colorimetric method of Sörensen (1909, 1909a) and Sörensen and Palitzsch (1910, 1910a) was employed in all determinations of the hydrogen-ion concentration of the sea-water. During the summer of 1918 the buffers and indicators as suggested by Clark and Lubs (1917) for bacteriological work were used. It was with great difficulty that the buffers were prepared at the station. Specially was this true of the sodium hydroxide solution. To avoid any error due to a possible inaccuracy in the stock solutions for the buffers each mixture was compared

* Studies from The Department of Zoology, The University of Nebraska, No. 127.

for its hydrogen-ion concentration with Hynson, Westcott and Dunning's set for the colorimetric determination of the hydrogen-ion concentration of the blood.* During the summer of 1919 both the Hynson, Westcott and Dunning's set, and the indicators and buffers used in 1918 were employed. In comparing samples with the standards, they were held in diffused day-light in the box with a white background supplied for the purpose, or against a white paper. Ten cc. of sea-water was placed in a small test tube, and after adding the indicator, was compared with ten cc. of the standard buffer solution with the same amount of indicator added and contained in a similar tube, or to the standard buffer set. When possible both methods were used, the more reliance being placed in the standard buffer set. The standard buffer solutions were always kept stoppered to prevent contamination from the air and the carbon dioxide of the observer's breath. All readings were corrected for the "salt error" of Sörensen (1909) and Sörensen and Palitzsch (1910). McClendon (1917, 1918) was followed in making this correction. The chlorine of the sea-water was not taken daily to determine the concentration but the same correction was made (Shelford and Powers 1915) for all determinations for both 1918 and 1919. The readings are given in the second decimal but no great accuracy can be claimed for the second decimal place, since the same factor was used in correcting for the "salt error" for all determinations; and the second decimal place was always an estimation, as the standard buffers read only to the first decimal place. No corrections were made for the variation in temperature (Sörensen and Palitzsch 1910, Henderson and Cohn 1916, and McClendon 1917, 1918) as the temperature variations were slight in most cases and corrections for this factor were considered within experimental error. In most cases where the temperature was high there was also a low hydrogen-ion concentration which was generally outside the range of the standard buffer set, thus making the second decimal less dependable; and without exception the correction for the temperature factor would have been in the second decimal place. The temperature at different depths was taken with a Nagretti-Zambra reversing thermometer, and at the surface by a chemical thermometer reading to one tenth of a degree. Samples from the surface and near the surface were collected for oxygen determinations by connecting a small glass bottle by means of a rubber hose to a large bottle from which the air was exhausted, thus eliminating any possible leakage of air into the sample. Samples from greater depths were taken by means of a water bottle open at both ends and automatically closed by sending down a messenger. Great care was taken to avoid contact with air. Both the hydrogen-ion concentration and the oxygen content were determined from the same sample.

* The writer wishes to thank Professor C. M. Child for the use of his hydrogen-ion colorimetric set in standardizing buffers used in the summer of 1918.

The oxygen content was determined by the Winkler method (Birge and Juday 1911).

2. Experimental data

During the progress of the work more than 500 samples were taken. In the tables are given only those determinations which are characteristic and can be subjected to comparison. The pH is used to express the hydrogen-ion concentration of the sea-water or the hydroxyl-ion concentration, if you choose to call it so, instead of the C_H or C_{OH}. The pH is used by most workers on the hydrogen-ion concentration of sea-water (Sörensen and Palitzsch 1910, Palitzsch 1911, McClendon 1916, Henderson and Cohn 1916 and others). The error resulting from the correcting of the so-called "salt error" to which Gaarder (1917) has called attention is within that of the method used. The oxygen content is expressed in cc. per liter. Determinations were made only over a very limited area of the Sound and were confined mainly to the vicinity of the Station. All determinations on parenthetical dates were made during the summer of 1919 and all the numbers expressing the time of determinations made in the forenoon are likewise in parentheses. An asterisk (*) after the date in the tables indicates that it was clear weather when the determination was made. The symbol (') indicates cloudy weather and the double symbol ('') indicates cloudy weather with rain. The symbol (o) indicates that the tide was going out; (i), tide coming in; (h), high tide; (l), low tide.

3. Hydrogen-ion concentration and oxygen content of surface sea-water

On July 25 and 29, 1918, determinations were made of the surface waters over a wide area of the Sound. By an examination of table 1 it is seen that the pH varied from 7.77, station V, to 8.17, stations N and P.

Table 1. *The hydrogen-ion concentration and oxygen content of the sea-water at a depth of 30 centimeters below the surface in different regions of the Sound.*

Date	Station	Time	Tide	Temperature Centigrade	pH	Oxygen cc. per liter
7/25''	L	(10:50)	o	12°	7.97	5.97
7/25''	L	(11:15)	o	12°	7.87	7.54
7/25''	L	(11:30)	o	12°	7.87	7.33
7/25'	M	2:30	i	14°	8.02	7.46
7/25'	N	3:30	i	14.25°	8.17	7.47
7/25'	N	4:30	i	14°	8.12	7.05
7/25'	O	5:15	i	13°	7.97	5.91
7/25'	P	5:45	i		8.17	5.35
7/29*	R	(10:25)	o		7.84	3.69
7/29*	S	12:20	o	16°	7.78	
7/29*	T	3:30	i	16.5°	7.98	7.16
7/29*	U	3:50	i	11.5°	7.83	4.47
7/29*	V	4:10	i	12°	7.77	5.20

The lowest oxygen content was 3.69 cc. per liter, station R (see also table 2) and the highest was 7.54 cc. per liter, station L. The pH of the surface water in these observations does not parallel the oxygen content when the stations are distantly located (see map, page 372). But on the other hand when the stations are more closely located there is more agreement between the variation of the pH and the oxygen content of the water, i. e., a higher oxygen content is found where there is a high pH. There is an

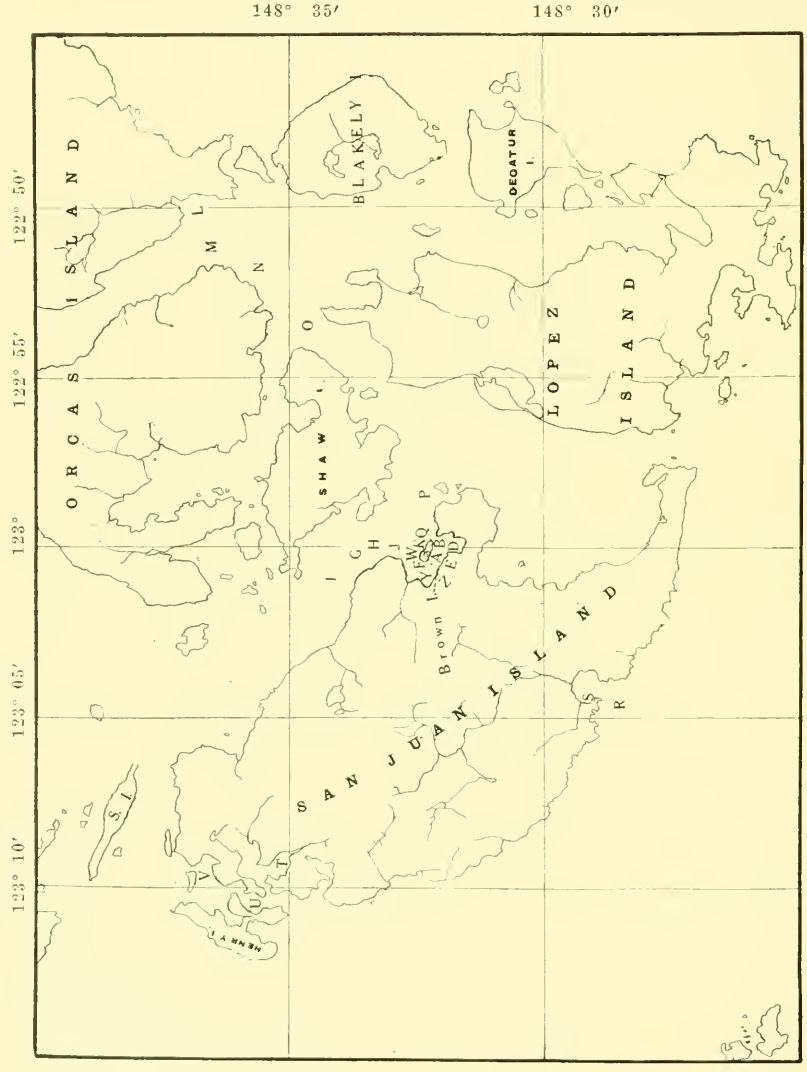

Plate 64. Points where water samples were taken; E, Biological Station; Z, Friday Harbor.

apparent exception to this relation in the first determination made at station L. This determination was made not more than 300 meters from the shore, in reality, in the embayment, when the tide was still going out (see map). The second sample of sea-water was taken 600 meters out and the third 1200 meters out. The condition of the water at the first point was affected perhaps more by the condition of the water of the embayment than that in the open. The water in the embayment close to shore [compare with table 7 determinations for (7/23) made before 6:45 A. M.] had a lower oxygen content, which was perhaps due to the time of day and the cloudy weather with rain the night before. Also the drainage from the village of Olga emptied into this embayment, which would act as a contaminating factor. The high oxygen content of the sea-water in this portion of the Sound without an accompanying high pH as compared with others is not understood. It might be explained by the fact that the tide was going out to a very low tide, —1.0 ft., and the surface water was composed, at least partly, of the water that had been bathing the vegetation along the margin of the shore of the embayment which extends back cutting the island almost in two. (See map, page 372.) This can be seen better by referring to a map of the Puget Sound region*. This explanation is in keeping with observations made at the same time in the eel-grass (*Zostera marina*) in the small embayment near station L. At 10:35 A. M. the water bathing the eel-grass had a pH of 8.35 and an oxygen content of 5.16 cc. per liter. This had a higher pH by 0.05 and a lower oxygen content by 1.48 cc. per liter than open water near by at the same time. At 1:45 P. M. water bathing the same eel-grass had a pH of 8.4 and an oxygen content of 8.72 cc. per liter which was 0.53 pH and 1.39 cc. of oxygen per liter higher than water at station L proper. The water bathing the eel-grass showed an increase of 0.05 pH and 3.56 cc. of oxygen per liter in three hours and ten minutes despite the cloudy weather and a light rain. The low pH of the water at station L as compared with the oxygen content might be explained by the presence of accumulated by-products of decaying substances due to the incomplete circulation of the water of the embayment, a condition that is suggested by its physiography. This would also favor the accumulation of oxygen, provided that the excess oxygen produced by the photosynthetic action of the plants would not all be used up by the metabolism of the animal and plant organisms and the decaying organic matter of the embayment. This lower pH could not have been due to the presence of hydrogen sulphide as was found by Palitzsch (1911, 1911a, 1912) to be the case in the deeper water of the Black Sea. The presence of hydrogen sulphide in the sea-water is an indication of a deficiency of oxygen (McClendon 1917). Palitzsch found

* See Coast and Geodetic Survey map, Washington Sound.

that the pH of the water of the Black Sea fell very rapidly at a depth of about 180 meters, always being accompanied by a rapid fall of the oxygen content of the water; this, however, was the only locality at which he found a direct relation between the pH and the oxygen content of the sea-water.

4. Hydrogen-ion concentration and oxygen content of water at different depths

A few determinations were made at different depths which indicate that there is no uniformity with the variation of the hydrogen-ion concentration and depth. This does not agree with observations of other

Table 2. *The variations in the hydrogen-ion concentration and oxygen content of the sea-water with depth.*

Date	Station	Time	Tide	Depth in Meters	Temperature Centigrade		pH	Oxygen cc. per liter
7/19'	G	(8:30)	i	0.3			8.03	6.76
7/19'	G	(8:55)	i	40			7.78	4.50
7/19'	G	(8:35)	i	80			7.63	3.80
7/19'	G	(8:45)	i	120			7.63	3.62
7/19'	H	(9:05)	i	40			7.76	4.39
7/19'	H	(9:15)	i	80			7.77	4.86
7/19'	I	(9:25)	i	80			7.67	5.46
7/19'	I	(9:35)	i	120			7.58	3.12
7/20'	H	(9:50)	i	20	11°	2:10	7.82	3.43
7/20'	H	(10:05)	i	40	10.5°	2:25	7.77	3.40
7/20'	H	(10:50)	i	60	10.25°	2:35	7.75	3.59
7/20'	H	(11:00)	i	80	10.2°	2:40	7.67	
7/20'	H	(11:20)	i	100	10.15°	2:45	7.57	4.26
7/20'	H	(11:40)	i	120	10°	2:50	7.57	4.64
7/20'	J	3:00	i	20	10.75°	4:00	7.77	4.20
7/20'	J	3:05	i	40	10.5°	4:15	7.77	5.15
7/20'	J	3:10	i	60	10.5°	4:30	7.76	4.43
7/20'	J	3:15	i	80	10.3°	4:40	7.78	4.78
7/20'	J	4:55	o	140			7.79	4.30
7/22*	H	(9:10)	h	0.3			7.67	
7/22*	H	(9:25)	i	20			7.77	4.52
7/22*	H	(9:35)	i	40			7.75	4.22
7/22*	H	(9:45)	i	60			7.72	4.00
7/22'	H	(10:20)	i	80			7.70	3.42
7/22'	H	(10:40)	i	100			7.72	3.80
7/22'	H	(11:00)	i	120			7.74	4.16
7/22'	H	(11:25)	i	160			7.77	4.46
7/22'	H	1:30	i	0.3			7.82	
7/22'	H	1:45	i	20			7.79	4.23
7/22'	H	2:30	i	40			7.78	4.10
7/22'	H	2:50	i	60			7.77	4.09
7/22'	H	3:05	i	80			7.77	
7/22'	H	3:20	i	100			7.77	4.10
7/22'	H	3:35	i	120			7.77	4.30
7/22'	H	3:55	i	140			7.77	4.25
7/22'	H	4:20	i	160			7.77	
7/22'	H	4:35	i	180			7.77	4.20
(7/11')	G	(8:40)	o	20	11.75°		7.87	3.96
(7/11')	G	(9:00)	h	40			7.97	3.98
(7/11')	G	(10:00)	i	60	10°		7.95	2.33
(7/11')	G	(10:40)	i	80			7.84	3.88
(7/11')	G	(11:00)	i	100			7.69	3.00
(7/11')	G	(11:45)	i	140	9.3°		7.87	3.86
7/29*	R	(10:25)	o	0.3			7.82	3.69
7/29*	R	(10:50)	o	20			7.87	
7/29*	R	(11:00)	o	40			7.79	3.45

workers (Natterer 1892, 1893; Palitzsch 1911a, 1912; Henderson and Cohn 1916; Gaarder 1917). These have found a decrease in the pH or an increase in the hydrogen-ion concentration with depth. On July 19, 1918, when the tide was coming in, stations G and I showed a more or less uniform decrease of the pH with depth and a still more uniform decrease in the oxygen content. On the other hand, at station H the relations both in pH and oxygen content were reversed, though the differences were far less than those found at stations G and I. On July 20, 1918, station H showed a uniform decrease in the pH with depth but the oxygen content was reversed, while station J showed at first a decrease in the pH with depth which was followed by a rise at greater depths. The oxygen content showed no uniformity whatever. Again on July 22, 1918, at station H, there was a decrease in both the pH and the oxygen content down to 80 meters and then a rise in both down to 160 meters. By the afternoon of the same day there seemed to have been a general mixing of the water at all depths as shown by the pH and oxygen content. At station G, on July 11, 1919, there was a rise in the pH down to 40 meters with the oxygen content within experimental error, the lower being toward the surface. Downward this was followed by a decrease in the pH down to 100 meters, then a rise down to 140 meters. The oxygen content showed still less uniformity. The only conclusion to be derived from these observations is that the water at different levels has different sources. That is, the water from different portions of the Sound, for example from different embayments, at times may become more or less stratified, due to the physiography of the region. The specific source of a given layer of the water depends upon a number of factors, chief of which are the wind, its velocity and direction, and the stage of the tide. Again the condition of the water from these various sources depends upon their locality, the weather, the tide in its relation to day and night, and the season of the year (Moore, Prideaux and Herdman 1915). The condition of the water depending upon the tide in its relation to day and night will be better understood after we have discussed the effect of vegetation upon the hydrogen-ion concentration and oxygen content of the water. Finally, at certain stages of the tide the water of the channels of the Sound may be more or less completely mixed at all depths. This conclusion is borne out both by the observations made on the pH and oxygen content of the water, and by the fact that at times there are wellings up of the water thruout the main channels of the Sound. From the observations made, no curve could be formulated to show the relation of the pH and oxygen content at different depths, and other factors. The controlling factors are very complex.

5. Hydrogen-ion concentration and oxygen content of surface sea-water, and their relation to tide

The hydrogen-ion concentration and in most cases the oxygen content of the surface sea-water at stations Q, D and A were taken at intervals thruout the course of the investigation (see tables 3, 4 and 5, and map on page 372). When these tables are examined to determine the relation of the pH and oxygen content of the surface water to the stage of the tide there seems to be no general conformity. On July 23, 1919, the pH

Table 3. *The hydrogen-ion concentration and the oxygen content of the surface sea-water at station Q.*

Date	Time	Tide	Depth in Centimeters	Temp. Centigrade	pH	Oxygen cc. per liter	Remarks
7/27*	3:30	i	10	9.75°	7.78	4.41	Intervals of sunshine
7/31*	5:25	i	10		7.80	4.30	
7/31*	5:30	i	500		7.80	4.28	
8/6*	7:00	o	10	9.8°	7.85		
(7/7*)	7:45	i	10	11.3°	7.87		
(7/8*)	9:45	i	10	10.5°	7.97		Near shore
(7/8*)	9:52	i	10	10.4°	7.95		
(7/9*)	7:30	o	10	10.3°	7.89		H_2O welling up
(7/9*)	7:35	o	10	10.6°	7.95		Near preceding
(7/9*)	7:40	h	10	10.6°	7.97		Farther away
(7/16*)	4:15	i	10	13.8°	8.12	4.23	
(7/16*)	4:20	i	500		7.92		
(7/17*)	4:10	i	10	14.1°	7.99	4.71	
(7/17*)	4:15	i	500		7.99	4.39	
(7/21*)	3:45	o	10	13.2°	7.87	4.18	
(7/21*)	3:52	o	500		7.98	4.74	
(7/22*)	3:50	o	10	13.2°	7.97	4.77	
(7/23*)	(10:40)	i	10		7.92	4.61	

Table 4. *The hydrogen-ion concentration and the oxygen content of the surface sea-water at a depth of* 10 *centimeters at station D.*

Date	Time	Tide	Temperature Centigrade	pH	Oxygen cc. per liter
7/15*	5:15	i		7.77	
7/17*	4:25	h		7.82	
(6/23*)	8:00	i	10.4°	7.97	
(6/24*)	5:40	o	11.2°	7.64	
(6/25*)	1:15	l	10.2°	7.52	
(6/26*)	4:25	i	10.2°	7.77	
(6/26*)	7:45	o	10°	7.82	
(6/27*)	8:00	o	9.6°	7.87	
(7/1*)	4:40	i		8.07	
(7/2*)	(11:37)	o	10.4°	7.78	
(7/3*)	4:15	i	10°	7.77	
(7/5*)	4:10	h	9.8°	7.77	
(7/7*)	7:20	i	10.2°	7.97	
(7/8*)	9:22	i	8.4°	7.94	
(7/9*)	3:45	o	11.5°	7.87	
(7/9*)	8:00	i	10.7°	7.87	
(7/10*)	4:40	l		7.97	
(7/11*)	8:05	o	12.1°	7.97	
(7/12*)	(10:33)	i	11.9°	7.84	
(7/12*)	7:05	o	12.6°	7.87	
(7/13*)	9:10	o	11.8°	7.92	
(7/14*)	9:15	o		7.84	
(7/17*)	5:05	i	14°	8.02	5.00
(7/21*)	4:40	i	13.2°	8.02	4.46
(7/22*)	4:25	o	13.3°	7.98	4.32

TABLE 5. *The hydrogen-ion concentration and the oxygen content of the surface sea-water at station A.*

Date	Time	Tide	Depth in cm.	Temperature Centigrade	pH	Oxygen cc. per liter
7/15*	4:15	i	10		7.77	
7/16"	3:55	i	10	11.9°	7.52	4.85
7/16"	4:20	i	500		7.47	4.62
7/31*	4:45	i	10		7.47	4.72
7/31*	5:00	i	500		7.47	4.57
(6/30*)	3:00	i	10	10°	7.67	
(7/1*)	(11:45)	o	10	9.9°	7.79	
(7/7*)	8:07	i	10	10.4°	7.97	
(7/10*)	3:15	i	10	12.2°	7.87	
(7/11*)	7:25	o	10		7.97	
(7/12*)	(9:35)	o	10	11.6°	7.90	
(7/12*)	7:20	o	10	12.7°	7.84	
(7/12*)	9:00	o	10	11.9°	7.87	
(7/14*)	2:00	i	10	12.4°	7.97	4.07
(7/17*)	3:20	i	10	13.5°	7.94	4.95
(7/21*)	(9:10)	i	10	13.3°	7.82	4.30
(7/21*)	3:20	o	10	12°	7.97	4.34
(7/22*)	3:10	o	10	13.5°	8.03	4.53
(7/23')	(5:50)	o	10	12.8°	7.87	4.26
(7/23')	(6:15)	o	10	12.8°	7.92	4.41
(7/23')	(7:00)	h	10	12.8°	7.94	4.45
(7/23')	(10:10)	i	10		7.97	4.46
(7/23')	2:50	i	10		8.02	4.58

of the surface water at station A was determined at intervals from 5:50 A. M. until 2:50 P. M. The pH rose continuously thruout the day regardless of the stage of the tide. This was doubtless due to the effect of light upon the vegetation. At station D, on June 26, 1919, at 4:25 P. M., when the tide was coming in the pH was 7.77 and at 7:45 P. M. when the tide was going out the pH was 7.82, with two tenths of a degree lower temperature. On July 12, 1919, station A, all determinations were made when the tide was going out. The lowest tide, 9:35 A. M., showed the highest pH, 7.90; the next lowest tide, 9:00 P. M., gave the next highest pH, 7.87. Station D, July 9, 1919, showed no difference between low and high tide. These are isolated cases and too much confidence cannot be placed in them as sometimes samples taken only a few feet apart on the surface of the water showed a difference in the pH with practically the same temperature. (See determinations made at station Q, July 9, 1919, table 3.) On June 25, 1919, four samples were taken between station D and Brown Island (station B) within a radius of 50 meters and with only fifty minutes elapsing between the taking of the first and last sample. The following are the determinations with the appended notes: 2:30 P. M., cloudy and drizzling rain, just southwest of Brown Island, pH 7.52, 9.7° C.; 2:50 P. M., just south of last point, no oil on surface, but few drifting Ulva, pH 7.67, 9.6° C.; 3:05 P. M., just south of second in drift of algae and oil, pH 7.55, 9.6° C.; 3:20 P. M., just south of third in clear water, pH 7.55, 9.5° C.

To avoid the errors due to isolated samples the averages of all determinations at each of the three stations were compared. It was found that the pH and oxygen content at station Q averaged 7.92+ and 4.46 cc. per liter; station D, 7.87 (and 4.59 cc. per liter from an average of only three samples); and station A, 7.83 and 5.50 cc. per liter. The open water at station Q showed a higher pH than either station D or A, and station D a higher pH than station A. The average oxygen content of the water at stations Q and A was just the reverse. If the averages of all determinations made at the surface, i. e., a depth of 10 centimeters, when the tide is coming in, are compared with the averages when the tide is going out, it is found that the pH and oxygen content at station Q are 7.93— and 4.45 cc. per liter for the incoming tide, and 7.91— and 4.48 for the out-going tide. However, the oxygen for the out-going tide was from an average of only two determinations, one of which was very low, 4.18 cc. per liter. Both the oxygen content and pH were higher at a depth of 500 centimeters (see table 3). It is interesting to note that the surface water north of Brown Island at 3:30 P. M. had a pH of 7.96 and an oxygen content of 4.19 cc. per liter. Station D showed an average of 7.92— for the incoming tide and 7.85— for the outgoing. Station A gave 7.85+ for the incoming tide and 7.87—for the outgoing. The oxygen content was the reverse, the incoming tide being 4.50— cc. per liter and the outgoing being 4.48— cc. per liter. The pH and oxygen content were both almost within the limits of experimental error but are averages of a large number of determinations. There seems to be a different relation of the pH to the tide at station D and A. It is worthy of note that the pH for the incoming and outgoing tides at station A does not go much higher than that of the outgoing tide at station D, and that the pH for the surface water near the village of Friday Harbor, station Y, was found to be at least 0.1 pH lower than the surface water in the open channel near by. Thus, it seems that the condition of the water at station A is influenced by that of the water at both stations D and Y. Station B perhaps shows better the effect of an embayment on the condition of the water than either station D or A. The average pH for all determinations at station B was 7.88. The average for the incoming tide was 7.96 and the outgoing 7.76. This shows a greater difference than that at station D, and much greater than that at station A. With few exceptions the pH and oxygen content were raised in passing from station A toward Brown Island, and from station D toward the beach. This fact is emphasized by determinations made at station F, July 21, 1919, at 10:05 A. M., when the tide was coming in. At this time there were two currents meeting at station F. The two currents showed a difference of 0.9 of a degree temperature and

a difference of 0.21 pH and 0.15 cc. of oxygen per liter. The higher temperature, pH, and oxygen content were from water which had just bathed eel-grass near station A and Brown Island. The other current had its source from the main channel north and east of Brown Island. The two samples were taken not more than 10 meters apart. From these observations and also those made at the surface at different regions of the Sound (see table 1), it seems that the water passing into an embayment of comparatively deep water reduces the pH but increases the oxygen content of that of the open water when entering. The pH of the water bathing the vegetation as well as the oxygen content is materially increased during daylight (see table 6): but the rise in the pH is completely counteracted, and the oxygen content largely so, by the water of the embayment proper, possibly due to decay taking place at the bottom. This is true of water only of the deeper portions of the embayment. Water that has bathed the vegetation along the shallower portions may flow out of the embayment without mixing with that of the deeper portion and thus still retain its high pH and oxygen content, provided that it is daylight. Evidence which will be given later will show that probably this is not true of water bathing the vegetation during the night. The observations made at station Q as well as those at stations D, A, B, F and Y point to the probability that the water entering the Sound at high tide has a higher pH and a lower oxygen content than when flowing out at low tide. This point needs further investigation. Station Q was not located in the center of the main channel as it was dangerous there at times in a row boat. Thus the water at station Q might have been affected more by the water of the embayments than that in the main channel proper. The pH and oxygen content of the ocean water in the vicinity of the Puget Sound region is not known.

6. Hydrogen-ion concentration of sea-water of a small lagoon

The lagoon chosen for these observations is located in the embayment (station D) near the Biological Station. The communication with the open water was completely cut off at about half tide. The bottom was more or less completely covered with *Ulva*. This *Ulva*, however, was often disturbed by seining for the viviparous perch and otherwise, as it was the chosen bathing resort for the children of the village of Friday Harbor. This lagoon without exception showed a high pH and was supersaturated with oxygen. One determination gave 10.70 cc. of oxygen per liter. No determinations were made during the early hours of the morning, but it is presumed that the pH and oxygen content were materially reduced during the night [see determinations made (7/23), table 7].

Table 6. *The hydrogen-ion concentration of a lagoon at a depth of 10 centimeters.*

Date	Time	Tide	Temperature Centigrade	pH	Remarks
(6/26*)	4:45	i	16.2°	8.38	
(6/26*)	4:50	i	13°	8.07	At mouth of lagoon
(6/26*)	5:06	o	11.4°	7.89	20 meters from mouth
(6/26*)	8:05	o	14.2°	8.37	
(7/9*)	8:10	i	18.2°	8.62	
(7/11*)	7:35	o	16,2°	8.62	
(7/11*)	7:45	o	13.4°	8.17	20 meters from mouth
(7/12*)	(10:22)		17.1°	8.57	
(7/13*)	9:55	o	17.8°	8.07	
(7/17*)	5:30	i	19°	8.54	

Table 7. *The hydrogen-ion concentration and oxygen content of sea-water bathing vegetation*

Date	Time	Depth in cm.	Temperature Centigrade	pH	Oxygen cc. per liter	Remarks
(7/17*)	1:45	15	16.8°	9.3 +1.	12.13+6.79	Ulva 30 cm. deep
(7/17*)	2:00	100	15.8°	8.57+0.27	5.54 - 0.01	Ulva 200 cm. deep
(7/23*)	(6:25)	20	13.3°	7.77 - 0.16	3.57 - 0.84	Ulva 50 cm. deep
(7/23')	(9:25)	40		8.95+1.00	11.45+6.09	Ulva 70 cm. deep
(7/23')	3:10	380		8.27+0.25	5.02+1.44	Ulva 400 cm. deep
(7/23')	3:20	20		8.17+0.15	4.78+0.20	Ulva 400 cm. deep
						Eel grass
8/6*	3:40	10	11.3°	7.72+0.10	6.05+0.49	" " 500 cm. deep
8/6*	4:00	450		7.73+0.11	6.25+0.69	" " 500 cm. deep
(7/16*)	2:20	10	15.1°	8.67+0.70	6.75+2.78	" "
(7/23*)	(6:45)	20	12.9°	7.80 - 0.08	4.22 - 0.19	" "
(7/23')	(9:35)			8.37+0.40	4.81+0.35	" "
(7/16*)	3:35	20	13.4°	7.97 - 0.50	4.81+0.64	Kelp
(7/17*)	3:50	20	14°	8.00+0.02	4.76 - 0.98	Kelp
(7/21*)	(10:35)	20	12.8°	8.03+0.05	4.45 - 0.02	Kelp
(7/22*)	3:30	20	13.3°	7.97 - 0.15	4.54 - 0.06	Kelp

7. Effect of vegetation and animal organisms upon hydrogen-ion concentration and oxygen content of sea-water

Table 7 gives a few of the determinations made of the sea-water bathing vegetation. By comparing this table with all other tables it is found that the pH and oxygen content of the water bathing vegetation are very materially raised during daylight by the photosynthetic activity of the vegetation. This is in agreement with other workers (Palitzsch, McClendon, Gaarder and others). There are some exceptions shown in the case of the kelp, *Nereocystis luetkeana*. These exceptions can possibly be explained by the fact that the kelp selected for these observations was located on the side of Brown Island nearest station J; and the water bathing the kelp and that a short distance away might have had an entirely different source due to the current. Determinations made early in the morning of water bathing the Ulva and eel-grass showed that both the pH and oxygen content were lowered below that of the surrounding water during the night. The plus sign in the table indicates that the pH or the oxygen content was higher than that of the surrounding water, and the minus means that it was lower. On July 23, 1919, at 6:25 A. M. the water

bathing Ulva had a pH of 7.77 and an oxygen content of 3.57 cc. per liter, or a pH of 0.16 and an oxygen content of 0.84 cc. per liter lower than that of sea-water in the main channel only a short distance away at the same time. But in three hours despite the cloudy weather the pH had increased to 8.95 and the oxygen content to 11.45 cc. per liter of water bathing Ulva in the same locality with only 20 centimeters greater depth. The same relation was shown by the eel-grass. These observations and those made at stations Q, D and A will help to explain the non-conformity of the pH and oxygen content at different depths.

Table 8 gives the determinations made in *Upogebia pugetensis* holes at low tide. The pH was always found to be below normal and the oxygen content was very materially lowered, the highest found being 0.75 cc. per liter and the lowest 0.15 cc. per liter. This is in keeping with experiments performed on the respiration of marine invertebrates by Moore, Edie, Whitley and Dakin (1912) and by Henze (1910) when animals were kept in closed vessels.

TABLE 8. *The hydrogen-ion concentration and the oxygen content of the water in Upogebia holes at low tide*

Date	pH	Oxygen cc. per liter	Date	pH	Oxygen cc. per liter
7/27	7.77	0.34	(6/30)	7.22	0.59
7/27	7.82	0.75	(7/23)	7.27	0.25
(6/30)	7.27	0.43	(7/23)	7.22	0.15

8. DISCUSSION

In recent years much work has been done showing the great importance of the hydrogen-ion concentration in physiological and chemico-physiological processes in general. Bacteriologists and those working on enzymatic and fermentation processes have long since learned the necessity of taking the hydrogen-ion into consideration. The sea-water can be considered as a vast medium in which the physiological processes of the marine organisms are taking place. Or it can be considered as a biological fluid with a buffer action somewhat less than that of the blood of vertebrates. One can hardly doubt that if one is going to make an exhaustive study of the biology of the sea or sea-water that the hydrogen-ion concentration is one of the important factors with which it is necessary to reckon. Attention has been called to the importance of the hydrogen-ion concentration of the sea-water by Loeb (1903, 1906), Moore, Roaf and Whitley (1905), Delage (1906), Herbst (1906), Loeb and Wasteneys (1911), Warburg (1914) and others for the first stages of development of certain of the marine animals. Each organism seems to have a definite range of hydrogen-ion concentration within which development can take

place. The range perhaps varies for different species of animals. McClendon (1918) claims that there is no optimum pH for plants but that the range of pH compatible with the life of sea weeds is rather broad and may be different for different species. The foregoing observations show that the range of the pH differs markedly for different habitats or rather different classes of habitats. The fixed plants and the animals of this plant habitat, unless they have a diurnal migration, must tolerate large and sometimes very rapid changes of the pH every 24 hours, at any rate, during the most vigorous growing season of the plants. Perhaps the animals that have to tolerate the greatest as well as the most rapid change in both the pH and the oxygen content of the water are those which live in burrows among the Ulva above low tide level. For example, when the tide comes in, the first water that comes in contact with *Upogebia pugetensis,* which is bathed with water perhaps with as low a pH as 7.22 and oxygen content of 0.15 cc. per liter, may have a pH as high as 9.3 and be supersaturated with oxygen. Or the change in pH may be as great as 1.98, and in oxygen content sufficient in itself to supersaturate the water. However, the rapidity with which these changes take place can be controlled largely by the burrow-living animals. The animals of the open water or the plankton do not have to tolerate such great variations in pH and oxygen content. Bethe (1909) found that the rhythmic movements of medusae are hastened if the reaction of the water is changed from a Cn of 10^{-8} to 10^{-7} or 10^{-6}, while a concentration of 10^{-9} or 10^{-5} will diminish or paralyze the movements. McClendon (1916) found that all marine animals tested by him died more quickly if the pH was changed beyond the limits of 6 to 8.25. The oxygen consumption of certain marine invertebrates is known to vary with the pH (McClendon 1917) and the oxygen tension (Moore, Roaf and Whitley 1905, Henze 1910, Loeb and Wasteneys 1911, McClendon 1917b and 1918, and others). While an exhaustive study has not been made of the fauna of the Puget Sound region by the writer, the luxuriance of the fauna at certain localities, and the comparative average sizes of individuals of the same species, as for example the barnacles at different localities, seem to suggest that the specific pH range has more to do with the compatability of their habitat than other factors such as the oxygen content of the water. This needs further investigation. The foregoing observations also suggest that Puget Sound and similar regions are ideal natural laboratories for the study of the causes of vertical migrations of plankton organisms. Here are found natural variations in the relations of light, carbon dioxide and oxygen tensions of the water without the objections used as argument against purely laboratory observations.

9. Acknowledgments

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On the Action of Certain Substances on Oxygen Consumption. IV. Further Experiments on the Action of Potassium Cyanide on Invertebrates

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This paper is a report of some further experiments on the effect of potassium cyanide on the rate of oxygen consumption. It has already been shown for a number of organisms that this substance lowers their respiratory rate. The literature dealing with this subject has been referred to in previous papers (Hyman, 1916, 1919a, 1919b) and need not be again considered here; and in these papers are represented data showing the depressing effect of potassium cyanide on the oxygen consumption of some of the lower invertebrates. The present paper merely attempts to extend these observations to members of other invertebrate phyla, namely, the echinoderms, mollusks and arthropods, concerning which no data have as yet, to my knowledge, been published.

This work was carried out at the Puget Sound Biological Station, Friday Harbor, Washington, where the University of Chicago kindly provided me with a research room.

1. Method. The method of procedure in such experiments is relatively simple and is described in detail in a previous paper (1919a). The animals to be tested are placed in wide-mouthed bottles of about 500 cc. capacity; these bottles are filled air-tight with sea-water of known oxygen content and the animals allowed to respire in them for a given length of time; a sample of the water is then drawn off from the bottle and its oxygen content determined; and the difference between this and the original oxygen content of the water gives the amount of oxygen consumed by the animal. The oxygen content was determined by Winkler's method. After determining the rate of oxygen consumption of the animal in normal sea-water, a certain amount of potassium cyanide is added to the sea-water and the respiration of the animal in the cyanide-containing sea-water determined. In all cases, two independent determinations of the normal respiration and the respiration in the presence of cyanide were made. During the experiment the bottles containing the animals were suspended in the water at the laboratory docks in order that the temperature might remain fairly constant during the five hours occu-

pied by each experiment and in order that this temperature might be the same as that in which the animals normally live. The temperature variation during such an experiment was generally about one degree Centigrade, sometimes more, sometimes less, but never more than two degrees. The temperatures recorded during the summer of 1920 varied from 10.5 to 13° C.

2. *Experiments on sea-urchins.* Small individuals of *Strongylocentrotus drobachiensis* and *S. franciscanus* were used, usually one individual being placed in each experimental bottle. The results are tabulated in table 1. Experiment 1 was performed on *S. franciscanus;* the other experiments on *S. drobachiensis.*

3. *Experiments on starfish.* Small individuals of *Asterias victoriana* were employed, one in each bottle. The results are given in table 1.

4. *Experiments on sea-cucumbers.* These experiments were performed on small individuals of *Cucumaria chronhjelmi* (Nos. 1 to 3), *Stichopus californicus* (Nos. 4 to 6) and *Cucumaria japonica* (No. 7.) The results are tabulated in table 2.

5. *Experiments on chitons.* The species used was *Catherina tunicata.* The data are given in table 3.

6. *Experiments on bivalves. Mytilus edulis* was employed, generally two individuals in each bottle. The animals were placed in the experimental bottles the day before the tests were carried out in order that they might fasten themselvs to the glass. The results are given in table 3.

7. *Experiments on nudibranchs.* Three species of nudibranchs were used, of which only one was identified. The species identified was *Triopha carpenteri,* employed in experiment 1, table 4. The form used in experiment 2 was a large white Dorid, probably an Anisodoris. The third species, used in experiments 3 to 5, was the little brightly colored Eolid, very common on the eel grass at Friday Harbor. As this species is rather small, five or six individuals were placed in each experimental bottle. The data on the nudibranchs appear in table 4.

8. *Experiments on crabs.* The common purple shore crab, *Hemigrapsus nudus,* was used. Two or three individuals were placed in each experimental bottle. This species was found to be remarkably susceptible to cyanide. The results are given in table 5.

TABLE 1. *Action of potassium cyanide on the oxygen consumption of sea-urchins and starfish.*

	Sea-urchins					Starfish		
No. of Exp.	1	2	3	4	5	1	2	3
Oxygen consumed, cubic centimeters per hour.								
1st hr. normal	0.50	0.42	0.45	0.42	0.46	0.48	0.81	0.70
2nd hr. normal	0.59	0.46	0.42	0.43	0.47	0.55	0.76	0.80
Conc. of KNC	1-2000 mol.		1-5000 mol.					
1st hr. KNC...	0.26	0.15	0.32	0.37	0.34	0.20	0.34	0.17
2nd hr. KNC..	0.29	0.19	0.30	0.36	0.30	0.19	0.31	0.20

TABLE 2. *Action of potassium cyanide on the oxygen consumption of sea-cucumbers.*

No. of Exp.	1	2	3	4	5	6	7
Cubic centimeters of oxygen consumed							
	per 2 hours			per 1½ hours			per hr.
1st period normal	0.13	0.11	0.27	0.39	0.31	0.29	0.37
2nd period normal....	0.13	0.13	0.30	0.37	0.36	0.24	0.27
Conc. of KNC	1/5000 mol.			1/2500 mol.			$^1/_{1000}$ mol
1st period KNC.....	0.08	0.08	0.11	0.13	0.14	0.13	0.15
2nd period KNC.....	0.10	0.07	0.10	0.09	0.12	0.14	0.10

TABLE 3. *Action of potassium cyanide on the oxygen consumption of chitons and bivalves*

	Chitons			Mytilus		
No. of Exp.	1	2	3	1	2	3
Oxygen consumed, cubic centimeters per hour						
1st hour normal	0.30	0.64	0.47	1.13	1.86	1.35
2nd hour normal.....	0.42	0.71	0.63	1.38	2.04	1.67
Conc. of KNC	1/5000 mol.			1/5000 mol.		
1st hour KNC.....	0.20	0.30	0.20	0.32	0.26	0.29
2nd hour KNC.....	0.21	0.28	0.22	0.31	0.27	0.11

Table 4. *Action of potassium cyanide on the oxygen consumption of nudibranchs*

No. of Exp.	1	2	3	4	5
Oxygen consumed, cubic centimeters per hour					
1st hour normal	0.75	1.23	0.60	0.61	0.92
2nd hour normal	0.65		0.72	0.55	0.90
Conc. of KNC	$^1/_{1000}$ mol		1/10000 mol.		
1st hour KNC	0.17	0.59	0.10	0.25	0.17
2nd hour KNC	0.17	0.59	0.13	0.32	0.17

Table 5. *Action of potassium cyanide on the oxygen consumption of the shore crab*

No. of Exp.	1	2	3	4	5	6
Oxygen consumed, cubic centimeters per hour						
1st hour normal	1.46	1.84	1.41	1.41	2.13	1.29
2nd hour normal		1.60	1.24	1.02	1.72	1.08
Conc. of KNC	1/5000 mol.			1/20000 mol		
1st hour KNC	0.19	0.21	0.15	0.15	0.27	0.16
2nd hour KNC				0.24	0.07	0.14

9. *Results and discussion.* As shown in the tables the oxygen consumption is in all cases markedly reduced in the presence of potassium cyanide. This result is in agreement with that previously found for a number of other organisms. The question arises whether this depression of the oxygen consumption may not be due, in part at least, to some effect of the cyanide on the degree of muscular activity of the animal or the degree of extension of respiratory structures. Particular note was made of these matters during the experiments. In the case of the shore crabs only could any difference in the amount of movement be detected in the presence of cyanide as contrasted with movement in normal sea-water. These animals, to my surprise, were very susceptible to cyanide and showed signs of anaesthesia in the 1-5000 molecular solution. Owing to this circumstance they were left in this solution only one hour. In order to eliminate the possibility that the depression of oxygen consumption observed in the case of these animals was due to the anaesthesia three experiments were run using a much more dilute solution, 1-20000 solution.

In this dilution, anaesthesia did not occur; nevertheless the oxygen consumption again fell during the exposure to cyanide.

Particular note was also made of the condition of respiratory processes and structures during the experiments. No differences were observed in the degree of extension of the tube feet and branchiae of the starfish and sea-urchins, the tentacles of the holothurians, or the gills of the nudibranchs in normal and cyanide-containing sea-water. In the case of Mytilus, it seemed to me that the siphons were not so widely open during the exposure to cyanide as before. This may account for part of the very great decrease of oxygen consumption observed in the case of these animals.

In general, then, it may be said, both from these and from previous experiments, that the decrease in the rate of oxygen consumption which occurs in the presence of potassium cyanide is not due to any effect of the cyanide upon either muscular activity or respiratory mechanisms but must be ascribed to some direct action of the cyanide upon the respiring protoplasm. The nature of this action has not yet been ascertained with certainty but the opinion of the majority of investigators favors the idea that it is chemical, i. e., that the cyanide unites with the protoplasm.

That the action of the cyanide is reversible has already been shown in previous experiments (Hyman, 1916, 1919a, 1919b). After the animals are returned to normal water, the oxygen consumption returns to the normal value, provided that other factors have been eliminated. When, however, animals are removed from their environments and kept in captivity for experimental purposes, their rate of oxygen consumption falls rather rapidly at first. This fall is probably due to starvation. The materials used in the present experiments were all freshly collected animals and it was therefore impractical to determine whether or not the oxygen consumption returned to the normal value after exposure to cyanide since the oxygen consumption would fall anyway owing to starvation or other factors. These factors would not affect the oxygen consumption within the four or five hours required for each experiment but would be in evidence by the following day. Owing to these circumstances, the oxygen consumption on the day following exposure to cyanide may or may not be as high as before such exposure. In some tests made in connection with the present experiments results of this nature were obtained. In some of the individuals the oxygen consumption had returned to the original figure on the following day, while in others it was lower than originally. As already stated this latter result commonly occurs whether the animals have been exposed to the cyanide or not. Not much attention was paid to the question of the reversability of the action of cyanide in the present experiments as the reversibility has already been sufficiently demonstrated in

previous experiments. It may be stated that the animals after the cyanide treatment appeared entirely normal in all respects.

10. *Conclusion.* Potassium cyanide was found to cause a marked decrease in the rate of oxygen consumption of some echinoderms, mollusks, and a crustacean. This result is in agreement with a large body of previous work. Potassium cyanide may therefore be used as an agent to depress the respiratory rate of organisms for experimental purposes.

Citations

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1916. On the action of certain substances on oxygen consumption. I. The action of potassium cyanide. Amer. Jour. Physiol., Vol. 40, pp. 238-248.

1919a. On the action of certain substances on oxygen consumption. II. Action of potassium cyanide on Planaria. Amer. Jour. Physiol., Vol. 48, pp. 340-371.

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Plant Migration Along a Partly Drained Lake

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A few years ago a canal was cut along the southern boundary of the campus of the University of Washington thus connecting Lake Washington with Lake Union. This lowered Lake Washington about 11 feet (3.4 m.). There was thus left a shore largely unoccupied, largely of excellent soil, in certain localities indeed almost wholly of decomposing vegetation. This offered a fine opportunity for the observation of the adjustments of the vegetation to the new water level.

Three chief lines of observation were followed: (a) What is revealed by the uncovering of the submerged lake margin? (b) What will happen to the plants along the old water level and below it? (c) What gets established on the unoccupied area, and why?

The recession of the water revealed the following plants as the commoner: *Nuphar polysepala* (yellow water lily), *Brasenia schreberi* (water shield), *Scirpus occidentalis* (tule), *Elodea canadensis* (water weed), *Utricularia vulgaris* (bladderwort), *Ceratophyllum demersum* (hornwort), *Myriophyllum* sp. (water milfoil), *Potamogeton natans* (pondweed), *Polygonum amphibium* (water smartweed), *Isoetes echinospora* (quillwort).

Of these the following did not reappear above the new water line: Elodea, Utricularia, Ceratophyllum and Myriophyllum. All are attenuated plants which dry easily. Only Ceratophyllum, of the four, was observed the first year below the new level. Elodea alone of the four has not been seen since.

Brasenia and Potamogeton reappeared in the old location, but did not reach maturity. Potamogeton has again appeared below the new level, but Brasenia has not.

Isoetes was abundant in suitable edaphic situations 8 to 14 feet (2.5 to 4.3 m.) below the old water level at the time of recession. It grew well the first year even where uncovered. But the winds of the next winter caused waves which eroded the new shore, and with it went all the Isoetes. Neither in the old location nor under the new level has it been found since. It might, however, be fairly abundant 8 to 14 feet (2.5 to 4.3 m.) below the new level without one finding it.

Nuphar reappeared in the old location for about 2 years, the time varying with the dampness of the location. The leaves were not over half size and the petioles not over 2 inches (5 cm.) long. It is reappearing along the new level. The dense growth of other plants on the old location surely must have been a factor in the disappearance of Nuphar above the water line, although not the only one. Parenthetically it may be remarked that the first year after and 8-foot (2.5-meter) rise in Trout Lake at Friday Harbor, Washington, petioles of Nuphar were observed 9 feet (2.8 m.) long, bearing nearly normal blades.

Scirpus continued its subterranean shoots and thus grew out to the new water level, when the uncovered shore was not over 75 feet (23 m.) wide. A more gentle slope usually held enough water for the growth of the plants to continue in the old habitat. Where the water was insufficient the plants high up on shore died in a few years.

Thus the most delicate plants died at once. Certain perennials with greater ability to withstand dryness, or with more stored food as possible factors, persisted for a year or more. One was washed away. One with a very large rhizome grew for more than one year. One extended its rhizomes into the new habitat. All left the old habitat or were crowded out, within three years, unless the location was such that springs, streamlets or almost level muck kept the soil soggy.

The constantly damp regions of gentle slope and much decomposing vegetation were the most completely revegetated the first year. These are the only ones which will be considered here concerning the new plant covering, whose most abundant species are given below. The first in abundance is Typha. It is marked 100 in the scale so that other plants may be numerically compared with it in this respect.

Typha latifolia (cat tail)	100
Bidens cernua (Spanish needle)	90
Scirpus occidentalis (tule)	70
Juncus bufonius (toad rush)	50
Eleocharis obtusa (spike rush)	30
Sparganium androcladium (bur reed)	25
Oenanthe sarmentosa (water celery)	20
Epilobium paniculatum (fire weed)	12
Polygonum hydropiperoides (water pepper)	10

In Union Bay the water receded for more than a quarter of a mile (about 400 m.). How did the seed get there so abundantly?

Typha and Epilobium are distributed by wind, so their reaching the area in abundance is not surprising. One autumn a strong wind from this

area, during one night, sowed seeds of cat tail on the wet roof of a house over 1¼ miles (2 km.) away, so thickly that there was a seed for about every 3 square inches of roof.

Scirpus, Eleocharis, Sparganium and Oenanthe are usually considered as plants having their seeds distributed through their floating on water. These might drift ashore as the level recedes thus sowing the area; or birds might carry them on their feet; or the sunken seeds might lie on the bottom and grow when uncovered.

Bidens is plainly fitted to have its seeds carried by animals. Surely it could not be thus carried as if sown, over a large area of fairly thin mud. One is inclined to look for some other method of dispersal.

How Polygonum and Juncus are distributed is somewhat uncertain.

Tests were made on the floating power of seeds. Many different kinds were gathered, specially those which could be secured from the newly overgrown areas. The naturally attached parts were left on, so the floating power of the seed in the condition in which it might fall into the water would be determined. These were put into dishes of water and left to see how long they would float. The dishes were shaken every day to simulate the natural roughness of water, and to be sure that the seeds did not float by mere surface tension. Of those plants mentioned above as most abundant on a wet flat, all except Juncus floated for 30 days, at which time the experiment was discontinued. *Juncus bufonius* grew mostly near the former level and along streamlets, from which one might expect dispersal by animals following the streamlets, or by flowing water.

Since the seeds of Bidens floated for at least 30 days, it is quite likely that water is one of the instruments of its dispersal. In fact Typha, Bidens and Epilobium are on an equality with water-dispersed plants, and have the wind or animal dispersal in addition.

A test of the seeds of many dry land plants showed that almost 80 per cent floated for at least 30 days, while only about 5 per cent sank within the first 5 minutes. Floating seeds are very much more common than is usually supposed. When seeds are fitted for distribution by animals, by wind or by some other means, it is no evidence that they may not also be distributed by water. Ravn's contention that shore plants have floating seeds might be extended by saying that the same is true of most plants. One should not conclude that dry-land plants are wanting along wet shores because the seeds have not the same advantages of distribution as those of shore plants; indeed many, and perhaps most of them, have better means. Those also fitted for wind dispersal have a powerful aid in getting to the water.

The fruits of the following plants did not sink within 30 days, the duration of the experiment:

Bidens elata
Bidens cernua
Epilobium paniculatum
Typha latifolia
Alnus oregona
Aster eatoni
Spiraea douglasii
Hypochaeris radicata
Achillea millefolium
Mentha piperita
Rumex persicarioides
Erigeron canadensis
Ranunculus pennsylvanicus
Rumex conglomeratus
Rumex obtusifolius
Eleocharis obtusa
Polygonum hydropiperoides
Scirpus occidentalis
Roripa paulstris
Sparganium androcladium
Oenanthe sarmentosa
Holcus lanatus
Polygonium persicaria

The fruits of *Holcus lanatus* germinated floating and continued to grow on the surface until the end of the 30 days. The hulled seeds of *Polyganium persicaria* did not float so well; only 60 per cent remained afloat for 30 days.

The fruits of the following did not all float for 30 days: *Lactuca pulchella,* 10 per cent sank; *Rumex occidentalis,* 20 per cent sank; *Plantago major,* all sank; *Anaphalis margaritacea,* 30 per cent sank and germinated on bottom, 20 per cent germinated floating, 50 per cent floated without germinating; *Sisymbrium altissimum,* all sank within 5 minutes; *Juncus bufonius,* all sank at once. This last plant is a water loving one, growing in wet places and close along fresh water shores, yet its seeds do not float at all.

No experiments were made to test the germinating power of seeds which had been afloat for 30 days. Experiments designed to show whether the seeds might lie in the mud on the bottom, and germinate when the water recedes and uncovers them, were not conclusive; the indications are that the seeds in the mud are not sufficiently abundant to explain the distribution, although some are present.

One other observation should receive attention. The seeds of *Carduus lanceolatus* and *Carduus edulis,* both common thistles in western Washington, may be distributed in a manner which seems not to be recorded. They roll on quiet water like a tumbleweed on a prairie. The pappus is not easily wetted and hence surface tension prevents sinking. Where these plants are abundant one can find ponds whose margins appear from a distance to have a line of froth. Examination proves it to be a ridge of thistle seeds as much as 2 inches (5 cm.) high. The rate of rolling depends upon the rate of the wind. The most rapid observed was about that of a good walker perhaps 5 miles (about 8.1 km.) per hour.

Summary

1. Shore plants must migrate with the water level or perish when the level falls permanently.

2. Some make the change easily, others with difficulty.

3. Erosion by waves is a factor in keeping some plants below a certain depth in the water.

4. Floating seeds are so general that it is doubtful whether this characteristic is any considerable factor in determining the habits of shore plants.

5. The seeds of some thistles roll like tumbleweeds on smooth water.

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