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# A STUDY OF THE REPRODUCTIVE BIOLOGY OF THE RED ABALONE, *HALIOTIS RUFESCENS* SWAINSON, NEAR MENDOCINO, CALIFORNIA<sup>1</sup>

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The reproductive cycles of two subtidal populations of the red abalone, *Haliotis rufescens*, were studied at Point Cabrillo Lighthouse Station and Van Damme State Park near Mendocino, California. From June 1972, through March 1974, gametogenesis was monitored histologically. Both populations spawned during spring and early summer. Not all members of either population spawned during a season. Fecundity was estimated for females ranging in shell lengths 134.00 to 198.5 mm (5.3 to 7.8 inches). The lowest and highest estimates were 619,000 and 12,575,000 ripe oocytes per ovary. The minimum size at sexual maturity was investigated. The smallest male was 84.5 mm (3.3 inches) and the smallest female was 39.5 mm (1.6 inches). Females matured at a smaller size than males. A possible mode of gamete resorption was noted.

## INTRODUCTION

The purpose of our study was to determine minimum size at sexual maturity, to measure fecundity, and to monitor histologically the reproductive cycle of two populations of the red abalone, *Haliotis rufescens* Swainson, for 2 years near Mendocino, California.

Early investigators believed that the red abalone spawned during late winter and early spring (Heath 1925, Bonnot 1930, and Croker 1931). Boolootian, Farmanfarmaian and Giese (1962) used a gonad index to detect spawning in a red abalone population at Pacific Grove, California. Their gonad index is the ratio of the cross-sectional area of the gonad, at a fixed location, to the shell length times 100. The index allows for detection of reduction in gonad size during spawning. No definite spawning cycle was detected and ripe gametes were present the year round. Young and DeMartini (1970) detected the presence of mature gametes throughout the year in red abalones near Fort Bragg, California. Additionally, they found necrotic oocytes in females. Shibui (1971) studied red abalone imported to Japan from California and found gonadal maturation optimal at temperatures ranging from 14 to 20 C (57 to 68 F).

Leighton (1974) noted that southern California red abalones spawned in the laboratory every month of the year. Price (1974), using a gonadal bulk index to monitor a natural population of red abalones in southern California, found that spawning occurred in April, with possible minor spawnings in January and September.

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Giese (1959) noted that periodic histological examination of gonads over several years is an excellent method for determining the time and duration of reproductive cycles in many marine invertebrates.

#### METHODS AND MATERIALS

From June 1972 through April 1973, 10 red abalones were collected monthly from a subtidal population at the Point Cabrillo Lighthouse Station, Mendocino, California. In April 1973, sampling of a second population was initiated at Van Damme State Park, Mendocino, California. Subsequently, these two populations will be referred to, respectively, as the Point Cabrillo and Van Damme popula-

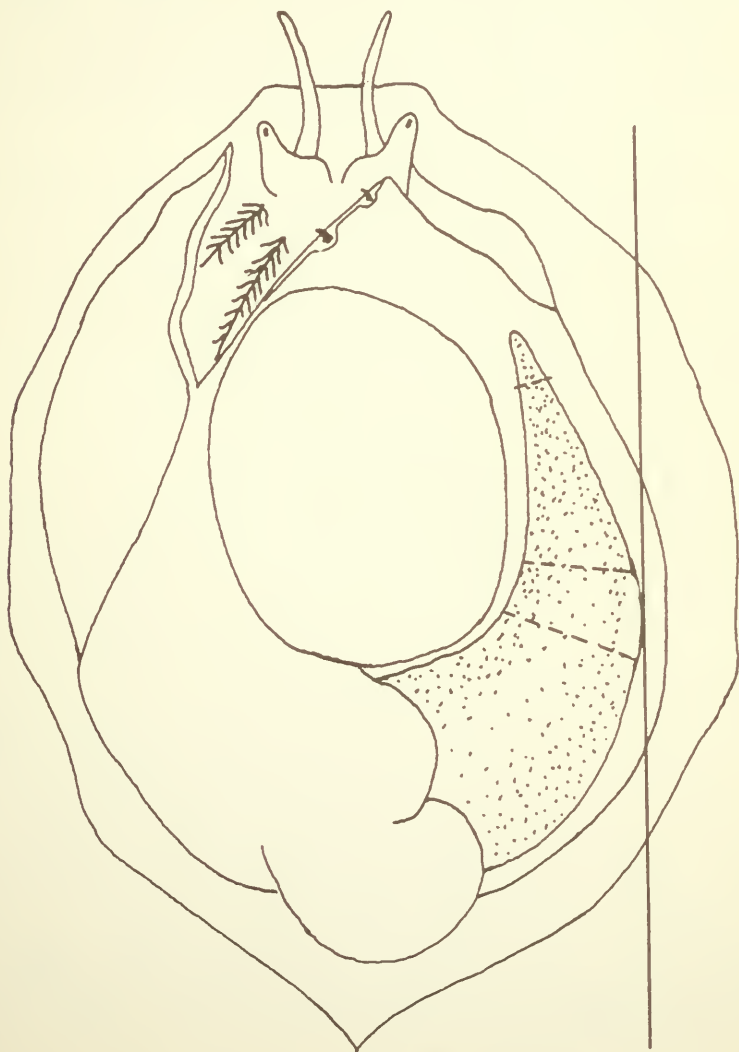


FIGURE 1. *Haliotis rufescens* with shell removed, showing conical appendage (stippled) and sampled areas of conical appendage (broken lines). From Young and DeMartini (1970).



tions. Sampling continued through April 1974. In May 1973, the sample was increased from 10 to 15 abalones per population. Monthly sampling was often interrupted by adverse conditions, especially during winter. Abalones were procured using scuba at depths between 7.5 and 15 m (25 and 50 ft). We collected 321 specimens during this study.

Shell length in millimeters, total weight, shell weight, and shucked body weight in grams, sex, and remarks concerning macroscopic features were recorded for each specimen. From each specimen, two pieces of conical appendage, consisting of digestive gland and surrounding gonad, were excised from the tip and the midportion. The latter section was determined by locating the tangent of the conical appendage parallel to the longitudinal axis of the abalone as done by Young and DeMartini (1970) (Figure 1). Excised pieces were fixed and stored in a mixture of formalin, ethanol, and glacial acetic acid (FAA). Tissue was processed in an Autotechnicon. At least three slides were processed for each piece of gonad and examined histologically.

Ninety-nine animals were collected at Point Cabrillo Lighthouse Station during summer 1974 to determine minimum size at sexual maturity. Shell length, total weight, and shucked weight were recorded for each specimen. The sex was recorded for those specimens displaying gonadal pigmentation, the ovaries green and the testes cream-colored. Shell lengths ranged from 21.5 to 204.5 mm (0.8 to 8.1 inches). The entire conical appendage was removed and fixed for specimens up to approximately 120 mm (4.7 inches). Apical and lateral pieces of gonad were excised from larger specimens and tissues were prepared as previously mentioned. Slides were examined then to assess gametogenic activity.

Thirty-three females were collected at Point Cabrillo for fecundity estimates on December 3, 1973. Twenty-five specimens, a minimum of 134.0 mm (5.3 inches), were used for counting oocytes because smaller specimens had thin ovaries which were not readily separated from the digestive glands. The entire conical appendage was excised and fixed in F.A.A., and subsequently split longitudinally and slashed every few centimeters to assure thorough fixation. After a few days, the F.A.A. was replaced by 70% ethanol. Later, the ovary was dissected from the digestive gland and weighed to the nearest 0.1 g. One piece, weighing approximately 0.03 to 0.06 g, was excised from each of three portions of the gonad, the tip, midportion and base. This method is similar to Newman's (1967). Oocytes were then teased from the trabeculae of each piece with a small coarse paint brush. Next, oocytes from each piece were dispersed into 100 ml of tap water, a procedure similar to Poore's (1973). The beaker and its contents were placed on a magnetic stirring plate. Subsamples of 2 ml were pipetted from the beaker while the liquid was agitated. The subsample was placed in a watch glass and larger oocytes (160 to 250 microns in diameter) were counted using a dissecting microscope. The subsample was then returned to the beaker and two more subsamples were drawn and counted, yielding three counts per piece of excised ovary. Because the variation about the mean for the subsamples was slight, the means were used to calculate the total number of large oocytes in each ovary.



## RESULTS AND DISCUSSION

### Reproductive Cycle

Histological examination of specimens collected during summer 1972 indicated that part of the Point Cabrillo population spawned during the preceding spring and early summer. Some specimens contained only early gametogenic stages, while others were full of apparently mature gametes. Still other specimens contained both early stages and ripe, residual gametes.

During autumn 1972, some specimens were still full of either spermatozoa or large oocytes (160 to 250 microns). Many of the oocytes were necrotic. By the end of autumn, up to about 90% of the oocytes in a cross-section of ovary would be necrotic, while other specimens contained several gametogenic stages. Testes contained stages from spermatogonia through spermatozoa. Ovaries contained oogonia and a spectrum of oocytes up to 250 microns in diameter. Large oocytes in some of these females were necrotic, and were probably residuals from the preceding spawning.

By winter 1972-73, all specimens contained maximum densities of either spermatozoa or large oocytes. Necrotic oocytes were present in some of the females and the quantities varied individually. Up to about 95% of the oocytes in a cross-section of ovary would be necrotic.

During spring 1973, spawning occurred in both the Point Cabrillo and Van Damme populations. As in spring 1972, only a portion of either population spawned. Of the 19 Point Cabrillo males collected from April 1973 through June 26, 1973, four contained maximum densities of spermatozoa, two lacked spermatozoa and displayed intense proliferation of early spermatogenesis and 15 displayed intense proliferation with few spermatozoa. Of the 33 females inspected during this period, 14 were ripe and the other 19 contained few large oocytes and displayed intense proliferation of small oocytes up to 40 microns. Necrotic oocytes were still present in some females. Van Damme specimens had similar gametogenic conditions.

During the summer and autumn 1973, both populations displayed the same variety of gametogenic events as were noted from the Point Cabrillo specimens in summer and autumn 1972.

By January 1974, all specimens contained maximum densities of either spermatozoa or large oocytes. Additionally, necrotic oocytes were present in some ovaries as noted for winter 1972-73. In March 1974, one female displayed spawning; all others were full of gametes.

Field observations support the histological evidence of spring and early summer spawning. We observed eight males spawning on July 17, 1972. On March 24, 1974, Steven Schultz of California Department of Fish and Game (pers. commun.) observed at least 10 males spawning in approximately 6 m (20 ft) of water at Point Cabrillo. On March 25, 1974, we observed two females spawning at Point Cabrillo at a depth of 6 m (20 ft). However, these two abalones had been tagged and measured for growth the preceding day and the disturbance may have induced spawning. On April 25, 1974, we observed three males spawning in 3.5 m (12 ft) of water at Van Damme State Park. During April 1975, we observed numerous males and one female spawning at Point Cabrillo.

The evidence from histological preparations and field spawning observations indicates that only a portion of the population spawned during the spring and

early summer, a condition not peculiar to the red abalone. In British Columbia, Quayle (1971) found that only a portion of a pinto abalone, *Haliotis kamtschaticana*, population spawned during the spring of several years. Poore (1973) also observed only a portion of a population of *H. iris* spawning during 1969.

Some members of our populations only released a portion of their gametes; and thus, spawned incompletely, as noted for other haliotids (Crofts 1929; Newman 1967; and Poore 1973). The residual gametes from incomplete spawnings could account for the varying quantities of necrotic oocytes observed during the study. A third portion of the population did not spawn at all during a given season resulting in retention of all their gametes. Virtually all unspawned large oocytes were necrotic. These three spawning patterns henceforth will be referred to as types. Type I spawning pattern is defined as complete spawning, Type II as incomplete spawning, and Type III as nonspawning. Histological evidence only allows for diagnosing of a specimen's spawning pattern during the season preceding the sampling date.

### Type I Spawning Pattern

The annual reproductive cycle of Type I specimens was classified with modifications into the phases developed for the surf clam, *Spisula solidissima* (Ropes 1968), and for the gaper clam, *Tresus capax*, (Machell and DeMartini 1971).

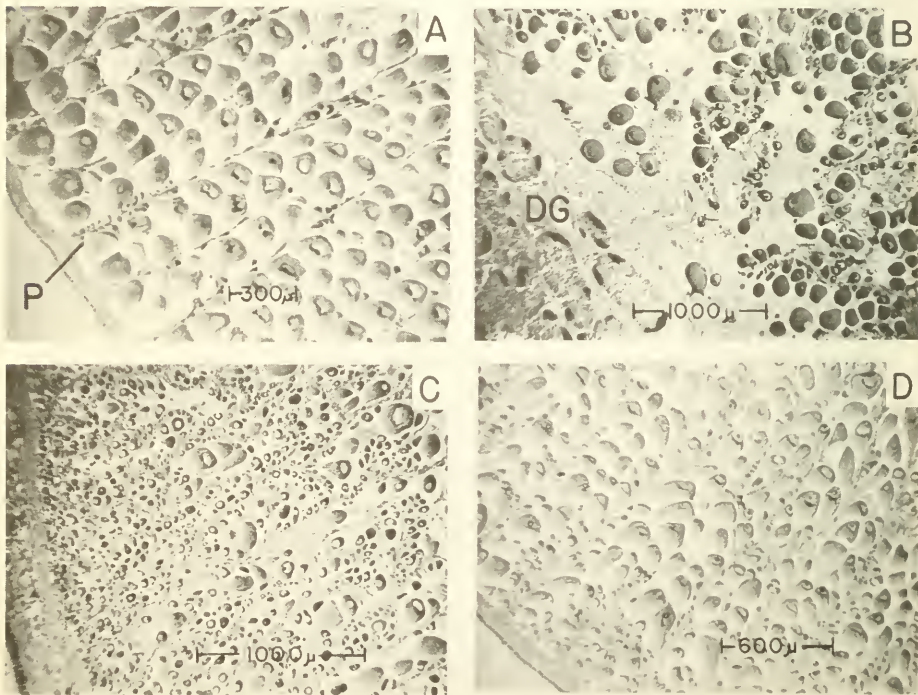


FIGURE 2. A, Ripe ovary of female displaying Type I pattern, collected March 5, 1974. P = proliferation near peripheral gonad wall. B, Partially spawned ovary of a female displaying Type I pattern, collected July 9, 1972. Note the free oocytes near the digestive gland = DG. C, Active ovary of female displaying Type I pattern, collected April 7, 1974. D, Advanced active ovary of female displaying Type I pattern, collected August 3, 1973.

*Ripe Phase:* An ovary was defined as ripe when virtually all primary oocytes were greater than 160 microns. Oocytes can reach a maximum diameter of about 250 microns. Slight proliferation of small oocytes less than 50 microns was still evident, especially near the peripheral wall of the gonad (Figure 2A). A ripe testis mainly contained spermatozoa. Few early gametogenic stages were present and were restricted to the area immediately surrounding the trabeculae (Figure 3A). Specimens were ripe during the winter with maximum ripeness attained in February.

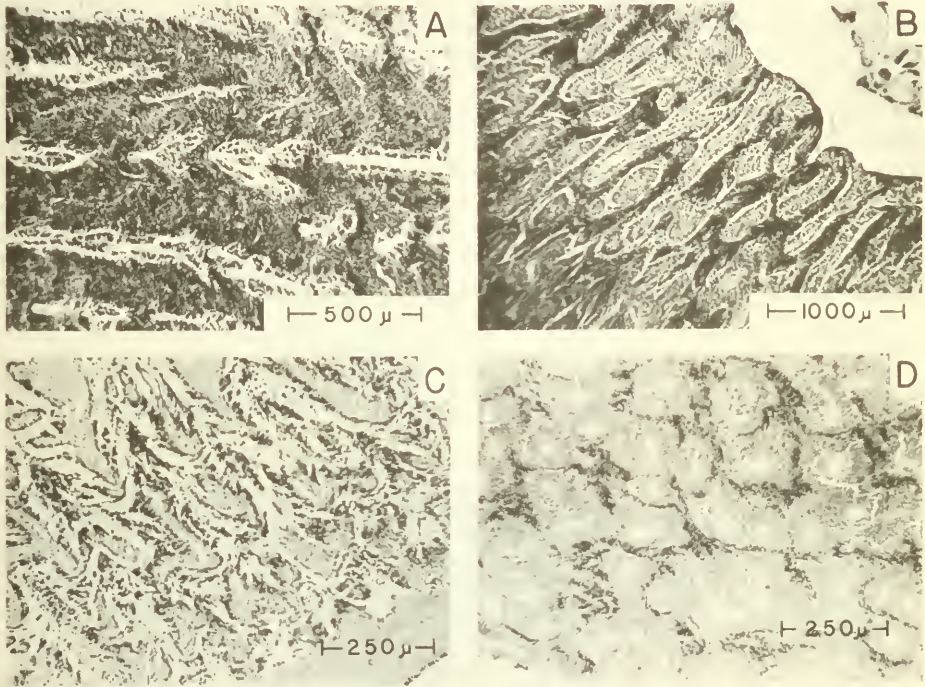


FIGURE 3. A, Ripe testis of male displaying Type I pattern, collected January 30, 1974. Trabeculae are light areas enveloped by a thin band of spermatogenic stages antecedent to spermatozoa. Spermatozoa dominate the remainder of the testis. B, Partially spawned testis of male displaying Type I pattern, collected April 3, 1973. Dark areas contain spermatozoa. C, Spent testis of male displaying Type I pattern, collected April 7, 1973. D, Active testis of male displaying Type I pattern, collected July 19, 1972.

*Partially Spawned Phase:* The partially spawned phase followed the ripe phase. Partially spawned gonads contained reduced densities of gametes relative to ripe gonads (Figures 2B and 3B). Histologically, partially spawned specimens could not be classified accurately as either Type I or Type II because we could not predict whether the specimen would have released all its gametes had it not been collected (Type I), or if it had finished spawning and was retaining residual, ripe gametes (Type II). The partially spawned condition was evident in specimens collected throughout the spring.

*Spent Phase:* The spent phase was characterized by a lack of ripe gametes and extremely slight gametogenic activity (Figure 3C). Macroscopically, the gonad was reduced greatly. The spent condition was observed only during spring. Few



spent gonads were observed indicating that few members of the population are Type I or that in most cases gametogenesis is initiated concurrently with or immediately after spawning. Webber and Giese (1969) noted initiation of gametogenesis immediately after spawning in a population of black abalone, *H. cracherodii*.

**Active Phase:** The active phase, which occurred during summer, is characterized by intense gametogenic activity. Ovaries contained primarily small oocytes less than 50 microns in diameter (Figure 2C). In testes, spermatogonia and primary spermatocytes dominated (Figure 3D). As the active phase progressed into autumn, oocytes continued to develop and increase in size. Later in autumn, ovaries contained a spectrum of oocytes ranging from about 10 to 250 microns in diameter in more or less equal proportions (Figure 2D). Near the end of autumn, large oocytes and spermatozoa were approaching maximum densities characteristic of the ripe phase.

### **Type II Spawning Pattern**

The Type II pattern (incomplete spawning) followed the same annual gametogenic and spawning cycles as the Type I pattern, but differed only by a partial release of gametes. During the summer, intense gametogenic activity indicative of the active phase was evident, but additionally, numbers of large ripe oocytes were still present. The quantity of residual oocytes varied individually. As autumn approached, residual oocytes became necrotic. By winter, there was a mixture of ripe and necrotic oocytes (Figure 4A). There was no evidence of necrosis of residual spermatozoa.

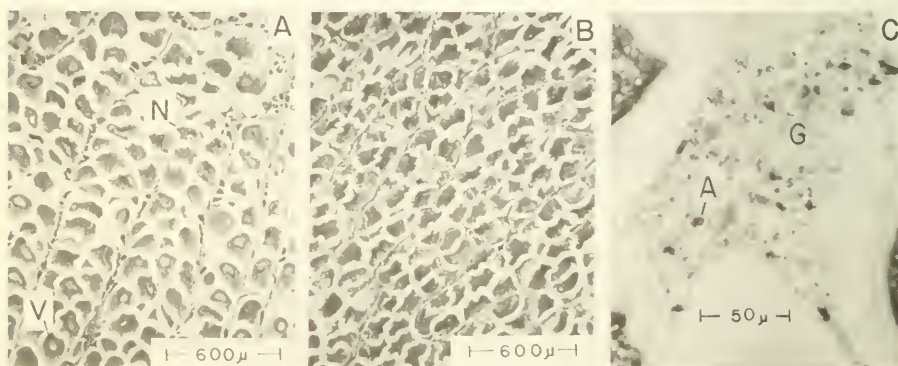


FIGURE 4. A, Ovary of female displaying Type II pattern, collected March 5, 1974. Note presence of necrotic = N and viable = V oocytes. B, Ovary of female displaying Type III pattern, collected January 28, 1973. Virtually all oocytes are necrotic. C, Female collected March 5, 1974. Granular substance = G and associated cells found among necrotic oocytes = A.

### **Type III Spawning Pattern**

Type III specimens, nonspawners, entered the spawning season with ripe gonads but did not spawn. Specimens sampled during summer were still ripe. By late summer, early necrotic stages appeared in the ovaries. No events resembling the active phase were apparent during summer as was the case for Type I and Type II patterns. Early spermatogenic events and transformations from oogonia to primary oocytes were relatively few. Quantities of unspawned

gametes may have inhibited gametogenesis until the residual gametes were either released or reabsorbed. Throughout autumn necrosis became extensive, and by winter, up to about 95% of the large oocytes in a cross-section of ovary would be necrotic in Type III specimens (Figure 4B).

Booolootian, *et al* (1962) postulated year round spawning for a red abalone population near Pacific Grove, California. Young and DeMartini (1970) concurred on this point for red abalones collected near Fort Bragg, California, because ripe gametes were present throughout their monthly samples. Either the Fort Bragg animals did not spawn during their year of study or Young and DeMartini's (1970) sample size was too small to detect the spawning we observed. We examined Young and DeMartini's (1970) histological preparations and found no gonads resembling the partially spawned or spent conditions that we had found in spring. We believe that their specimens did not spawn during their study. Poore (1973) observed that two New Zealand species, *H. iris* and *H. australis*, did not spawn during the 1968–69 season, while they did spawn during the 1967–68 season. But, because Young and DeMartini's (1970) sample size was 10 abalones per month versus our 30 abalones per month, they may have had a sampling error. There are some records in the literature of only a few or of a single animal in a population spawning. Because of this variability among invertebrates, investigators agree that a large sample is much preferred (Giese 1959).

Incompletely spawned gonads (Type II spawning pattern) are not uncommon among other haliotids. Crofts (1929) examined the ormer, *H. tuberculata*, after spawning and noted that gonads lacked marked signs of being spent. Newman (1967) observed incomplete spawning in the Midas abalone, *H. midae*, as did Poore (1973) for a population of *H. iris* in New Zealand. Price (1974) investigated a southern California population of red abalones and found substantial variation about the mean gonadal bulk index during the spawning period. We suggest that this variation may be the result of partially spawned and (or) non-spawning members present in the population which would display little or no reduction in the gonad bulk index over the previous sampling period.

### ***Exogenous Factors Affecting Reproduction***

Intensity and fluctuation of water temperature have long been considered dominant exogenous factors affecting invertebrate reproductive cycles (Giese 1959). Spawning occurred in a population of the disc abalone, *H. discus hannai*, from August through October when water temperature was maximum, 20 C (68 F) (Tomita 1967). A French population of the ormer spawned during summer and early autumn, when water temperature was maximum, 17 C (70 F) (Girard 1972). Similarly, red abalone imported to Japan, had optimal gonadal maturation and subsequent spawning between 14 and 20 C (57 and 68 F) (Shibui 1971).

Due to a series of thermograph malfunctions, we did not generate a continuous temperature record during the study. However, our high and low recordings were 7.6 and 13.9 C (46 and 57 F), respectively. Low temperatures occurred during winter and early spring, while higher temperatures occurred during late summer and early fall. Spawning, though often incomplete, did not correlate with high water temperatures. Newman (1967) noted low-intensity spawning in areas of low water temperature fluctuation for the Midas abalone. Annual water temperature fluctuation in our areas may not be great enough to stimulate complete spawning.

Photoperiod, physical disturbance, and food abundance also are known to affect invertebrate reproductive cycles, either independently or in concert (Giese 1959). Kelps (*Alaria*, *Hedophyllum*, *Nereocytis*) are the major foods in the diet of adult red abalones found near Mendocino, and are most abundant during summer months. During late fall and winter, there is virtually no available kelp. Abundant summer kelps correlate with intense gamete production. The abundance of kelp at this time apparently provides ample nutrition for both growth and gamete production. A similar correlation between gonad growth and abundant food was noted for two South Australian haliotids, *H. laevigata* and *H. Cyclobates* (Shepherd and Laws 1974).

### ***Necrosis***

Necrotic oocytes occurred in some ovaries in all monthly samples. Necrosis was first noted in the red abalone by Young and DeMartini (1970). They observed that the nucleus became eosinophilic, the nuclear membrane then broke down and numbers of eosinophilic vacuoles appeared in the cytoplasm, and the plasma membrane convoluted and eventually ruptured. Typically, only oocytes greater than 150 microns were necrotic. The number of necrotic oocytes present is a direct result of the spawning pattern followed the preceding season. Some winter specimens contained no necrotic oocytes (Type I), while in others, necrotic oocytes were present in varying amounts (Type II) to the point where virtually all large oocytes were necrotic (Type III). We believe that necrotic oocytes were autolysing residual gametes. Observations by Arthur Giese (pers. commun.) support our assessment. He noted degenerating mature gametes in abalones late in the breeding season and believes that degeneration removes unspawned gametes. He observed less intense oocyte degeneration than we observed. The occurrence of necrotic oocytes is not limited to haliotids. Harvey (1956) found some ovaries of the sea urchin, *Arbacia punctulata*, full of both degenerate and abnormal eggs which had not been spawned the previous season. Caddy (1967) observed autolysing eggs in spent ovaries of the bivalve *Macoma balthica*. In gaper clams, Machell and DeMartini (1971) observed cytolysis of residual oocytes in spent ovaries and the presence of leucocytes in association with the necrotic oocytes. In our specimens, a yellowish staining granular substance appeared in the lumen in areas of advanced necrosis. A distinct cell type appeared among the granules and is probably associated with them (Figure 4C). Granules and these associated cells appeared not only in the ovarian lumen, but also in the digestive gland and the wall separating the digestive gland from the gonad, leading us to believe that the associated cells are resorptive. Granules and these cells were sparse in ovaries containing large numbers of viable oocytes. However, the quantities of necrotic oocytes, granules, and associated cells increased concomitantly. Neither the granules nor associated cells were observed within the cytoplasm of unruptured oocytes. The granules and associated cells were noted in the testes, but not to the extent that they appeared in ovaries. The term "leucocyte" may categorize these cells associated with the granules, but we refrain from applying the term here since the terminology and criteria used to define and classify invertebrate leucocytes can be broad and confusing (Cheney 1971).



### Sex Ratio

Females of gonochoristic molluscan species tend to be more numerous than males and may become even more numerous as the increasing population age (Fretter and Graham 1964). They postulated that this may be a result of the early death of males. Females outnumbered males in the Point Cabrillo population, but not in the Van Damme population. Two hundred sixty-nine mature specimens (109 males and 160 females) were collected at Point Cabrillo and 115 (56 males and 59 females) at Van Damme for determining a sex ratio. The hypothesis that the sex ratio was 1:1 was tested for each population. A Chi-square value of 9.66 was calculated for Point Cabrillo while Van Damme had a value of .0781 ( $\chi^2$  p.05, 1 d.f. = 3.841). Therefore, the hypothesis was rejected for Point Cabrillo and accepted for Van Damme. The degree of human predation certainly has a significant effect on the age class structure of these two populations. For years the red abalone at Van Damme have been heavily fished by sportsmen, consequently, the larger, older individuals are constantly being harvested. However, the Point Cabrillo population has been closed to fishermen for many years, possibly allowing a natural age class structure and sex ratio to develop as Fretter and Graham (1964) note for populations comprised of older individuals.

### Minimum Size at Sexual Maturity

Unlike Newman's (1967) study, where only macroscopic coloration of the gonad was used to indicate sexual maturity, each of our specimens was inspected histologically. We define a sexually mature specimen as one having either spermatozoa or primary oocytes.

All specimens in size class 100.0 to 125.0 mm (3.9 to 4.9 inches) were sexually mature and females mature at smaller sizes than males (Table 1). The smallest mature female was 39.5 mm (1.6 inches), while the smallest male was 84.5 mm (3.3 inches). If both sexes have the same growth rates, then females mature earlier than males. All specimens with shucked weights greater than 100.0 g (0.2 lb) were sexually mature (Table 2).

**TABLE 1. Shell Length at Sexual Maturity and Frequency of Occurrence of Male and Female Red Abalones Collected on June 12, 26 and July 26, 1973, at Point Cabrillo Light-house Station.**

Length classes (mm)	Number of specimens	Number sexually mature	Number males	Number females	Total % sexually mature
0 - 25.0.....	2	0	-	0	-
25.1- 50.0.....	8	1	-	1*	12.5
50.1-75.0.....	14	8	-	8*	57.2
75.1-100.0.....	14	10	2	8*	71.5
100.1-125.0.....	10	10	7	3*	100.0
125.1-150.0.....	20	20	11	9**	100.0
150.1-175.0.....	8	8	2	6	100.0
175.1-.....	23	23	11	12	100.0
Total .....	99	80	33	47	

\* Size classes where all oocytes were less than 50 microns.

\*\* Size class where some specimens contained all oocytes less than 50 microns and other specimens contained oocytes both greater than and less than 50 microns.

**TABLE 2. Shucked Weight at Sexual Maturity and Frequency of Occurrence of Male and Female Red Abalones Collected on June 12, 26 and July 26, 1973, at Point Cabrillo Lighthouse Station.**

<i>Shucked weight (g)</i>	<i>Number of specimens</i>	<i>Number sexually mature</i>	<i>Number males</i>	<i>Number females</i>	<i>Total % sexually mature</i>
0 100.0 .....	36	17	3	14	47.2
100.1-200.0 .....	12	12	5	7	100.0
200.1-300.0 .....	16	16	7	9	100.0
300.1-400.0 .....	5	5	5	0	100.0
400.1-500.0 .....	1	1	1	0	100.0
500.1-600.0 .....	5	5	1	4	100.0
600.1-700.0 .....	6	6	2	4	100.0
700.1- .....	18	18	9	9	100.0
Total .....	99	80	33	47	

Typically, 50 microns was the maximum diameter of oocytes present in specimens smaller than 132.0 mm (5.2 inches). Specimens greater than 132.0 mm usually contained some oocytes up to 100 microns. Because the abalones were collected in June and July when oocytes would not be maximum size, the question arises, would these small oocytes mature by the following winter? In an attempt to answer this question a small sample of 10 abalones ranging in length from 99.5 to 139.5 mm (3.9 to 5.5 inches) was collected on December 3, 1973 (Table 3). Pieces of gonad were removed and oocytes were measured with an ocular micrometer. Food availability and growth are apparently greatest from mid-spring through early fall. Thus, these winter specimens were probably shorter during the previous June. Based on our unpublished growth studies, the 99.5 mm specimen (Table 3), which contained no oocytes larger than 160

**TABLE 3. Length, Whole Weight, Shucked Weight and Occurrence of Ripe Oocytes Greater Than or Equal to 160 Microns in Small Female Red Abalones Collected on December 3, 1973, at Point Cabrillo Lighthouse Station.**

<i>Length (mm)</i>	<i>Whole weight (g)</i>	<i>Shucked weight (g)</i>	<i>Occurrence of ripe oocytes</i>
99.5.....	155.8	114.8	-
112.0.....	284.1	207.3	+
112.5.....	326.1	234.1	+
120.0.....	368.4	257.6	+
123.5.....	291.3	223.3	+
125.0.....	317.8	210.4	+
134.0.....	424.6	301.4	+
136.0.....	409.2	296.9	+
138.5.....	557.1	431.1	+
139.5.....	494.6	343.9	+

microns, may well have been 80.0 to 95.0 mm (3.2 to 3.7 inches) long in June. If so, it probably had oocytes no larger than 50 microns (Table 1) during the previous summer. Two specimens, 112.0 and 112.5 mm (4.4 inches) long contained oocytes greater than 160 microns, with many near 250 microns (Table 3). During the summer months, these abalones were probably 95.0 to 108.0 mm (3.7 to 4.3 inches). Abalones of this size range, examined during the summer, contained only oocytes smaller than or equal to 50 microns (Table 1), indicating that some females containing only oocytes smaller than 50 microns during the

summer may contribute ripe oocytes to the following season. However, none were observed spawning in the field. Larger females, 100.0 to 140.0 mm (3.9 to 5.5 inches), collected during the summer, contained very few oocytes greater than 160 microns. The lack of ripe residual oocytes from previous seasons indicates either previous spawning activity or resorption of the residual oocytes. Resorption probably occurs after necrotic oocytes have lysed. Because virtually no necrotic oocytes were evident in specimens less than 150 mm (5.9 inches), resorption seems unlikely. These small abalone which do spawn can be classified as displaying the Type I pattern.

The presence of only small oocytes during the summer and large oocytes during the winter in abalone, less than 140 mm (5.5 inches) further supports the hypothesis of the annual gametogenic cycle, i.e., ripeness is attained during the winter.

Gonadal pigmentation was always associated with the presence of either spermatozoa or oocytes, but gametes can be present in the absence of pigmentation. Nine of the 24 specimens less than 75.0 mm (3.0 inches) contained gametes when examined histologically (Table 4). Only two of the nine were pigmented. The testes of the ormer, *H. tuberculata*, were unrecognizable until males were 4 cm (1.6 inches) long, but spermatozoa were obtained from specimens 2.8 cm (1.1 inches) long; spawning probably first occurred in animals 2 to 3 years old (Stephenson 1924).

**TABLE 4.** Shell Length at Sexual Maturity Comparing Frequency of External Gonad Pigmentation with Occurrence of Spermatozoa or Oocytes Within Red Abalone Gonads Collected June 12, 26 and July 26, 1973, at Point Cabrillo Lighthouse Station.

Length classes (mm)	Number of specimens	Number of specimens displaying macroscopic gonadal pigmentation	Number of specimens containing spermatozoa or oocytes
0 - 25.0.....	2	0	0
25.1- 50.0.....	8	0	1
50.1- 75.0.....	14	2	8
75.1-100.0.....	14	5	10
100.1-125.0.....	10	10	10
125.1-150.0.....	19	19	19
150.1-175.0.....	8	8	8
175.1-204.5.....	23	23	23

We observed no histological evidence of hermaphroditism. Girard (1972) noted successive hermaphroditism in a specimen of the ormer. Murayama (1935) observed a hermaphroditic specimen of the Japanese species, *H. gigantea*.

### Fecundity

As found by Newman (1967) for the Midas abalone, histological inspection prior to counting indicated that there were two broad size classes of primary oocytes. The larger oocytes, greater than 160 microns, were used for determining fecundity because these would ripen by the next spring. Additionally, precise counting of the smaller oocytes was impractical due to the smallness and adherence to the germinal epithelium.

Eight specimens with nearly the same shucked weight, approximately 800.0 g (1.8 lb) were chosen for fecundity estimates. Densities in oocytes per gram of ovary were determined for each of the three different locations on each

specimen (Table 5). The hypothesis that mean oocyte counts of different gonad locations was the same was tested by a one-way analysis of variance. An F value of 2.21 was calculated ( $F_{p.05, 2 \text{ and } 21 \text{ d.f.}} = 3.47$ ). Therefore, any position on the ovary can be sampled to determine total oocyte counts. We used pieces from the mid-portion for fecundity estimates. Fecundity estimates ranged from 619,000 to 12,575,000 oocytes per ovary (Table 5). Fecundity can vary substantially between specimens of the same length (Table 5). Such variability may be due to errors in technique while weighing the smaller pieces or making actual counts, but it is more likely due to individual variation. Specimens were collected in late autumn, and based on our observations of the reproductive cycle, we did not expect ovaries to contain maximum densities of large oocytes until the following winter. Thus, all specimens would not be expected to contain the same percentage of large oocytes because many were still growing. A more accurate estimate of fecundity could have been obtained by sampling just prior to spawning. Thus, our figures probably underestimate the actual fecundity.

**TABLE 5. Fecundity Estimates Using Oocytes Greater Than or Equal to Approximately 160 Microns, for Female Red Abalones Collected December 3, 1973, at Point Cabrillo Lighthouse Station.**

Shell length (mm)	Body weight (g)	Gonad weight (g)	Gonad sample weight (g)			Oocyte counts			Oocytes per gram using mid-portion	Fecundity $\times 10^6$
			Midpor-			Midpor-				
			Tip	tion	Base	Tip	tion	Base		
134.0	301.4	10.6	.039	.045	.056	4950	4340	4810	96444	1.0
136.0	296.9	7.6	.105	.043	.042	11620	3500	2710	81395	0.6
138.5	431.1	14.5	.048	.060	.048	4120	4310	4070	71833	1.1
143.5	405.1	9.6	.048	.056	.051	4590	4750	4000	84821	0.8
146.5	428.2	11.3	.064	.071	.083	4920	5110	4340	71971	0.8
148.5	417.9	17.0	.028	.059	.040	2360	3850	3910	65254	1.1
161.5	437.4	22.8	.054	.038	.040	6590	4350	3010	114473	2.6
162.0	701.2	50.6	.038	.052	.042	5190	5490	4190	105576	5.3
168.5	688.3	40.8	.041	.059	.064	4710	5860	6220	99322	4.1
169.5	652.0	55.0	.039	.055	.055	5100	5760	5660	104727	5.8
171.0	695.9	30.4	.035	.040	.039	4350	4360	4740	109000	3.3
171.5	786.9	22.5	.018	.044	.036	3150	5170	3380	117500	2.6
171.5	744.4	44.2	.039	.038	.045	4420	5350	4410	140789	6.2
172.5	580.6	32.7	.059	.061	.049	6400	5840	5040	95737	3.1
176.5	639.1	56.1	.043	.042	.062	4950	6360	6220	151428	8.5
180.5	745.6	30.2	.026	.059	.067	3750	7870	7600	133389	4.0
182.5	822.9	67.7	.039	.040	.038	5320	4330	3610	108250	7.3
185.0	836.4	48.2	.034	.045	.029	5120	3740	3700	83111	4.0
185.0	770.1	86.3	.029	.037	.044	2600	2800	3810	75675	6.5
190.5	1042.1	65.4	.044	.022	.037	4850	4230	2920	192272	12.6
192.0	887.1	60.4	.045	.061	.042	4850	6460	4840	105901	6.4
192.0	806.8	49.3	.049	.038	.071	5900	6920	6020	182105	9.0
198.0	940.9	47.1	.020	.024	.023	4150	2350	3960	97916	4.6
198.5	1008.6	52.3	.038	.028	.046	4400	1910	3180	68214	3.6
198.5	1066.1	76.0	.025	.058	.044	4230	6550	4550	112931	8.6

Another factor which affects the true fecundity is the presence of necrotic oocytes. While counting, necrotic oocytes were not distinguishable from viable oocytes. Necrotic oocytes could only be detected in histological preparations. Thus, there were no means to determine accurately the percentages of necrotic oocytes per ovary.



Gonads of specimens less than 125.0 mm (4.9 inches) were so thin that accurate estimates of fecundity were not determined. Based on fecundity estimates made for larger females, we estimate that females 100.0 to 125.0 mm (3.9 to 4.9 inches) can have at least tens of thousands, if not hundreds of thousands of oocytes, at the upper end of this size range. Even though the numbers of gametes, particularly of oocytes, is relatively low for these smaller specimens, very few necrotic oocytes were observed in histologic preparations. However, in some females greater than 150.0 mm (5.9 inches), about 75 to 95% of the large oocytes viewed in a cross-section of ovary were necrotic. Consequently, these smaller abalones are contributing substantial quantities of viable oocytes to the gametic pool.

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# COPPER TOXICITY EXPERIMENTS IN RELATION TO ABALONE DEATHS OBSERVED IN A POWER PLANT'S COOLING WATERS <sup>1</sup>

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Toxicity of copper as copper sulfate to adult and larval red abalone, *Haliotis rufescens*, and adult black abalone, *Haliotis cracherodii*, was determined by static bioassay in seawater at 14 C (57 F). Copper accumulation studies and histopathological analysis in digestive gland and gill tissues were conducted.

The TL<sub>50</sub>'s for adult red and black abalone were 65 ppb and 50 ppb copper, respectively. The TL<sub>50</sub> for larval red abalone was 114 ppb copper. Copper was found to accumulate in gill tissues of red and black abalone at 56 ppb copper concentration. Histopathological abnormalities in gill tissues occur at concentrations above 32 ppb.

## INTRODUCTION

Approximately 1,500 dead abalone (*Haliotis rufescens* and *H. cracherodii*) were estimated in the discharge area of the Diablo Canyon nuclear power plant following the testing of a cooling system (D. Gotshall, CF&G pers commun.) The tests were conducted after a period of shutdown, during which non-circulating seawater was in contact with copper-nickel tubing in the condensing system. It was apparent that large amounts of copper (Cu) dissociated from the tubing since the first pulse of discharge water contained 1,800 µg of Cu/liter. Concentrations rapidly decreased with flushing; however, even after 30 days, 20 µg Cu/liter were still found in the effluent waters versus 1 µg/liter entering the system.

Because of the well-known toxicity of Cu to almost all marine organisms, it was logical to assume that the abalone deaths were due to the elevated levels of this element in the discharge area. Nevertheless, for various financial and legal, as well as scientific reasons, it was necessary that these assumptions be corroborated by laboratory experiments. Thus, the purpose of this study was to evaluate the acute and chronic effects of Cu on red and black abalone.

## METHODS AND MATERIALS

### Adult Red and Black Abalone Experiments

Red, *Haliotis rufescens*, and black, *Haliotis cracherodii*, abalone were collected 1 mile south of Diablo Canyon, San Luis Obispo County, California, and maintained in flow-through holding tanks with unfiltered seawater for 2 weeks prior to testing. The animals were examined for physical injury to foot tissues and rejected for experiments if damaged. The animals were not fed during the holding period nor during the test exposure. All exposures were for 96 hr, a

<sup>1</sup> Contribution six of the Marine Bioassay Laboratory. Accepted October 1976.

standard for fish toxicity testing (Standard Methods 1971). Red abalone ranged in shell lengths from 173 to 204 mm (6.8 to 8.0 inches), and weights from 852 to 1342 g (1.9 to 3.0 lb); black abalone ranged in shell length from 62 to 170 mm (2.5 to 6.8 inches), and weights from 32 to 1,053 g (.07 to 2.3 lb). Abalones of the entire size range were tested at each concentration.

Red abalone were exposed to copper in 76 liter (20 gal) polyethylene buckets filled with 60 liters (15.8 gal) of unfiltered sea water. Black abalone were exposed to copper in 22 liter (5.8 gal) polyethelene buckets filled with 18 liters (4.8 gal) of unfiltered seawater. Water quality characteristics were: temperature,  $14 \pm 1^\circ\text{C}$  ( $57 \pm 2^\circ\text{F}$ ); dissolved oxygen,  $6.7 \pm 2.2$  mg/l; salinity, 33‰. Analysis of control seawater showed less than 5.0 ppb copper and was considered negligible. All glassware and plasticware used in the experiments were washed with Micro<sup>2</sup> laboratory cleaning solution, rinsed with seawater, acid rinsed with 5% HCl and triple rinsed with distilled water. All test containers were aerated with oxygen. Dead abalones (no touch response on oral surface) were removed from the test vessels; gill and digestive glands were taken for heavy metal analyses and frozen in polyethylene containers. Polyethylene gloves, forceps, and stainless steel scissors were used during the dissections. A small sample of gill tissue was also preserved for histopathological analyses.

Concentrated aqueous solutions of copper as  $\text{CuSO}_4$  were added to the test containers to attain the desired concentrations of 0 (control), 10, 32, 56, 100, and 180 ppb; seven animals were placed in each concentration. Test waters were changed at 24, 48, and 72 hr; temperature, dissolved oxygen, and number of mortalities were recorded.

Digestive gland and gill tissue were digested for heavy metal analyses as follows: 1–5 g of tissue were dried for 24 hr at 70C (158 F); the dried sample was digested in 70% redistilled  $\text{HNO}_3$ ; the solution was taken to dryness, and charred at 350C (662 F); the residue was redissolved in redistilled  $\text{HNO}_3$  and 30%  $\text{H}_2\text{O}_2$  was added until the solution remained clear; the solution was evaporated to 2 ml and diluted to 25 ml with distilled water. Samples were then analyzed by means of an atomic absorption spectrophotometer equipped with a deuterium arc background corrector to correct for non-atomic absorption.

### Red Abalone Larvae Experiments

For the larvae study, spawn was obtained from one male and female. The trochophore larvae were approximately 48 hr old at the initiation of the bioassay. Approximately 100 larvae were placed in 1 liter (1.1 qt) polypropylene beakers filled with 0.8 liters (0.9 qt) of water. The pH of test solutions were  $8.0 \pm 0.2$ . The temperature was  $13 \pm 1\text{C}$  ( $55 \pm 2^\circ\text{F}$ ), and the salinity was 30.4‰; the dissolved oxygen was greater than 8.0 mg/l at all times.

Concentrated aqueous solutions of copper as  $\text{CuSO}_4$  were added to the larvae test containers to achieve the following concentrations of copper: 0 (control), 10, 20, 40, 80, 160, 320, and 640 ppb. Five replicates of each concentration and 10 replicates of the controls were tested. All exposures were for 48 hr (Woelke 1972). At the termination of the experiment, 50 larvae were counted in each replicate, and the percent survival was calculated. The criteria for larval death were loss of epithelial ciliary activity and loss of cellular color.

<sup>2</sup> Mention of trade name does not constitute endorsement.

## RESULTS

## Toxicity

The numbers of adult abalone surviving the copper treatments decreased with increasing copper concentration (Table 1). One hundred percent survival for both species was observed in the control, 10 and 32 ppb copper solutions; 5/7 and 3/7 of red and black abalone, respectively, survived in 56 ppb copper; and only one black abalone survived at 100 ppb. All abalone of both species died in 180 ppb copper. Using these data, the 96-hr LD<sub>50</sub> was 50 ppb for black abalone and 65 ppb for red abalone.

TABLE 1. Survival of Adult Abalone in Various Concentrations of Copper in 96-Hr Static Bioassay (Alive/Total Tested).

Concentration	Survival	
	Red abalone	Black abalone
Control.....	7/7	7/7
10 ppb.....	7/7	7/7
32 ppb.....	7/7	7/7
56 ppb.....	5/7	3/7
100 ppb.....	0/7	1/7
180 ppb.....	0/7	0/7

The mean percent of red abalone larvae surviving after 48 hr in the copper solutions decreased from 93.6 in the controls to 90.4 in 10 ppb, 88.8 in 20 ppb, 87.2 in 40 ppb, 79.2 in 80 ppb, 23.6 in 160 ppb, and 0 in 320 and 640 ppb (Table 2). The 48-hr LD<sub>50</sub> calculated from these data was 114 ppb copper. The percent survival differed significantly between abalone larvae in controls and each of the treatments from 80 ppb to 640 ppb copper ( $P < .05$ ; Dunn's non-parametric multiple comparison test; Hollander and Wolfe 1973) indicating that significant mortality occurred in all concentrations 80 ppb and higher.

TABLE 2. Percent Survival of Red Abalone Larvae in Solutions of Copper.

Replicate #	ppb copper in solution						
	0 (Control)	10	20	40	80	160	320 640
1.....	94	92	90	82	82	32	0 0
2.....	92	90	94	80	80	20	0 0
3.....	94	88	84	92	82	16	0 0
4.....	92	88	88	90	88	88	0 0
5.....	94	94	86	94	64	30	0 0
6.....	94						
7.....	90						
8.....	96						
9.....	96						
10.....	94						
$\bar{X}$ .....	93.6	90.4	88.8	87.2	79.2	23.6	0 0
$\pm 95\%$ CL.....	1.3	3.2	4.8	7.6	11.2	8.7	0 0

## Accumulation Experiments

The mean copper concentration in the tissues of abalone that were exposed to copper in the toxicity experiments increased as the concentration in solution increased. The only exceptions were the copper concentrations in the digestive glands of abalone exposed to the higher concentrations of copper (Table 3).

**TABLE 3. Copper Accumulation in Red and Black Abalone Exposed to Five Concentrations of Copper for 96 Hr.**

	Concentration of copper added in seawater (ppb)						
	0 ppb (Control)	10 ppb	32 ppb	56 ppb	100 ppb	180 ppb	
PPM Cu in red abalone gills .....	7.4	12.1	20.2	47.1	129.9*	184.6*	
	6.2	17.0	31.6	64.6	89.7*	61.9*	
	9.4	32.9	33.2	75.4	92.6*	164.1*	
	19.8	14.1	24.9	55.1	95.9*	116.4*	
	11.6	17.6	39.2	31.8	119.9*	182.1*	
	8.3	14.4	—	98.2	114.9*	—	
	5.4	13.2	—	64.4*	120.1*	—	
	9.6	—	—	135.2*	—	—	
$\bar{X}$	9.7	17.3	30.9	71.5	109.0	141.8	
$\pm 95\%$ CL	3.8	6.6	7.5	27.1	14.7	65.1	
PPM Cu in black abalone gills .....	17.3	17.1	41.4	95.9	135.8*	114.2*	
	14.0	17.8	27.7	116.0	137.9*	92.0*	
	14.1	17.0	26.6	50.5	143.6*	205.3*	
	11.8	22.1	58.4	99.9*	142.5*	181.4*	
	16.6	16.0	30.9	115.0*	145.7*	212.5*	
	—	14.3	53.0	—	139.5*	291.3*	
	$\bar{X}$	14.8	17.4	39.7	95.5	140.8	182.8
	$\pm 95\%$ CL	2.8	2.7	14.3	37.1	4.0	75.9
PPM Cu in red abalone digestive glands .....	6.8	9.4	13.8	16.7	17.6*	17.2*	
	5.0	11.7	11.6	20.5	42.7*	15.8*	
	6.4	6.3	16.0	21.3	59.4*	17.7*	
	7.0	7.5	9.5	13.1*	16.5*	19.2*	
	8.8	9.8	14.9	51.4*	10.4*	17.2*	
	4.4	—	—	—	—	—	
	$\bar{X}$	6.4	9.0	13.2	24.6	29.3	17.4
	$\pm 95\%$ CL	1.6	2.6	3.2	19.0	26.0	2.8
PPM Cu in black abalone digestive glands .....	7.0	8.1	18.0	32.6	24.3*	15.3*	
	5.2	7.9	27.1	18.2	11.5*	12.1*	
	8.6	14.0	16.9	25.1	12.6*	23.2*	
	8.0	12.8	33.4	19.6	14.9*	15.1*	
	7.4	8.6	19.4	19.0*	25.3*	—	
	10.5	—	—	—	15.4*	—	
	$\bar{X}$	7.8	10.3	22.9	22.9	17.3	16.2
	$\pm 95\%$ CL	1.9	3.6	8.8	7.5	6.2	7.6

\* Tissue from abalone that died during the experiment.

The minimum concentration of copper that could be accumulated significantly by the abalone was determined by comparing the copper concentrations in the tissues of the controls to the concentrations in tissues of the treatments ( $P < .05$ ; Dunn's non parametric multiple comparison test; Hollander and Wolfe 1973). These tests indicate the gills of both species accumulated copper significantly in water that had as little as 56 ppb copper and the digestive glands accumulated copper significantly in as little as 32 and 56 ppb in black and red abalones, respectively.

The copper concentrations in tissues of abalone that died during the experi-

ment were significantly higher than in the tissues of the survivors ( $P < .01$ ; Mann Whitney U, Hollander and Wolfe 1973). The copper in surviving red abalone gills ranged from 5.4 to 98.2 and in black abalone 11.8 to 116.0 ppm. In gills of red abalone that died the copper ranged from 61.9 to 184.6 and in black abalone 92.0 to 291.3 ppm (Table 3).

The copper accumulation rates in tissues of abalone were tested for differences by comparing the slopes of the least square regressions of the copper in tissues (Y variable) as a function of the copper in solution (X variable) ( $P < .05$ ; Sokal and Rohlf 1969). The regression equations and their product moment correlation coefficients ( $r$ ) are:

$$Y = 7.9 + 910X, r = 0.856; \text{ for red abalone gills}$$

$$Y = 7.8 + 1,240X, r = 0.854; \text{ for black abalone gills}$$

$$Y = 6.4 + 227X, r = 0.919; \text{ for red abalone digestive glands}$$

$$Y = 8.4 + 325X, r = 0.804; \text{ for black abalone digestive glands}$$

All the correlation coefficients are significant ( $P < 0.05$ ; Sokal and Rohlf 1969). The slope comparison tests indicate that the gills of black abalones accumulate copper significantly faster than the gills of red abalone; the gills of both species accumulate copper at significantly faster rates than digestive glands.

Histopathological analyses of gill tissues showed minor loss of frontal and lateral gill cilia in red and black abalone, but the frequency and extent of the loss would not severely restrict respiration. Necrotic tissues were observed in higher concentrations of copper reflecting advanced post mortem changes. At 180 ppb copper concentration, the lamellar epithelium was distinctly necrotic and contained pyknotic nuclei. Portions of the connective tissue epithelium were also necrotic and showed signs of fragmentation. At sublethal copper concentrations (32 ppb or less), there were no significant histopathological abnormalities.

## DISCUSSION

The results presented above clearly show that copper can cause abalone mortality in larvae when present at 80 ppb and in adults when present at 50 ppb. These values are slightly lower than the previously reported  $LD_{50}$  of 100 ppb reported for green abalone (Marks 1938). Although the exact mechanism responsible for these deaths is unknown, the accumulation data suggest two possibilities. The higher concentrations found in the gills may indicate that copper was concentrating passively on the surfaces. Copper associated damage to gill and respiratory tissues have been reported for marine organisms (La Roche, *et al.* 1973; Baker 1969). For example, when fish are exposed to high concentrations of heavy metals, mucus from the gills combines with the metal to form a mucus-metal complex. This affords some protection as it is continuously sloughed off and lost to the environment, but in extreme conditions suffocation may occur (Dawson 1935; Douderoff and Katz 1953). Perhaps then suffocation may have been the cause for the abalone deaths if the increased concentrations of copper in the gills caused more mucus to be secreted than could be sloughed off.

From the increased accumulation in the digestive glands, it is also apparent that copper was entering the blood stream of these animals. However, the rate of increase in these tissues that normally function as detoxification organs, suggest that the animals may be unable to remove copper from the blood stream



at a rate high enough to prevent a critical buildup with consequent poisoning of vital enzyme systems.

The lowest concentrations of Cu in the gills of abalone that died in the 96 hr copper exposures were 61.9 and 92.0 ppm for red and black abalone, respectively. These values can thus be designated as the estimated detrimental accumulation levels (EDAL) for these species. This EDAL for red abalone is near the levels found in the gills of living red abalone collected from Diablo Canyon shortly after the previously mentioned mortality was observed ( $\bar{X}$  = 65, range 48–78 ppm Cu) indicating the abalone had accumulated potentially harmful amounts of copper. Of course, abalone can survive with gill concentrations higher than these levels with adequate times for acclimatization to high environmental copper levels. For example, Anderlini (1974) reported red abalone collected off Long Beach, California, with an average copper concentration in their gills of 123.5 ppm. Nevertheless, in a pulse situation, our experiments show that excess copper readily kills adult abalone, and in view of the large amounts released during the testing of the power plant cooling system, there can be little doubt that this element was responsible for the observed mortality. In order to prevent future occurrences of this nature, the copper tubing has been replaced with tubing constructed with titanium.

### ACKNOWLEDGMENTS

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# FACTORS INFLUENCING DISTRIBUTION OF FISH EGGS AND LARVAE OVER EIGHT 24-HR SAMPLINGS IN RICHARDSON BAY, CALIFORNIA<sup>1</sup>

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To determine the factors affecting the day-night distribution of ichthyoplankton, a series of eight 24-hr samplings was conducted in Richardson Bay, California. Slightly modified channel nets were fished in two locations and collections made for each half-tide stage. Of the different larval taxa collected, *Clupea harengus pallasii*, *Engraulis mordax* and Gobiidae comprised 98% of the total catch. The most speciose family was Cottidae.

Densities of larvae and eggs were positively correlated with tide stages of the fastest currents and with night conditions. Analysis of the catch results suggests the most determinant factor in the distribution of eggs and larvae is tidal currents.

Interesting occurrences of 10 species of offshore spawned larvae is reported, and a possible explanation for their presence within the bay is discussed.

## INTRODUCTION

Ichthyoplankton surveys along the west coast have centered around the CalCOFI (California Cooperative Oceanic Fisheries Investigations) cruises which began in 1949 and concentrated on the ichthyoplankton of the California current (Ahlstrom 1965). Only geographically scattered, recent studies have attempted to understand the inshore, estuarine ichthyoplankton (Eldridge and Bryan 1972; Blackburn 1973; Percy and Myers 1974). The only study in the central California coastal area was that of Chadwick (1958) who sampled in the Sacramento-San Joaquin Delta area, upstream from San Francisco Bay. This work dealt principally with the eggs and larvae of striped bass, *Morone saxatilis*, and fresh-water species. Percy and Myers (1974) are the only researchers to date to study the diel distribution of ichthyoplankton in a Pacific coast estuary. Their study took place over three 24-hr periods, in a comparatively narrow Oregon estuary.

Public and governmental concern over the deteriorating environmental quality of San Francisco Bay principally was responsible for a series of three comprehensive studies which began in 1964 (Pearson, Storrs, and Selleck 1970; Kaiser Engineers 1969; Brown and Caldwell 1971). The main objective of these studies was the evaluation of water quality, primarily the physico-chemical conditions throughout the bay. Research on the finfish population in San Francisco Bay has been limited to juvenile and adult fishes and confined to deeper channel stations (Aplin 1967). Studies were begun by staff members of the Tiburon Laboratory in 1972 in Richardson Bay, a small embayment within San Francisco Bay, to provide comprehensive information on the finfish populations of a shallow, estuarine tidal basin. The project included sampling phytoplankton, ichthyoplankton, and juvenile and adult fishes. Part of the ichthyoplankton survey was

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concerned with temporal distribution of fish eggs and larvae over short time periods. This work is the subject of the present paper. The major part of the ichthyoplankton survey covered the entire Richardson Bay at weekly intervals over a 1-year sampling period. The results of that survey will be reported later.

Diurnal and nocturnal rhythmic behavior has been documented in adult estuarine fishes (Gibson 1969). Likewise, larvae have exhibited varying diel distribution patterns in open-ocean waters (Isaacs 1964; Bridger 1956) and inshore areas (Lewis and Wilkens 1971; Lewis and Mann 1971; Richards and Kendall 1973; Percy and Myers 1974). Since day-night differences in distribution can bias survey results, an effort was made to determine factors affecting distribution of Richardson Bay ichthyoplankton. This paper reports the results of eight 24-hour samplings conducted at two locations from March 23, 1972, to February 9, 1973.

### DESCRIPTION OF THE STUDY AREA

Richardson Bay is approximately 11 km (8.0 miles) north of the San Francisco Peninsula (Figure 1). Its total area covers approximately 12.9 km<sup>2</sup> (4.9 miles<sup>2</sup>). This surface area changes 10–13% during spring tide conditions indicative of the shallow depth of the bay which averages only 0.45 m (1.4 ft). The only channel of notable depth averages 6m (19.2 ft) and is a minor ship channel adjacent to the city of Sausalito.



FIGURE 1. Map of Richardson Bay, California, showing the two sampling stations used in the study of the diel distribution of fish eggs and larvae. Short-dashed lines represent the 10-m contours. Long-dashed lines represent water level at zero feet tide level.

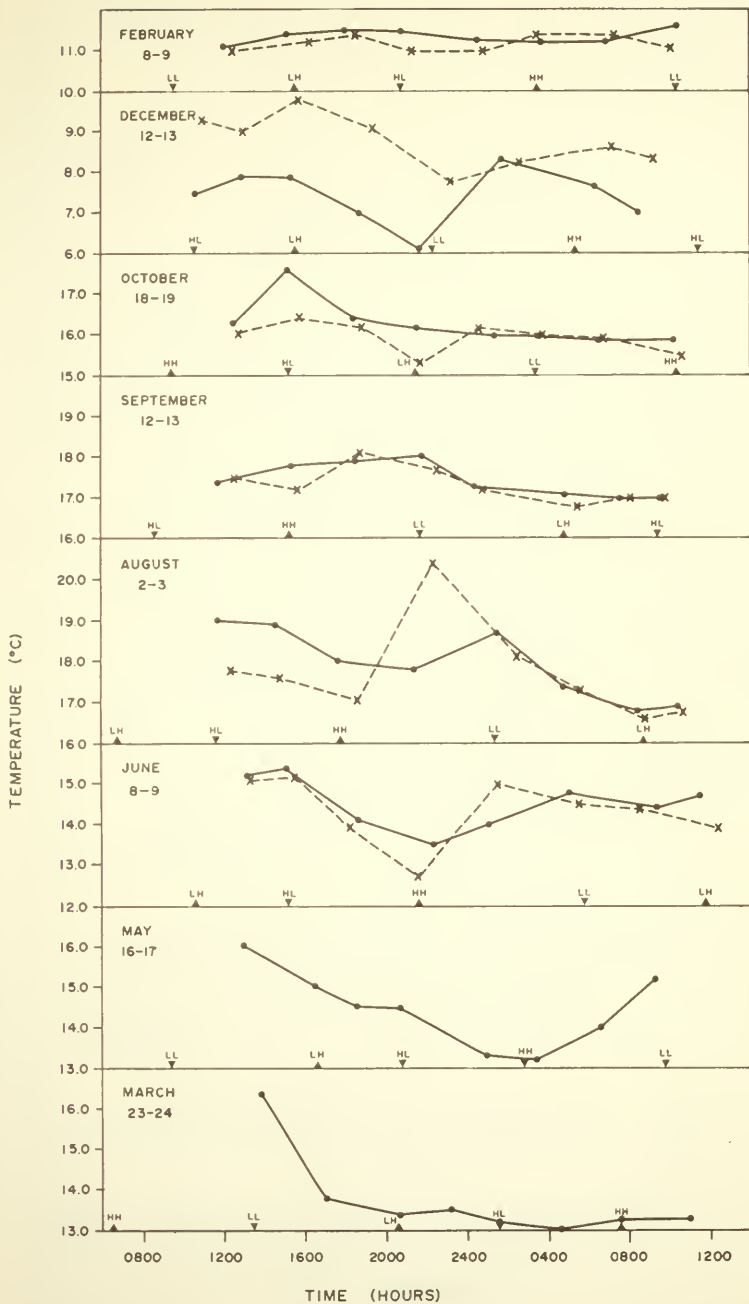


FIGURE 2. Water temperature measured in the study of fish eggs and larvae of Richardson Bay, California. Solid line represents Station 2 and dotted line Station 1. Different tide stages are indicated by triangles with appropriate symbols (LL = lower low tide; HL = higher low tide; LH = lower high tide; HH = higher high tide).

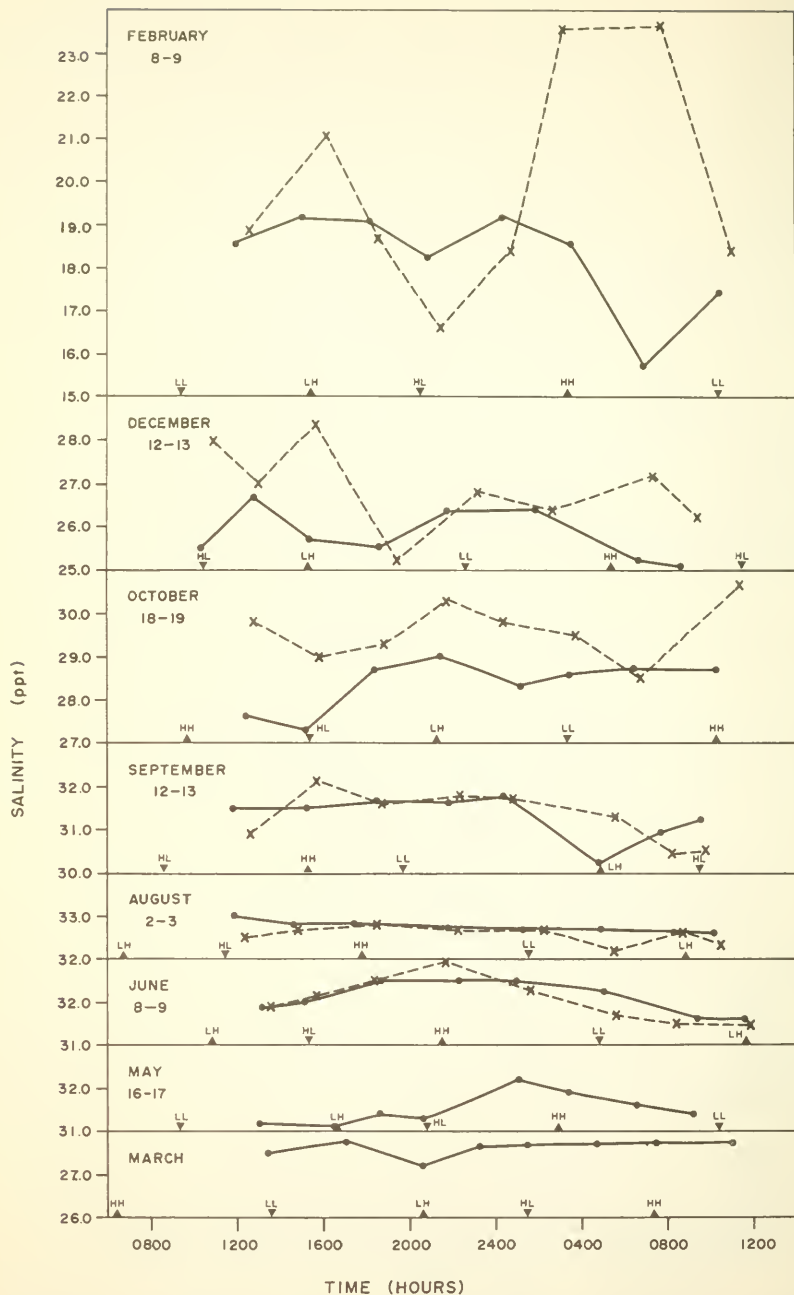


FIGURE 3. Salinities measured in the study of fish eggs and larvae of Richardson Bay, California. Solid line represents Station 2 and dotted line Station 1. Different tide stages are indicated by triangles with appropriate symbols (LL = lower low tide; HL = higher low; LH = lower high; HH = higher high).

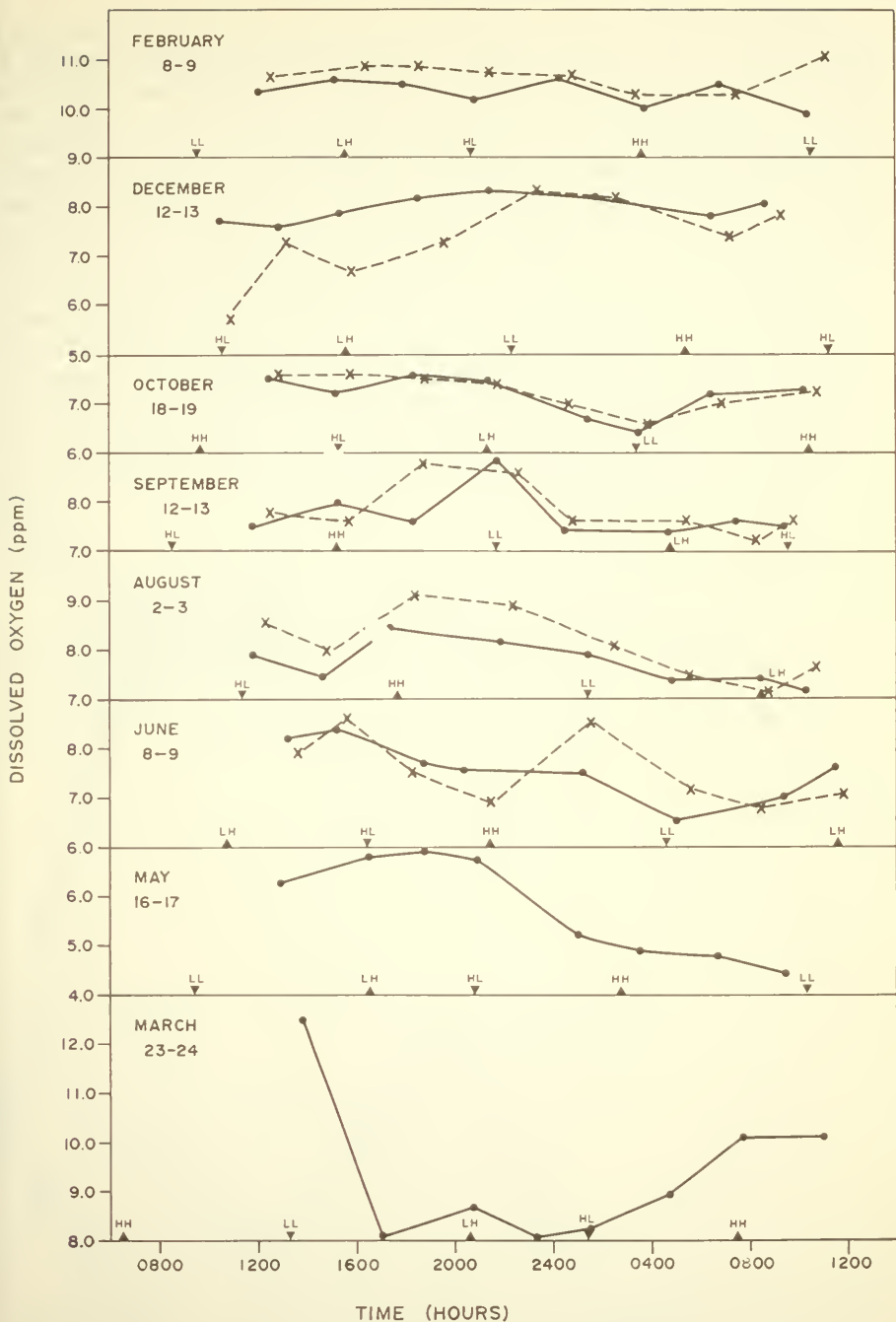


FIGURE 4. Dissolved oxygen concentrations measured in the study of fish eggs and larvae of Richardson Bay, California. Solid line represents Station 2 and dotted line Station 1. Different tide stages are indicated by triangles with appropriate symbols (LL = lower low tide; HL = higher low; LH = lower high; HH = higher high).

Frolander (1964) considered an estuary to be two-layered or stratified when conductivity, as a measure of salinity, changed by four or more units within a 1-m (3.3-ft) depth. By that definition Richardson Bay would be considered a vertically well-mixed estuary. Only during periods of high winter rainfall, near the entrance to the bay, did any evidence of stratification occur. Freshwater inflow into the bay comes mainly from two small creeks which drain a watershed area of approximately 45.7 km<sup>2</sup> (17.6 miles<sup>2</sup>). Also two sewage treatment plants discharge  $11.67 \times 10^8$  gallons of primary treated effluent per year into the bay.

Temperature, salinity, and dissolved oxygen levels were monitored throughout the sampling period (Figures 2, 3, and 4). The overall physico-chemical conditions were a function of the tide, season, and freshwater runoff. In winter the marine water entering the bay tended to moderate the colder, turbid upstream water. Conversely, in summer upstream water temperatures were generally warmer, and salinities were at their annual highs. At times the bay became a "hypersaline" or "negative" type estuary as described by Frolander (1964).

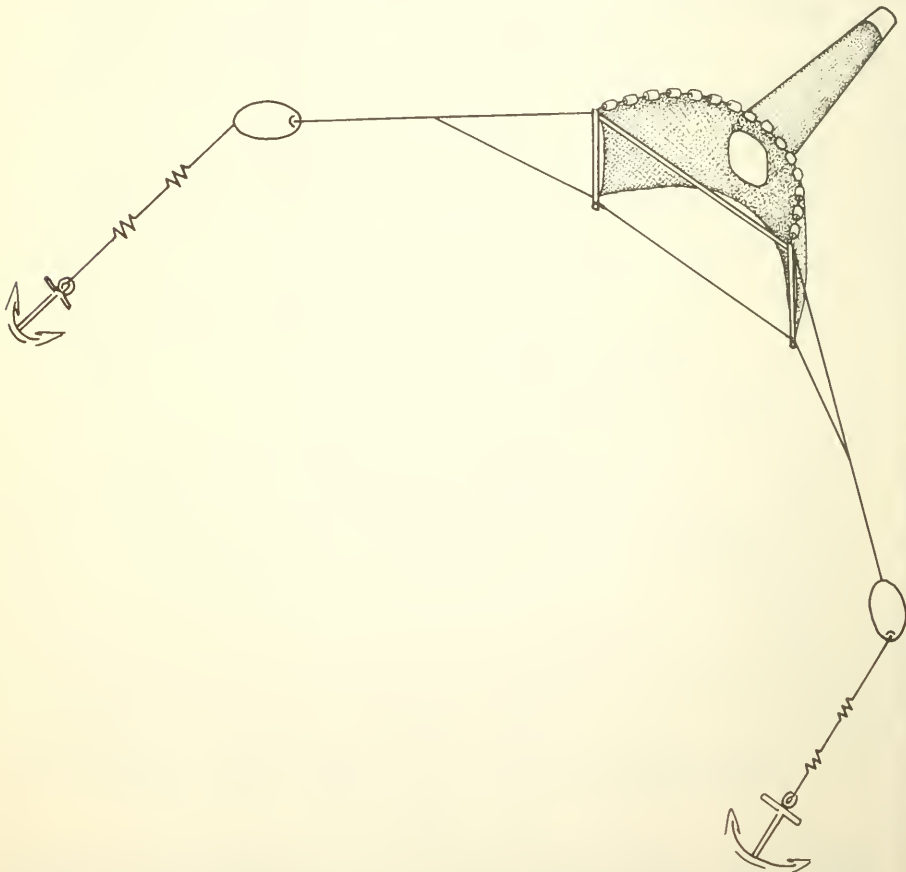


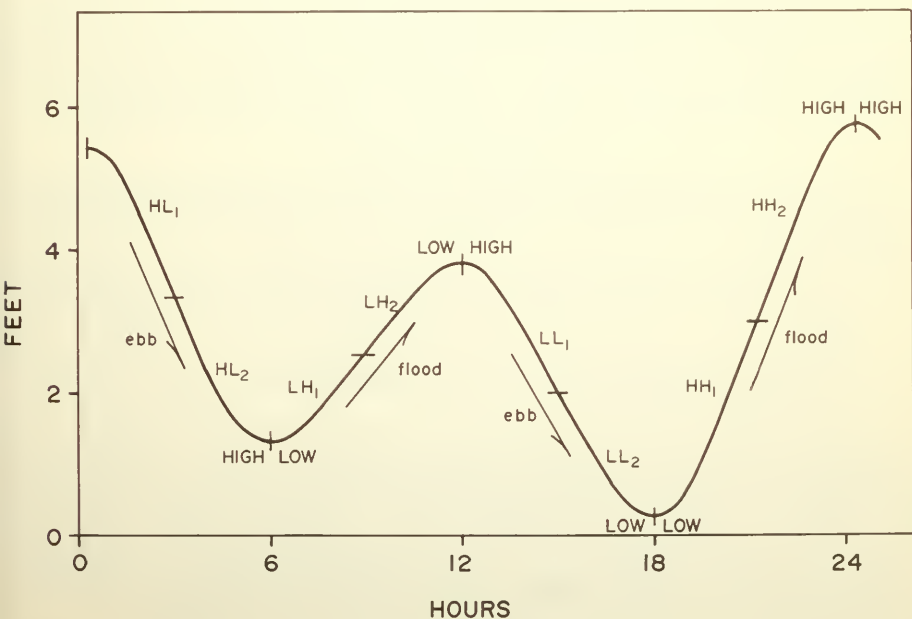
FIGURE 5. Anchored channel net used in the study of fish eggs and larvae. Flow meter (not shown) was mounted eccentrically in round 0.5 m net opening.



## METHODS

Sampling of the ichthyoplankton was carried out during eight 24-hr periods (Table 1) using a modified version (Figure 5) of a channel net (Lewis, et al. 1970). The net was fished in a stationary position, anchored in place, facing the tidal current. The tail bag consisted of a 0.5-m (1.7-ft) plankton net of  $333 \mu$  mesh. The wing dimensions were approximately  $1 \times 3$ -m ( $3.3 \times 9.9$  ft) with  $\frac{1}{8}$ -inch square nylon webbing. Digital flowmeters (Model 2030, General Oceanics, Inc.<sup>2</sup>) were mounted eccentrically in the mouth of each net to estimate the amount of water filtered. In the samplings of May 16, 17 and February 8, 9, numerous difficulties were encountered, i.e., clogging of the flowmeters, malfunctions, etc. Because many volume measurements of these two studies were questionable, I decided not to use the data for comparisons of catch (numbers of larvae/ $m^3$ ). Nets were placed in two locations in Richardson Bay (Figure 1); one in the ship channel in the middle part of the bay (Station 2), and the other near the entrance (Station 1). The water depths ranged from 1.0 to 2.0 m (3.3 to 6.6 ft) at Station 2 and 2.0 to 4.4 m (6.6 to 14.5 ft) at Station 1. For the first two studies (March 23–24 and May 16–17) only Station 2 was fished. To remove bias with respect to time (and weather conditions) the sampling dates were randomly selected ahead of time by means of a random numbers table, choosing two dates per season.

The sampling schedule for each 24-hr period was divided according to tide stages; the objective being to sample separately the first and second halves of each tide stage (Figure 6). The net was allowed to fish for the entire half-tide stage, then it was washed down, the plankton sample removed, and the net replaced for the next half-tide stage. For simplification in the succeeding text,



the different half-tide stages are represented as follows: high high tide stage =  $HH_1$  (first half) and  $HH_2$  (second half); low high =  $LH_1$  and  $LH_2$ ; high low =  $HL_1$  and  $HL_2$ ; and low low =  $LL_1$  and  $LL_2$ .

Concurrent to net samplings, the following physico-chemical parameters of bay water were measured: dissolved oxygen, temperature, salinity, pH, and turbidity. Vertical profiles of all parameters were made in front of each net. The values presented in Figures 2, 3, and 4 represent measurements taken in the mouth of each net. The ichthyoplankton samples were preserved in 5% buffered formaldehyde. Processing of samples followed procedures described by Kramer (Kramer, et al. 1972).

Component factor analyses (Rummel 1970) were used to determine the different relations between dependent and independent variables. This statistical method first calculated a correlation coefficient matrix of all possible combinations of variables. Then by matrix rotation it organized groups of related variables into distinct factors, which are independent of each other. Combined data for the entire year and data within each study were analyzed in this manner. Related variables were subsequently analyzed by analysis of variance.

## RESULTS

### Species Composition and Abundance

Among the eight different sampling periods a total of 38,226 larvae were caught, representing 39 separate taxa (Table 1). Twenty-six larval types were identified to species. Of the remaining larvae, two were identified to genus and 11 to family. *Clupea harengus pallasii* (Pacific herring), *Engraulis mordax* (northern anchovy), and Gobiidae larvae comprised 98% of the total catch. The next most abundant types were *Cynoscion nobilis* (white seabass), *Leptocottus armatus* (staghorn sculpin), and *Hypsopsetta guttulata* (diamond turbot).

The most speciose family was Cottidae (seven species) followed by Pleuronectidae with five species. Large catches which occurred mostly in the fall and winter studies were due to Pacific herring spawning. When the herring were not spawning, Gobiidae replaced them as the most abundant type. Gobiid 1, the unidentified Gobiid larvae, was the only type caught in all eight samplings.

### Temporal Distribution

Results of factor analysis and correlation analyses of all eight studies combined throughout the year showed the following significant relations between variables within factors:

- i. the number of larvae positively correlated ( $P = .05$ ) with ebbing tides.
- ii. the number of species showed a significant ( $P = .01$ ) increase on ebb tides.
- iii. the catch (number of larvae/ $m^3$ ) was significantly ( $P = .05$ ) higher during ebb tide conditions.

Within individual 24-hr samplings similar analyses indicated varying results. During March ebb tides the plankton volumes and the number of species were the highest. The catches (number of larvae/ $m^3$ ) in June consisted mostly of Gobiidae. The number of eggs and the number of species were directly related to catch (number of larvae/ $m^3$ ). In August, during flood tides, the number of species and the number of eggs increased, and the catches of *Hypsopsetta*

TABLE 1. Numbers of Larvae of Different Fish Species Caught According to Location (Station 1/Station 2), date, and half tide stage.

Taxa	March 23, 24	May 16, 17	June 8, 9	Aug. 2, 3	Sept. 12, 13	Oct. 18, 19	Dec. 12, 13	Feb. 8, 9	LL 1	HL 2	LH 1	HH 2	Total				
Clupeidae																	
<i>Clupea harengus pallasi</i> ....	-/1094						857/14,800	171/3,795	2,116	1,860	291	746	218	51	9,346	89	20,717
Engraulidae																	
<i>Engraulis mordax</i> .....	-/4		1/0	1/4/20	95/72	95/72	1,810/65	365/27	1,097	349	480	501	29	56	58	135	2,705
Osmeridae																	
<i>Spirinchus thaleichthys</i> .....		1/0					1/0	2	2								2
<i>Allosmerus elongatus</i> .....							1/0	1	1								1
Osmerid 1.....	-/6				0/1		1/1	1	3	1	2	1	1				9
Osmerid 2.....		1/0											1				1
Batrachoididae																	
<i>Porichthys notatus</i> .....						0/1	2/0	1	1	1	1						3
Ophidiidae																	
<i>Brosmophycis marginata</i> ..		2/0		2/0				2					2				4
Atherinidae																	
<i>Atherinopsis californiensis</i>	-/1		1/0	1/0		0/3	1/0	2	2	1							3
<i>Atherinops affinis</i> .....							9/3	1	10		2			2			15
Syngnathidae																	
<i>Syngnathus leptorhynchus</i>					1/1	13/23	4/1	1/0	27	10	1	1	4	1			44
Sciaenidae																	
<i>Cynoscion nobilis</i> .....	-/1				1/0	37/17	9/3	54/5	67	7	18	2	3	21	9		127
Embiotocidae																	
<i>Cymatogaster aggregata</i> ....			1/1														2
Clinidae																	
Clinid 1.....	-/1		1/0					1/0	2	1							3
Clinid 2.....								1/2	1		1		1				3
Sichaeidae																	
Sichaeid 1.....		-/1			3/0	1/0		1/0	1		3	1					5
Sichaeid 2.....											1						1
Pholidae																	
Pholid 1.....								1/0			1						1
Ammodytidae																	
<i>Ammodytes hexapterus</i> ....							7/0	4		2				1			7

TABLE 1. (Continued)

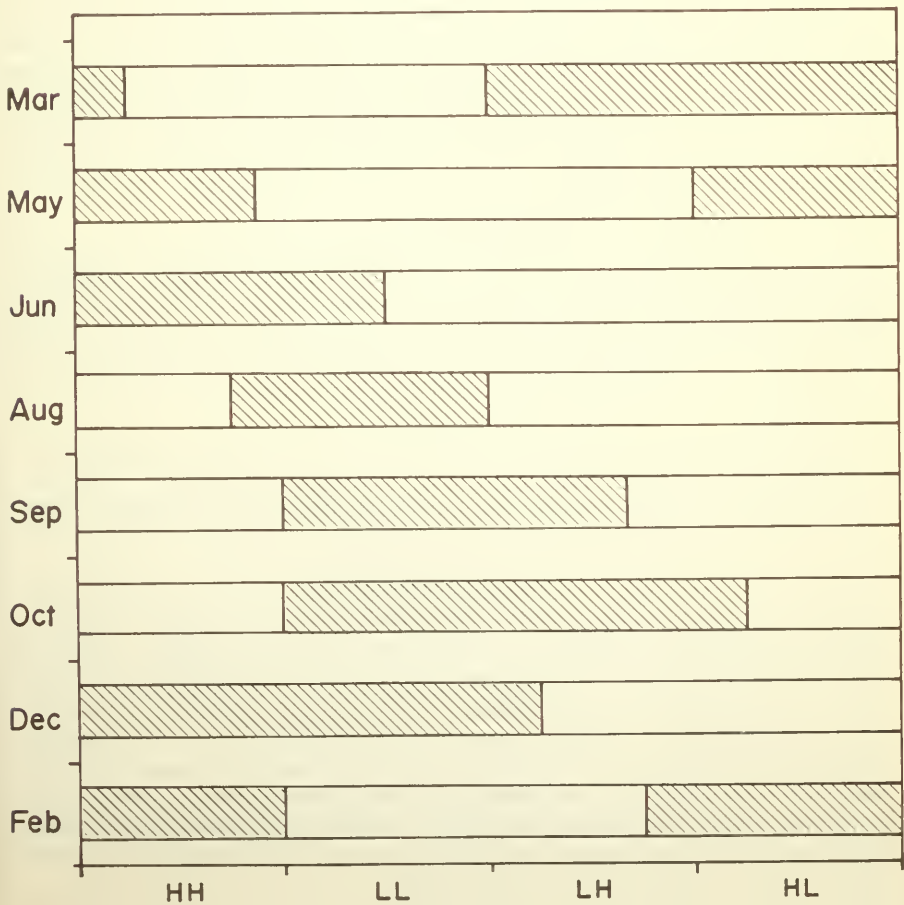
Taxa	March 23, 24	May 16, 17	June 8, 9	Aug. 2, 3	Sept. 12, 13 0/3 0/3	Oct. 18, 19 0/4	Dec. 12, 13 0/25	Feb.	LL	HL	LH	HH	Total				
Gobiidae									2	1	2	1	2				
<i>Cleavelandia ios</i> .....									1	2	1	2	32				
<i>Ilypnus gilberti</i> .....									3	1	3	23	1				
Gobiid 1.....									3	3	3	237	3				
Stromateidae									2,975	1,097	1,345	1,274	14,187				
<i>Peprilus similimus</i> .....					331/248	2,180/2,055	548/299		4,781	706	1,772	1,274	237				
Scorpaenidae						1/0						1	1				
<i>Sebastes</i> spp.....			1/0		14/6	4/1			10	9	7	5	2				
Hexagrammidae									4	1	2		7				
<i>Ophiodon elongatus</i> .....					7/0				1	2			2				
Cottidae																	
Scorpaenichthys																	
<i>marmoratus</i> .....					4/0				1	1			2				
<i>Leptocottus armatus</i> .....	-/21				38/14	1/0			46	16	25	13	4				
<i>Oligocottus maculosus</i> .....	-/2				4/0				1	1	4		8				
<i>Oligocottus snyderi</i> .....						1/0			1	4	2		1				
Cottid 1.....						0/1							1				
Cottid 2.....					2/1				1	1	2	1	1				
Cottid 3.....									1	1			3				
Bothidae													3				
<i>Paralichthys californicus</i> .....						11/0			10	1			11				
<i>Citharichthys</i> spp.....				0/1	7/1	6/6			6	1	2	5	22				
Pleuronectidae																	
<i>Lyopsetta exilis</i> .....													1				
<i>Parophrys vetulus</i> .....					1/0				1				1				
<i>Hypsopsetta guttulata</i> .....			1/0	6/13	5/3	24/17			36	5	4	12	80				
<i>Platichthys stellatus</i> .....			0/2						1	1	2	1	7				
<i>Psetichthys melanostictus</i> .....	-/5								6	10	3	23	43				
Total.....									8,228	11,269	1,910	2,015	2,077	1,484	10,763	490	38,236

*guttulata* were higher. Larval catches in number of larvae/m<sup>3</sup> correlated directly with the Gobiidae and *Engraulis mordax*. Catches of December's and February's samplings were comprised mostly of *Clupea harengus pallasii* and the highest numbers were caught during HH<sub>1</sub> stage. The greatest number of species (14 taxa) occurred on the LL<sub>1</sub> stage in February.

TABLE 2. Summary of Number of Larvae, Percentage of Total Catch, and Catch in Number of Larvae/m<sup>3</sup> According to Tide Stage

	Ebb				Flood			
	LL <sub>1</sub>	LL <sub>2</sub>	HL <sub>1</sub>	HL <sub>2</sub>	LH <sub>1</sub>	LH <sub>2</sub>	HH <sub>1</sub>	HH <sub>2</sub>
No. of Larvae .....	8,828	11,269	1,910	2,015	2,077	1,484	10,763	490
Percent of Catch .....	21.5	29.5	5.0	5.3	5.4	3.9	28.1	1.3
No./m <sup>3</sup> .....	6.109	11.262	1.761	1.458	5.030	1.479	9.926	.439

An examination of the results for the entire year according to half-tide stages (Table 2) demonstrates that both highest numbers of larvae captured and the highest density of larvae took place in LL<sub>2</sub>, HH<sub>1</sub> and LL<sub>1</sub>, respectively. Combin-





ing the catches of the two halves of each tide stage, 51% of all larvae were taken at the LL tide stage. These results indicate that larval densities were highest during the periods of high current, either HH flood or LL ebb.

In six of the eight 24-hr samplings in which accurate density measurements could be compared, much greater larval densities occurred during night conditions than in daylight (Table 3). The LL stage usually occurred at night (Figure 7). Two exceptions to these concurrences were March and June's samplings. Low low tide (LL) happened during daylight hours in March, and one-half of June's LL tide occurred during daylight. Interestingly enough, the only times when there were greater larval fish catches during the day were in March and June. This suggests that larval densities were related to current velocities. There is also a demonstrated positive relation between density and night condition.

TABLE 3. Comparison of Larval Fish Catches (Number of Larvae/m<sup>3</sup> at Night Versus Day)

	March	May	June	August	Sept.	Oct.	Dec.	Feb.	Overall Average
Day .....	3.1517	-	0.791	0.4889	4.0960	0.5393	1.1098	-	1.4612
Night .....	2.2138	-	0.6315	3.4370	7.330	13.7850	10.9410	-	7.2566

Conclusions about seasonal distribution of eggs and larvae must be considered preliminary and generalized due to the infrequency of sampling with only two samples per season. The number of species was greater in the winter samples. Winter sampling times were characterized by periods of high fresh-water inflow and precipitation, low water temperatures, low salinities, and high dissolved oxygen concentrations. The catch also was highest in winter during the periods with the greatest tide range.

### Spatial Distribution

Only limited conclusions can be drawn about spatial distribution because of infrequent sampling and because Station 2 only was fished in the March and May studies. In the six paired studies, more species were collected at Station 1, near the entrance of the bay. Higher catches of specific fishes occurred as follows:

- i. In June more *Citharichthys* spp., and *Hypsopsetta guttulata* were found at Station 1.
- ii. In September, both the catch and the number of species were higher at Station 1.
- iii. October and February's studies showed higher catches of *Sebastes* spp., and *Scorpaenichthys marmoratus* at Station 1.

### Individual Species

*Clupea harengus pallasii*—Adult *C. h. pallasii* historically have entered San Francisco Bay for spawning from November through March, and they spawn on the rocky shorelines near the entrance to Richardson Bay (Eldridge and Kaille 1973). I have noted the demersally spawned eggs on vegetation in upstream portions of the bay as well, but these spawnings were minor in both density and area of spawn. Catches of larvae occurred only during winter months (March, December, and February) and were correlated with the lowest temperatures recorded in all 24-hr samplings. The highest numbers of *C. h. pallasii* were caught on HH<sub>1</sub> (Table 1), indicating that the larvae were carried in on flood tides from areas outside or adjacent to Richardson Bay.

*Engraulis mordax*—Pelagic eggs of *E. mordax* have been collected every month of the year in the California current (Frey 1971) with peaks of abundance in late winter and early spring and another minor peak in early fall. Adult *E. mordax* normally are found in San Francisco Bay in greatest abundance from midsummer through early fall. Larvae were netted in samplings from August through March. The highest density occurred in December. The majority (91%) of larvae were captured at Station 1, suggesting that the main spawning occurred outside or near the entrance to Richardson Bay.

*Gobiidae*—A minimum of seven gobiid species have been known to inhabit San Francisco Bay (Aplin 1967; Ruth 1969; U.S. Fish and Wildlife Service 1970; Green 1975). There was difficulty in identifying this study's gobiid larvae because of a limited number of larval descriptions and the overlap of larval characters. Although most of the goby larvae could not be identified with certainty to species, the majority were very likely *Clevelandia ios*, the most commonly found goby in Richardson Bay.

Gobiid larvae comprised the only taxon found in all eight studies. They were also evenly distributed between the two stations (52% of all gobiid larvae were from Station 1). Over half (54%) of all Gobiidae were caught on LL ebb tide.

*Others*—Both *Syngnathus leptorhynchus* and *Leptocottus armatus* are common, year-round residents of San Francisco Bay. Both species of larvae were caught in the LL ebb currents (85% and 78%, respectively). There have been eight species of adult *Sebastes* caught in San Francisco Bay (Ruth 1969). Their larval presence in Richardson Bay was seasonal (all but one in October, December, and February) and they evidently entered the small bay from outside; 83% of all *Sebastes* larvae were from Station 1.

Three fishes (*Cynoscion nobilis*, *Hypsopsetta guttulata*, *Psettichthys melanostictus*), which most likely spawn pelagic eggs outside San Francisco Bay, were netted in fair numbers (Table 1) in Richardson Bay. *Cynoscion nobilis* and *Psettichthys melanostictus* were caught principally at Station 1 (72% and 88%, respectively) while *Hypsopsetta guttulata* appeared more generally dispersed between the two stations. In no San Francisco Bay surveys, to my knowledge, have sexually ripe adults of these three species been found.

## DISCUSSION

From the catches of all larvae and individual species, and from plankton volumes and numbers of species, it appears that the single most influential factor determining the density of fish larvae was tidal current. The highest larval densities occurred when tidal currents were the fastest; that is, on LL ebb and HH flood (Table 2). The fact that larval fish catches were markedly higher at night would appear to conflict with the tidal factor until one realizes the concurrence of strong LL ebb currents and night conditions. It is altogether likely and probable that light is a factor affecting distribution of larval fishes. Lewis and Wilkins (1971) found occurrences of three estuarine fishes (*Leiostomus xanthurus*, *Brevoortia tyrannus*, and *Logodon rhomboides*) had highly significant relations with the amount of light and tidal currents. Results of this study, however, indicate that between tidal and light factors tidal influence is more determinant with regard to distribution of fish eggs and larvae.

Associations of larvae with various physico-chemical parameters were found to correspond with variables which characterized either an upstream water mass

being carried out with ebb tide or outside marine water entering with flood tide. For instance, May's flood tide water was associated with high salinity, low temperature, and high amounts of stratification detected in vertical profiles. In winter, December's lower salinity and temperature values occurred on ebbing tides. This resulted from heavy runoff from local rains and low air temperatures.

An interesting correlation which arose was a highly significant ( $P = < .01$ ) negative correlation between the number of species and the coastal upwelling index. The indices used in this comparison are those described by Bakun (1973). A negative upwelling index signifies downwelling of surface water at the coast and a net shoreward movement of surface water. Coastal upwelling values were obtained from NMFS, Pacific Environmental Group (Monterey, California) and averaged for the 2-week periods prior to each study date. The types of larvae which were associated with negative upwelling indices are those which were most likely spawned offshore or, at least outside the entrance to San Francisco Bay. They were: *Cynoscion nobilis*, *Sebastes* spp., *Paralichthys californicus*, *Citharichthys* spp., *Lyopsetta exilis*, *Parophrys vetulus*, *Hypsopsetta guttulata* (weakly associated), *Platichthys stellatus*, and *Psettichthys melanostictus*. Four of these species were caught in such small numbers, it is most likely their presence is a fortuitous occurrence. This is especially true for *Peprillus simillimus* whose population centers off southern California (Hart 1973).

Although there have been few larval fish studies along the estuaries and coastal areas of the U.S. Pacific coast, two surveys (Eldridge and Bryan 1972; Percy and Myers 1974) compare well with this study in terms of species composition and catch (Table 4). *Clupea harengus pallasii*, gobiid and cottid species appeared abundant in all three locations, and *Cottidae* was the most speciose family. *Engraulis mordax* is the single most abundant adult fish both in biomass and individual numbers in San Francisco Bay (Aplin 1967). The pelagic eggs and larvae were evidently not a major constituent of the ichthyoplankton in the other, more northern bays.

TABLE 4. Results of Three Larval Fish Studies in Estuaries of the Pacific North Coast Area of the United States

	Number taxa	Number families	Most speciose family	Three most abundant taxa
Richardson Bay, California (Eldridge 1977) .....	39	20	Cottidae	<i>Clupea harengus pallasii</i> <i>Engraulis mordax</i> <i>Gobiidae</i>
Humboldt Bay, California (Eldridge and Bryan 1972) .....	37	17	Cottidae	<i>Lepidogobius lepidus</i> <i>Clupea harengus pallasii</i> <i>Leptocottus armatus</i>
Yaquina Bay, Oregon (Percy and Myers 1974) .....	45	17	Cottidae	<i>Clupea harengus pallasii</i> <i>Lepidogobius lepidus</i> <i>Cottus asper</i>

The limited scope of this particular study in space and time does not permit any conclusions regarding the degree or extent of dependence on the estuary by fishes. Results show that at least 39 different fish taxa including many species with offshore dwelling adult stages, were present in the bay. Among the three most abundant larval types, *C. h. pallasii* and *E. mordax* spawned in or near Richardson Bay and represent commercially fished species. Percy and Myers

(1974) concluded that although Yaquina Bay was an important nursery for the young of several marine fish species, their larval fish survey showed only *C. h. pallasi* to be abundant. This study's results differed principally in the relative abundance of *E. mordax*. An extensive survey conducted in oceanic areas adjacent to San Francisco Bay and simultaneous sampling inside the bay would establish whether the center of the anchovy population was located in the bay or outside.

### ACKNOWLEDGMENTS

Each 24-hr study required a minimum of six persons. The working conditions were often uncomfortable, the hours long, and the weather inclement.

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## NOTES

### SEA OTTER PREDATION ON PISMO CLAMS IN MONTEREY BAY

Although the sea otter, *Enhydra lutris*, has been reported to feed on bivalves (Ebert 1968; Vandever 1972; Hennessy 1972), only recently, since the otters expanded their range from rocky coast to sandy beach, have they been observed feeding on Pismo clams, *Tivela stultorum* (Wild and Ames 1974). The first observation of otters feeding in large numbers on Pismo clams was in Monterey Bay during April 1973 at well known beds some 500 m (1650 ft) south of Sandholt Pier at Moss Landing. During that same period, broken Pismo clam shells were first noticed on the surface of the sand intertidally and subtidally. Because Pismo clam shells are quite thick and resistant to breakage by the surf, it seemed probable that the increase in numbers of broken clam shells was due to predation by sea otters. Quantitative density surveys had been conducted during summer 1972 prior to the arrival of the sea otters so several beaches in Monterey Bay were resampled to obtain estimates of the effect of sea otter predation on the density of Pismo clams.

#### METHODS AND MATERIALS

The sampling design consisted of 20 randomly selected 1 m (3.3 ft) square samples within a 18.2 x 24.2 m (60 x 80 ft) grid placed so the lowest edge was at approximately the -1.0 ft tidal level. Each square meter was raked with a small hand rake set with tines that were spaced 12 mm (0.5 inch) apart and that penetrated 150 mm (6 inches) deep. A 0.25 m<sup>2</sup> area was subsampled by taking thirteen .018 m<sup>2</sup> cores that penetrated 150 mm into the substrate. The contents were emptied into a sieve box and sieved in the surf. The rakes gave adequate samples for clams greater than 39 mm (1.5 inches) in length as these clams could not fit between the tines sideways. The cores obtained quantitative samples for clams from 39 mm (1.5 inches) down to about 10 mm (0.4 inch). The lower limit of 10 mm was determined by the mesh size in the sieve box. Each clam was measured, the quadrat of occurrence noted, and then reburied. The grid was positioned from a landmark on the beach so that repeated samplings could be made in exactly the same spot. Observations on sea otter feeding were made during September 1973 with a 15-60× zoom telescope. During each observation the time of feeding, location of feeding, prey, and estimate of the size of clam were recorded.

#### RESULTS AND DISCUSSION

Three stations were sampled to obtain estimates of the densities of large Pismo clams (> 39 mm in length) (Figure 1). The first sightings of otters actively feeding on Pismo clams were at Moss Landing in April 1973. A maximum of 10 otters at one time was seen foraging there during the summer of 1973. At the station further north at Zmudowski State Beach, otters were not reported feeding on Pismo clams until 7 February 1974, when two otters were observed, and California Department of Fish and Game biologists observed 17 sea otters foraging off Palm Beach (adjacent and north of Zmudowski) in April of 1974 (Miller, Hardwick, and Dahlstrom 1975). At the station furthest north, Monterey Bay Academy, no otters had been reported until late January 1975. The station at

Moss Landing has therefore been exposed to sea otter predation the longest, Zmudowski the next longest, and Monterey Bay Academy not at all during this study.

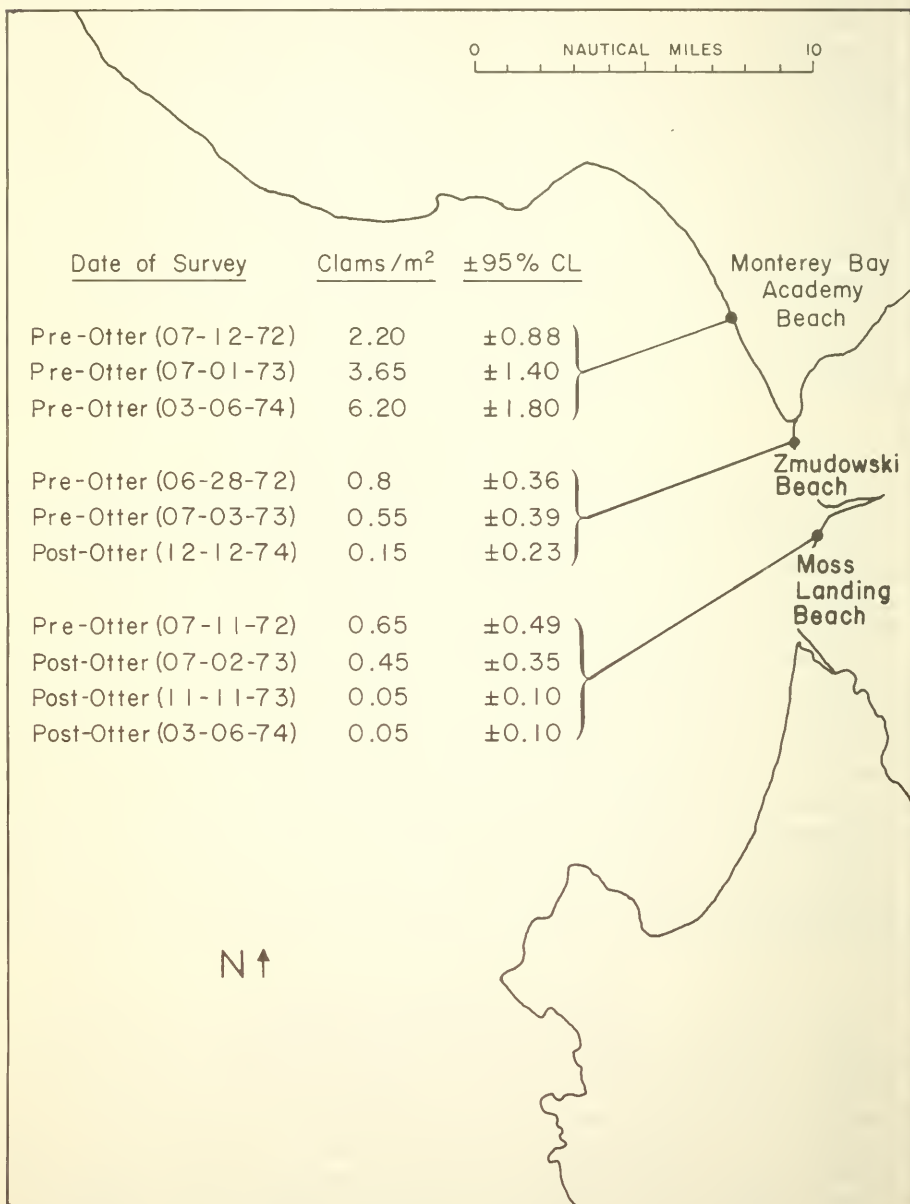


FIGURE 1. Location, date of survey, density, and 95% confidence limits of Pismo clams greater than 39 mm in length collected from Monterey Bay.

The densities of large Pismo clams ( $> 39$  mm in length) decreased from 6.5 to 0.5 clam/m<sup>2</sup> at Moss Landing during the period when otters were foraging there (Figure 1). At Zmudowski State Beach the density of large Pismo clams was 0.8 and 0.55 clam/m<sup>2</sup> in surveys made before sea otters were reported foraging, and decreased to 0.15 clam/m<sup>2</sup> in the survey made after sea otters were reported foraging at this beach. At Monterey Bay Academy, where no otters had been reported during the course of this study, the density increased from 2.2 to 6.2 clams/m<sup>2</sup>. The data could not be analyzed statistically due to the low densities found in some of the surveys. Human predation is probably not responsible for the observed declines in clams at Moss Landing and Zmudowski because 99% of the Pismo clams reported in this survey are sublegal, or less than 126 mm (5 inches) in length, and should not be affected substantially by clamming pressure.

Clammers were interviewed during the 1973–74 season at Moss Landing to determine clamming success after the sea otter was reported foraging there. Eleven clammers obtained one legal-size clam in 13 man days for a mean catch/effort of 0.08 clam/man/day. When asked about their clamming success the previous season before otters were reported there, they reported they obtained 137 clams in 28 man days, for a mean catch/effort of 4.9 clams/man/day. At Zmudowski Beach the catch/hour decreased from 2.12 in January 1974, before otters were reported, to 0.07 clam/hour in January 1975, after the otters had been reported foraging on this beach for a period of 11 months (Miller, Hardwick, and Dahlstrom 1975). The catch/hour at Monterey Bay Academy beach where otters were not reported until January 1975 was 2.72 in October 1974, 2.6 in November 1974, 2.23 in December 1974, 1.93 in January 1975, and 2.25 in February 1975 (Miller, Hardwick, and Dahlstrom 1975), indicating the catch per unit effort has not declined like those at Zmudowski and Moss Landing.

Other evidence of the decline of Pismo clams at Moss Landing is available from my monthly catch per unit effort observations during collection of clams for reproductive studies. From February 1972 to April 1973, two divers could collect 20 clams subtidally each month at Moss Landing within 45 min. During the summer of 1973, clams became progressively more difficult to obtain. Finally, during September 1973, divers could collect only one clam in 3 hr and 30 min, and the reproductive study had to be abandoned. The sand bottom contained large numbers of broken shells at this time, although prior to April 1973 broken shells were observed only rarely.

Factors other than sea otter predation that might have caused a decline in Pismo clam stocks at Moss Landing and Zmudowski, such as lack of recruitment, were probably not responsible for these declines. These decreases occurred suddenly, in less than 1 year's time. Furthermore, surveys during 1972 indicated that recruitment had occurred at these beaches over the previous 5 years.

Several direct observations were made on sea otters feeding on Pismo clams. On one occasion a single otter ate 24 Pismo clams and two spiny mole crabs, *Blepharipoda occidentalis*, in 2 hr and 15 min, and the average size of the clams consumed was estimated to be 100 mm (4.0 inches). On another occasion an otter fed on 14 Pismo clams and 37 spiny mole crabs. The average size of the clams was estimated to be 125 mm (5.0 inches) and the feeding period 4 hr. Another otter ate 22 Pismo clams in 2 hr at Atascadero State Beach (Wild and Ames 1974). Miller *et al.* (1975) estimated the number of Pismo clams eaten

in Monterey Bay from dietary requirements of otters and otter counts to be between 520,000 and 700,000 clams in 1 year.

### CONCLUSION

The density and catch per unit effort data indicate that the numbers of large Pismo clams have declined in areas that have been exposed to sea otter predation. Observations on the feeding of sea otters on Pismo clams and increased numbers of broken shells on the beach indicate that otters have contributed substantially to the decline.

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## THE UNDERWATER FORAGING HABITS OF THE SEA OTTER, *ENHYDRA LUTRIS*

The sea otter, *Enhydra lutris*, is a member of the nearshore community along the central California coast. The sea otter feeds on a wide variety of invertebrates including mussels, sea urchins, abalones, crabs, snails, and annelids (Vandever 1969). Faro (1969) and Houk and Geibel (1974) have described methods used by sea otters to remove abalones from substrate. No other detailed accounts of sea otter foraging have been reported. During the summer of 1974, Charles Totman and I made some brief observations on these foraging habits.

Our first observation was made within a dense forest of giant kelp, *Macrocystis* spp., in the vicinity of Hopkins Marine Station, Pacific Grove, at a depth of 7 m (23 ft). A large male otter was observed patting the vertical surface of a large granitic boulder with its forepaws. The otter swam approximately 1 m (3.3 ft) and stuck both forepaws into a crevice, appeared to feel around, but did not remove anything from the crevice. The sea otter then swam to a *Macrocystis* plant, reached around the holdfast and collected two snails (species unidentified). More boulders were quickly patted and the sea otter returned to the surface, consumed the two snails within 8 seconds, and dove again but was



not sighted. The sea otter worked very quickly underwater, feeling over three boulders, into one crevice, and around a giant kelp holdfast in approximately 15 seconds. The otter did not, in our opinion, appear to be searching visually for food. While patting boulders and reaching into the crevice the sea otter's head was held back, away from the working forepaws.

On 12 August, 1974, an otter was observed foraging at Stillwater Cove, Carmel. The water was 4 m (13 ft) deep and the substrate was silt and cobbles. When first observed underwater, only the otter's hind flippers could be seen, the rest of its body working in a cloud of silt. When the sea otter surfaced, Totman and I moved to investigate. We found several holes 30 to 40 cm (12 to 16 inches) in diameter and approximately 20 cm (8 inches) deep. Several minutes later the sea otter returned and we were able to determine how the holes had been dug. While kicking its hind flippers to stay close to the bottom, the sea otter was digging with its forepaws and rooting with its head. The circular motion of the forepaws was such that the power part of the movement occurred when the paws were brought in towards the axis of the body and down towards the rear flippers. On at least two consecutive dives the otter returned to the same hole enlarging its length. The rectangular-shaped hole was approximately 50 x 30 x 20 cm (20 x 12 x 8 inches). The otter's average bottom time was 70 seconds and the average surface interval between dives was 20 seconds. Surface observations made by Judson Vandevere revealed that the otter was surfacing with *Urechis caupo*, the fat innkeeper worm. The tube building worm, *Eudistyla* spp. and the purple olive snail, *Olivella biplicata*, which were common in the holes were not consumed. Due to the turbidity of the water, it is doubtful whether the otter could have sighted the worms while digging. The sea otter may be able to follow tactually the U-shaped burrow of the fat innkeeper worm.

On 13 August, 1974, an otter was seen foraging in a small area within Stillwater Cove harbor. The water was 3 to 4 m (10 to 13 ft) deep and visibility was poor, about 2 m (7 ft). The bottom in this area was covered heavily with the leaves of *Laminaria* spp. The otter was located, but was followed only for a single dive. The otter was observed swimming under the dense cover of *Laminaria* leaves close to the substrate. Occasionally, the rear flippers of the otter were seen, but otherwise the otter appeared to be a large lump moving through the *Laminaria*. The sea otter's bottom time was 35 seconds during which it covered approximately 10 to 12 m (33 to 40 ft) in essentially a straight line. Surface observations revealed that the sea otter was feeding on crabs, *Pugettia* spp.

These observations reveal the variety of sea otter foraging behavior. Previous observations indicated tool use underwater to dislodge abalones, and our observations demonstrate sea otters also can dig, "root", and move through dense cover removing food items. These observations suggest that sea otters may find areas suitable to forage visually, but locate food items tactually.

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## FEEDING IN THE DECORATOR CRAB, *LOXORHYNCHUS CRISPATUS* (Brachyura: Majidae)

*Loxorhynchus crispatus* Stimpson, 1857, commonly known as the moss crab or decorator crab, is an inhabitant of subtidal rocks and pilings along the coast of California. O'Connell (1953) found it to be an important component of the diet of the cabezon, *Scorpaenichthys marmoratus*.

### METHODS

As part of a study on the behavior of the species, the feeding habits were studied at both the Allan Hancock Foundation, Los Angeles; and at Hopkins Marine Station, Pacific Grove. Direct observations of the crabs were made during 1974 and 1975 while SCUBA diving near Hopkins Marine Station, north of the mouth of San Jose Creek in Carmel Bay, and along the main breakwater and the Cabrillo Fishing Pier, San Pedro.

Six crabs were captured while diving on the breakwater at San Pedro and off Hopkins Marine Station, then promptly killed to determine the contents of both the stomachs and hindguts. An additional 12 crabs were isolated in buckets immediately after capture off Hopkins Marine Station. The feces of these crabs were collected. Smears of both the feces and the contents of the stomachs and hindguts were analyzed at 100X.

Fifty-three *L. crispatus* were kept in aquaria; those at the Allan Hancock foundation in closed-system refrigerated tanks, and those at Hopkins Marine Station in aquaria with running sea water. The aquaria contained rocks, hermit crabs (*Pagurus* spp.), shrimp (*Heptacarpus* spp. and *Alpheus dentipes*), and small fishes of the families Cottidae, Stichaeidae, Gobiesocidae, and Clinidae as well as the crabs.

The crabs were fed once a week on chopped fish, chopped mussels (*Mytilus edulis*), or pellets made of dried brine shrimp, *Artemia salina*. The crabs also were given red algae, green algae, and invertebrates smaller than 20 mm (0.8 inches) in greatest dimension. After a new alga or invertebrate was collected, it was placed within easy reach of the crabs. If the crabs ate the organism within 1 hr after its introduction into the tank, a record was made of the feeding. Daily observations also were made of the aquaria, so that organisms eaten after 1 hr could be recorded. When possible, the feces of the crabs were collected on the day after feeding was observed, and examined for undigested material.

Predation on the purple sea urchin, *Strongylocentrotus purpuratus*, was studied by placing sea urchins measuring 5 to 70 mm (0.2 to 2.8 inches) wide in the aquaria with the crabs. Activities involved in predation were analyzed by direct observation and by taking series of still photographs during feeding.

## RESULTS

A large male *L. crispatus* was seen in its natural habitat eating a sea urchin, *S. purpuratus*. Other divers have observed *L. crispatus* eating dead jellyfish, *Pelagia noctiluca* (D. Chivers, pers. commun.)

Due to the maceration of food by both the mandibles and the gastric mill, as well as the dissolution of soft parts by digestive juices, only hard parts and undigested soft materials could be identified in the feces, guts, and stomachs of the crabs. The most common items found were red algae (from 9 crabs), at least four species of sponges (from 9 crabs), small crustaceans (*Cancer* sp., *Mimulus foliatus*, *Pagurus* sp., a cypris larva, and harpacticoid copepods, from 5 crabs), branched bryozoans (from 4 crabs), bits of *S. purpuratus* (from 3 crabs), and bits of brown algae (from 2 crabs). Other organisms seen were an unidentified holothurian, an ascidian, and bits of surf grass, *Phyllospadix* sp. Fine sand grains were found in the digestive tracts of three crabs.

In aquaria, *L. crispatus* ate many sessile or slow-moving organisms: red algae, green algae (both *Ulva* sp. and *Cladophora* sp.), sponges, a scaleworm (Family Polynoidae), a limpet (*Collisella pelta*), ophiuroids, the sea stars *Pycnopodia helianthoides*, *Henricia leviuscula*, and *Leptasterias aequalis*; sand dollars (*Dendraster excentricus*), and the sea urchins *S. purpuratus* and *S. franciscianus*. Although the nudibranchs *Triopha maculata* and *Archidoris montereyensis* were consumed, the feces excreted by the crabs on the next day contained chunks of undigested tissue from the nudibranchs. The crabs did not eat live shrimp, adult hermit crabs, or any live fishes.

Organisms to be eaten usually were torn apart by the chelae, then held by the third maxillipeds while pieces were chopped by the mandibles. Sea urchins, however, were cracked open before being masticated. The sea urchin first was grabbed by both chelae. Using a twisting motion, the two chelae ripped open the test. Any crab could crack a sea urchin if the height of the test of the sea urchin either equalled or was less than the gape of the fingers of the chelae. Adult males, however, could crack larger sea urchins by stabbing the fingers of the chelae through the perioral membrane. After tearing out the jaw apparatus (Aristotle's lantern) of the sea urchin, the fingers were inserted inside the test, and the tissue pulled out. All parts of the small sea urchins (less than 30 mm wide), including the test and spines, usually were eaten. The well-smashed tests and spines later were excreted in the feces. The tests of larger sea urchins were not eaten, but often were cracked by the crabs.

## DISCUSSION AND CONCLUSIONS

The diet of *L. crispatus* differs somewhat from the diets of other members of the family Majidae. Although *L. crispatus* eats organisms similar to those found by Hartnoll (1963) in the diets of 11 species of majid crabs from the Isle of Man, it also eats sponges, which the Manx spider crabs were not observed to eat. *Loxorhynchus crispatus* does not eat much brown algae, which Knudsen (1964) found to be an important part of the diet of the kelp crab, *Pugettia producta*; nor does it appear to eat live fish, which Dixon and Dixon (1891) saw captured by the crab *Hyas araneus*.

Although the feeding of the sheep crab, *Loxorhynchus grandis*, has not been studied in detail, five individuals observed in aquaria during May 1975 appeared to be more predatory than *L. crispatus*. During one week, a female *L. grandis*

kept at the Cabrillo Marine Museum in San Pedro ate eight live hydrozoans, *Velella velella*; three arms of a living starfish, *Astrometis sertulifera*; a live *Den-draster excentricus*, and a live *Strongylocentrotus franciscianus*.

It is concluded that *L. crispatus* has a varied diet in its natural habitat. It seems to be a scavenger or a grazer on sessile organisms, rather than an active predator.

#### ACKNOWLEDGMENTS

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### OBSERVATIONS ON FOOD HABITS OF THE ANGELFISHES *POMACANTHUS ZONIPECTUS* AND *HOLACANTHUS* *PASSER* IN THE GULF OF CALIFORNIA

During the summer of 1971 we captured six each adult angelfishes (Chaetodontidae: Pomacanthinae) of the species *Pomacanthus zonipectus* (Gill) and *Holacanthus passer* Valenciennes at Guaymas, Sonora, Mexico, along the central mainland coast of the Gulf of California. We examined their stomach contents to determine food preferences, as part of a general behavioral study. We also conducted a laboratory experiment on feeding preferences of one juvenile *P. zonipectus*.

These two angelfishes are the only members of the subfamily Pomacanthinae known to occur widely in the Gulf; *H. clarionensis* occurs only in the Cape region of Baja California. Diving observations showed these species to be diurnal substrate feeders.

Results of the stomach content analysis showed that the diet of adult fishes consisted mainly of sponges (more than 50% by volume), with *H. passer* consuming a larger percentage of sponge (71-80%) than *P. zonipectus* (53-60%). The second most common food item for both species was algae, which formed a larger proportion of the diet of *P. zonipectus* (35-38%) than of *H. passer* (15-16%). Other food items for *P. zonipectus* (5-9%) included hydroids, bryozoans, fish eggs, a shell-less pulmonate snail and one ctenoid scale; for *H. passer*, this category (4-14%) included caprellid and other amphipods and a single small holothurian.

A 65-mm (2.6-inch) TL juvenile *P. zonipectus* was captured alive and placed in an aquarium. Given a choice of three sponge species, 12 algal species, *Porites* coral, and small planktonic crustacea, the fish took more than 50% of the total

number of bites at the algae during three 10-minute observation periods (50–70% algae, 29–46% sponge, 1–4% *Porites* and plankton). This experiment gives at least a semi-quantitative estimate of the food preference of juvenile *P. zonipectus*, although actual volume of each material consumed was not determined.

Randall and Hartman (1968) reported that in the West Indies angelfishes of the genus *Holacanthus* (*H. ciliaris* and *H. tricolor*) consumed over 95% sponge by volume, while sponges comprised over 70% of the diet of *Pomacanthus* spp. (*P. arcuatus* and *P. paru*). They also noted that young of both genera have been observed to feed mainly on algae and on crustacean ectoparasites of reef fishes. One 95-mm (3.7-inch) *P. arcuatus* had eaten 75% filamentous algae by volume, as well as parasitic and free-living copepods. The stomach of a 59-mm (2.3-inch) *P. paru* contained algae and copepods, while a 95-mm (3.7-inch) specimen contained only sponge. Lowe (1962) reported sponges as the primary food of *P. arcuatus* and *P. paru* along the coast of South America, while Randall (1963) found sponges as the major food of *Pomacanthus* and *Holacanthus* spp. in the Virgin Islands. However, Menzel (1960) reported that stomach contents of *H. bermudensis* in Bermuda consisted almost entirely of plant material during March and December.

We conclude that the feeding habits of *P. zonipectus* and *H. passer* in the central Gulf of California are similar to the feeding habits of their congeners, *P. arcuatus*, *P. paru*, *H. ciliaris*, and *H. tricolor* in the West Indian region. We confirm Randall and Hartman's (1968) finding that sponges form a relatively larger part of the diet of the *Holacanthus* species studied than of the *Pomacanthus* species studied. It also appears that sponges comprise a somewhat smaller, but nevertheless major, part of the diet of these angelfish genera in the Gulf of California than in the West Indies. Furthermore, juvenile *Pomacanthus* appear to consume a larger proportion of algae, and less sponge, than do the adults.

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## A NEW RECORD-SIZE BONITO SHARK, *ISURUS OXYRINCHUS* RAFINESQUE, FROM SOUTHERN CALIFORNIA

On August 28, 1972, Joseph Gorgita harpooned a large bonito shark 5 miles south of the west end of Anacapa Island (Figure 1). William Walker and John Prescott of Marineland of the Pacific informed me of this catch. Mr. Gorgita donated the specimen to the Natural History Museum of Los Angeles County (LACM 32667).



FIGURE 1. *Isurus oxyrinchus*, an 11-ft, 5-inch bonito shark, LACM 32667. Taken by Joseph Gorgita off Anacapa Island. Photograph by Larry Reynolds.

The bonito shark caught by Mr. Gorgita was a female with a total length of 11 ft, 5 inches (3,507 mm). It weighed 1,030 lb (468 kg) at Port Hueneme 28 hr after capture.

On the day of capture at 1215 hours, a swordfish harpoon was driven into the head of the shark, just behind the chondrocranium. The fish struggled fiercely for 30 min. Then a 30 lb weight and buoy were attached to the line, and the struggle continued for 2½ hr before the shark was subdued and landed at 1515 hours.

The following table of measurements (Table 1) compares this specimen to a previously reported bonito shark (Applegate 1966). In both specimens, original measurements were taken in inches converted to millimeters. After a careful search of LACM 32667, no spiracle was found, therefore, spiracle measurements were omitted. The proportions are given as the percentage of the length to the upper caudal origin.

Except for five measurement lengths (snout, mouth width, length of the base of the second dorsal fin, length of the anterior margin of the pectoral fin, and trunk width), it is evident that the Anacapa *Isurus* is larger in most respects than the Catalina *Isurus*. The different snout length could represent possible individual variation, as could the length of the dorsal fin base. The two sharks have different pectoral fin shapes. The variation in trunk width could be due to the spreading of the carcass as LACM 32667 was several days old when measured, and the Catalina fish had been frozen prior to measuring.



**TABLE 1. Measurements of Body Parts and Proportions in Length of Two Large Bonito Sharks. A, An 11-ft *Isurus oxyrinchus* (LACMVP F1059) from Santa Catalina Island. B, An 11-ft, 5-inch *Isurus oxyrinchus* (LACM 32667) from Anacapa Island.**

	<i>Fem. A</i> mm (%)	<i>Fem. B</i> mm (%)
Total Length.....	3,365 (118.7)	3,507 (119.3)
Length without caudal .....	2,835 (100.0)	2,939 (100.0)
Trunk width over pectoral origins .....	512 (18.1)	408 (13.9)
Mouth width .....	270 (9.5)	268 (9.1)
length .....	190 (6.7)	242 (8.2)
Eye diameter: horizontal.....	43 (1.5)	45 (1.5)
vertical .....	45 (1.6)	51 (1.7)
Preoral length .....	142 (5.0)	96 (3.3)
Prenarial length.....	128 (4.5)	128 (4.4)
Preorbital length .....	227 (8.0)	217 (7.4)
Internarial length.....	130 (4.6)	140 (4.8)
Length: 1st gill slit .....	350 (12.3)	370 (12.6)
2nd gill slit .....	320 (11.3)	351 (11.9)
3rd gill slit.....	310 (10.9)	335 (11.4)
4th gill slit.....	300 (10.6)	325 (11.1)
5th gill slit.....	300 (10.6)	312 (10.6)
1st dorsal fin: height .....	320 (11.3)	376 (12.8)
length base.....	320 (11.3)	351 (11.9)
length posterior margin .....	50 (1.8)	62 (2.1)
2nd dorsal fin: height.....	58 (2.0)	77 (2.6)
length base .....	38 (1.3)	32 (1.1)
length posterior margin.....	58 (2.0)	70 (2.4)
Anal fin: height .....	64 (2.3)	83 (2.8)
length base .....	35 (1.2)	38 (1.3)
length posterior margin.....	58 (2.0)	83 (2.8)
Pectoral fin: anterior margin .....	850 (30.0)	727 (24.7)
distal margin .....	560 (19.8)	669 (22.8)
length base .....	220* (7.8)	268 (9.1)
Pelvic fin: anterior margin .....	140 (4.9)	140 (4.8)
distal margin.....	210 (7.4)	242 (8.2)
length base.....	150 (5.3)	216 (7.3)
Caudal fin: length dorsal lobe .....	635 (22.4)	727 (24.7)
length ventral lobe.....	515 (18.2)	599 (20.4)
dorsal tip to notch.....	65 (2.3)	153 (5.2)
depth notch .....	15* (0.5)	25 (0.9)
Tip of snout to: 1st dorsal origin .....	1,338 (47.2)	1,454 (49.5)
2nd dorsal origin .....	2,434 (85.9)	2,658 (90.4)
anal origin .....	2,611 (92.1)	2,652 (90.2)
lower caudal origin .....	2,830 (99.8)	2,958 (100.6)
Distances between bases: 1st and 2nd dorsals .....	883 (31.2)	925 (31.5)
2nd dorsal and caudal.....	275 (9.7)	293 (10.0)
pectoral and pelvic .....	935 (32.9)	1,020 (34.7)
pelvic and anal.....	390 (13.8)	395 (13.4)
anal and caudal .....	235 (8.3)	274 (9.3)

\* These measurements were incorrectly reported in Calif. Fish and Game 52(3):204-207, 1966.

The Anacapa *Isurus* has 189 vertebrae. Of these, 110 are precaudal and 79 are caudal. This count is well within the range ascribed by Garrick (1967) for *Isurus oxyrinchus*.

Lengths of selected teeth measured at right angles from the center of a line across the ends of the roots to the tip of the tooth are:

2nd upper left anterior:.....	42 mm (1.7 inches)
1st upper left intermediate: .....	19 mm (0.7 inch)
1st upper left lateral: .....	28 mm (1.1 inches)
2nd lower left anterior:.....	47 mm (1.9 inches)
3rd lower left anterior: .....	33 mm (1.3 inches)
1st lower right anterior: .....	47.5 mm (1.9 inches)

The dental formula for the Anacapa *Isurus* is:

P3	L6	I1	A2	A2	I1	L6	P3
P4	L6		A3	A3		L6	P3

Counts from other specimens in the Natural History Museum indicate variation in the number of posterior teeth and lateral teeth in *Isurus oxyrinchus*. The posteriors range from 3 to 5, whereas the laterals range from 5 to 7.

In both adult females discussed in this paper, all of the anterior teeth show sharp blades that run from the tip of the tooth to the bottom corner of each crown. These teeth, contrary to Garrick's statement (Garrick 1967:679), are quite different from the long finned mako, *Isurus belyaevi*.

A large jaw in the museum collection without sex or size data, LACMVP F410, has a second lower anterior measuring 30.8mm (1.2 inches). The blades of the anterior in LACMVP F410 reach the base of the teeth except in the first lower anterior and the first upper anterior. The outer blades of the first lower anterior run approximately one-half the distance between crown tip and base of the crown. The blade runs three-quarters of the distance from tip to crown base on the outer edge of the first upper anterior.

A jaw from Baja California, LACMVP F361, has a second lower anterior tooth with a total length of 25.5mm (1 inch); the first lower anterior has an outer blade that runs for one-third the distance between the tip and the crown base. The second lower anterior has a blade that runs three-quarters of the way down the outer edge of the crown. The first upper anterior has a blade that runs one-quarter of the distance between tip and crown base.

These specimens, each jaw smaller than the other, suggest that blade length in these three tooth positions is related to tooth size and the amount of blade present is related to the length of the shark. A larger sample would be needed to confirm this observation. At least one morphological feature, that of blade length of the anterior teeth in these sharks, may change with age. In the same manner the number of lateral denticles as reflected in the teeth of *Heterodontus* indicates ontogenetic changes (Garman 1913).

Bigelow and Schroeder (1948) remark that the mako or bonito shark is believed to reach a length of 13ft (4m). The longest specimen actually reported by them was estimated at 12ft (3.7m). This length was calculated from the size of the jaws, a highly questionable procedure. The International Game Fish Association (1972) reports that the record *Isurus oxyrinchus* caught with rod and reel was 12ft, 2 inches (3738mm) and weighed 1061 lb (483 kg). It was captured in 1970 off of Mayor Island, New Zealand. A large bonito shark captured by a Russian vessel in 1972 measured 3800 mm (12ft, 6 inches) and weighed approximately 500 kg (1100 lb). It was captured in the east Indian Ocean (Gubanov 1974).

This most recent California specimen of *Isurus oxyrinchus* represents an eastern Pacific record. Since a good possibility exists that record-size *Isurus* are not rare in southern California, there still remains a chance that a world's record may be caught there.

### ACKNOWLEDGMENTS

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## **PARASITISM OF BARRED SURFPERCH, *AMPHISTICHUS ARGENTEUS*, BY *NEROCILA CALIFORNICA*: A FIT TO THE POISSON**

The Poisson distribution is widely used in a variety of situations where any given event has a small probability of occurring and each event is assumed to occur at random. Past applications, as mentioned in Sokal and Rohlf (1969) and Feller (1968), have included examining the distribution of radioactive disintegrations, flying-bomb hits on London during World War II, connections to wrong telephone numbers, chromosome interchanges in cells, bacterial and blood counts, parasitic infestations, and Bortkiewicz's (1898) classic example of soldier deaths due to horses in the Prussian army. We wish to present another example of the Poisson's usefulness based on information derived from biological systems.

While gathering information on the frequency of occurrence of various physical anomalies in marine fishes from the Californias (Valentine 1972, 1975) we noted that the ectoparasitic isopod, *Nerocila californica*, frequently parasitized the barred surfperch, *Amphistichus argenteus*. A total of 323 fresh and preserved specimens of barred surfperch were examined from central Baja California,

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Mexico, to central California (additional information on these collections is given by Valentine, et al. 1973). The only fish examined, though, which were parasitized by *Nerocila* came from Belmont Shore, located adjacent to the southern entrance of the Los Angeles-Long Beach Harbors in southern California.<sup>1</sup>



FIGURE 1. The ectoparasitic isopod *Nerocila californica* attached to the ventral part of the lateral surface of the caudal fin of the barred surfperch, *Amphistichus argenteus*. The parasite is approximately 21 mm long.

*Nerocila* were observed to parasitize barred surfperch only on the caudal fin, at roughly the juncture of the hypural plate and caudal fin rays (Figure 1). The incidence of parasitism by *Nerocila* is easily quantifiable. In instances where the parasite has, through natural or mechanical means, been detached from its host, a roughly elliptical series of small scars remains. These scars, as well as disturbances in scale pattern or deformities in skin overlying the hypural plate, are easily detectable using a low power dissecting microscope. *Nerocila* must be a tenacious parasite because many specimens remained attached to their host during collection (fish were captured with a beach seine) and preservation. In no case, though, was more than one parasite found attached to a host, although multiple parasitism, as indicated by scars, was common.

Data on the incidence of caudal fin parasitism by *Nerocila* on barred surfperch from Belmont Shore are presented in Table 1. The fit of the observed data to the theoretical Poisson is exceptional ( $\chi^2_3$ ,  $0.975 < p < 0.995$ ).

Because parasitism appears to be random, one would suspect that other parameters related to *Nerocila* infestation might also occur randomly. This assumption appears to be only partially correct. The hosts were divided into size

<sup>1</sup> Specimens referred in this report from Belmont Shores are in the ichthyological collection of the University of California, Los Angeles, accession numbers UCLA58-377, UCLA69-15, UCLA70-7.

(age) classes because one might argue that larger (older) fish would be more highly subject to parasitism, having been exposed to *Nerocila* longer than smaller (younger) fish. No such trend was seen,<sup>2</sup> although for very young fish this is probably true.

TABLE 1. Incidence of Ectoparasitism of the Caudal fin by *Nerocila californica* in Barred Surfperch from Belmont Shore, California, 1958 and 1969-1970\*

Number of scars per fish	Number of fish with scars	
	Observed	Expected
0	70	69.90
1	59	59.54
2	26	25.05
3	5	7.07
4	3	1.49

\* Expected incidence of ectoparasitism is based on the Poisson distribution:

$$P(x = x) = \frac{e^{-\lambda} \lambda^x}{x!} \quad \text{for } x = 0, 1, 2, \dots$$

$$= 0 \quad \text{otherwise}$$

where  $\lambda$  is estimated by sample mean

$$\bar{x} = 0.85.$$

One might also inquire whether there is a tendency for *Nerocila* to favor either the right or left side of the caudal fin for attachment. The answer is apparently negative, as 66 scars were found on the right and 72 scars on the left sides of the caudal fin of barred surfperch. (Table 2,  $\chi^2$ ,  $0.75 < p < 0.50$ ). There is an indication, however, that the preferred site of attachment is on the upper half of the caudal fin. Of the 138 scars observed, 88 were on the top half of the caudal fin, 47 on the bottom, and only 3 in the center ( $\chi^2$ ,  $p < .005$ , ignoring the three parasites on the center of the caudal fin).

TABLE 2. Side and Location of Attachment of the Ectoparasite *Nerocila californica* on the Caudal Fin of Barred Surfperch from Belmont Shore, California, 1958 and 1969-1970

	Side		Totals
	Right	Left	
Top	45	43	88
Center	1	2	3
Bottom	20	27	47
Totals	66	72	138

Little is known concerning the biology of *Nerocila californica*. Most references are fairly obscure taxonomic reviews, with some additional distributional information being available. Barred surfperch, though, do not migrate far within short periods of time and are found almost exclusively in and near the surf zone (Carlisle, et al. 1960). Thus, the lack of *Nerocila* in other barred surfperch collections may be tentatively attributed to an elevated concentration of parasites in the Los Angeles-Long Beach Harbors. The reason for the apparent concentration of *Nerocila* at this location is unknown, but may have something to do with the apparently degraded marine environment in these harbors (Fay 1972).

<sup>2</sup> Size classes of individuals in millimeters, sample size (N), number of parasitic scars observed per size class and mean incidence of parasitism are as follows: 75.0-99.9 (103), 83, 0.81; 100.0-124.9 (44), 45, 1.02; 125.0-199.9 (16), 10, 0.62.



The data presented suggest that caudal fin parasitism of barred surfperch by *Nerocila californica* with respect to size (age) of fish parasitized, number of parasitic attacks per fish and side of caudal fin parasitized is random. *Nerocila* do, though, exhibit a strong preference for the top of the caudal fin over other possible attachment points.

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## RANGE EXTENSION OF TWO MARINE FISHES TO THE MONTEREY BAY AREA

Recent nearshore collecting of fishes in the Monterey Bay area has resulted in many specimens of new or unusual species to be brought to the ichthyology collection of the Moss Landing Marine Laboratories. The following accounts are of two species previously unknown in this area; one range is extended from the south, the other from the north.

On the morning of January 24, 1975, while assisting Steinhart Aquarium biologists Dave Powell and Tom Tucker make a scuba collection of rosy rockfish south of Carmel, California, I collected a specimen of the zebra goby, *Lythrypnus zebra* (Gilbert). This species reportedly ranges from Clarion Island, Mexico, lat 18°20'N, long 114°45'W to Lion Rock, San Luis Obispo County, lat 35°12'N, long 120°52'W (Miller and Lea, 1972). This collection represents a total latitudinal range extension of 130 km (81 mi) northward or 155 km (96 mi) along the coastline.

The specimen, an adult female 32.5 mm (1.3 inches) SL, deposited in the ichthyology collection of the California Academy of Sciences (CAS 31994) was taken from a cave about 1 m (3.3 ft) deep in the steep, granodiorite side of Carmel canyon. The collection was made by dip net after the application of the anesthetic quinaldine at a depth of 37 m (121 ft). The area is approximately 100 m (330 ft) from the shore just north of San Jose Creek beach (locally known as Monastery beach). Two rosy rockfish (*Sebastes rosaceus*) and about fifteen blackeye gobies (*Coryphopterus nicholsi*) were also collected. No other fishes were taken from this cave nor were other *L. zebra* seen on this or a subsequent dive.

The color of the live specimen at the surface was brilliant orange with sixteen chocolate colored vertical bars, instead of cherry red with blue bars as given in the original description (Gilbert, 1890) and later by MacDonald (1972). Zebra gobies from southern California are orange to brick red with blue bars (Peter L. Haaker, pers. comm.).

Meristic counts are as follows: D VI+I,13; A I,9; Vert. 9 precaudal + 17 caudal; Pect. 19; Pelvic 6; Caudal 29; GR 1+8. This dorsal fin count increases the range of meristics given by Miller and Lea (1972).

This collection is interesting in that *Lythrypnus* is mostly a tropical-subtropical genus (Böhlke and Robins, 1960) and the occurrence of *L. zebra* in the cool, deep waters of Carmel canyon (bottom temperature about 11°C) is unexpected. The bluebanded goby, *Lythrypnus dalli*, is known from the temperate waters of Morro Bay, California, and this zebra goby collection indicates *L. dalli* may occur farther north than known at present. The secretive, territorial nature of these short-lived species, as well as the fact that this specimen of *L. zebra* was an adult indicates it is probably not a straggler and that a substantial population might be found inhabiting the rocky caves of Carmel Bay.

In early August, 1976 rockfish surveys by the National Marine Fisheries Service of Seattle, Washington on the R/V John N. Cobb resulted in the capture of 71 eulachon, *Thaleichthys pacificus* (Richardson) in the central and northern part of Monterey Bay. This collection extends the range of this species 193 km (120 mi) along the coastline from Bodega Head (Miller and Lea, 1972).

Fishing a large, commercial-mesh otter trawl off the Salinas River mouth at lat 36°45.1'N long 121°54.2'W in 91 m (50 fms), two individuals were captured on August 9. On August 16, 69 specimens were taken in two trawls in 97–102 m (53–56 fms) but only five were saved from a tow beginning at lat 37°02.8'N long 122°23.9'W. The other 64 came from a nearby tow off Davenport at lat 36°55.2'N long 122°14.7'W.

Meristics of the southernmost seven specimens are as follows: D 12–13; A 17–21; Pect. 11; LL pores 71–76; GR 4–5 + 13–15; Pelvic 8. Two specimens with 17 anal fin rays increases the range of counts for *T. pacificus* given in Miller and Lea (1972) and Hart (1973). The specimens (all males) ranged from 141–170 mm (5.55–6.69 inches) SL. A few had been feeding on euphausiids and small decapod shrimp.

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## BOOK REVIEWS

### **A Field Guide to the Common and Interesting Plants of Baja California**

By Jeanette Coyle, and Norman C. Roberts. 1975. Natural History Press, La Jolla, California. 206 pp., 2 maps, 189 color figures. \$8.50 (paper), \$11.00 (cloth).

In the world of natural history literature there arises at great intervals, a star of startling brilliance. Such is the field guide (aptly named) now shining amidst widely scattered constellations of botanical works relating to southwestern flora.

This large pocket-sized manual (either hard or soft binding) covers several hundred of the plants found in Baja California. Approximately 180 plants are described and illustrated with an individual color plate, while another 75 are mentioned. You are told that of the 5,000 plants found in this plant province those described in the manual can be seen without leaving the immediate vicinity of the transpeninsular highway. Most species illustrated or described are trees, shrubs, and cacti. Ephemerals are not shown.

Basic features of the peninsular environment are described in vignettes, which are supplemented by line map sketches. Eight phytogeographic areas (Shreve and Wiggins described three for this region) are aligned parallel to the long axis of the peninsula. These are all arid regions, and the authors point to certain climatic conditions that influence plant distribution.

Phylogenetic categorization follows Shreve and Wiggins but with elaboration. Endemism is treated cursorily. A ten-page list of 60 families is given as well as a family key. The familial listing follows the order in which the families appear in the text. Phytogeographic communities are described, and glossaries of botanical and Spanish terms are included. Throughout the guide, the metric system is used.

There is a more-than-generous selection of color plates. In the printing, reds and yellows appear not to be rendered realistically, but even with that, the plates are of an overall excellence several orders beyond black-and-white illustrations. Certain of the full-page plates are suitable for framing. Dallas Clites, photographer, deserves particular mention for his superb work.

For my liking, the book is a smidgin too large (6" x 9"), but it is otherwise convenient for field work. The paper appears to be of durable stock. The 'tooth' is sufficient to pick up both pencil and ink. Plant illustrations are all on the right-hand side and most margin-mounted, which makes for ease in locating strangers.—Robert Eberhardt, Instructor, San Diego Community Colleges District, San Diego.

### **Modern Fly Dressings for the Practical Angler**

by Paul Jorgensen; Winchester Press, N.Y., 1976; xv + 224 p., illustrated with both black and white and color photos. \$15.00.

The fly leaf for *Modern Fly Dressings for the Practical Angler* (MFDPA) says the book ". . . joins a very small group of truly original books that have accumulated over the centuries. It is an essential book for the serious modern angler."

I think I would have to take exception to that definition; it *is* an excellent book but only time will make it a classic.

Basically, MFDPA is a book for the expert. It is beautifully done. The patterns illustrated are all useful, the illustrations are clear and easy to follow, and the materials used are easy to obtain.

Illustrated, step-by-step photos detail the tying of mayfly nymphs, spinners, and duns, caddis larvae pupae, and adults, stonefly nymphs and selected terrestrials. Recipes are given for additional patterns. Detailed instructions are given for tying with the recently popular latex/marking pen technique.

This one I can recommend. I've enjoyed the book and tied some of the patterns; the flies should take fish.—K. A. Hashagen, Jr.

### **Fishing with Light**

By M. Ben Yami, FAO Fishing Manuals, Fishing News Books Ltd., Surrey, England, 1976; ix + 121 p. illustrated.

Authored by one of the foremost fishing technologists in the world, "Fishing with Light" is a fishing manual intended for persons interested in using light for commercial or experimental capture of marine fish species. The author has drawn on his wide experience and knowledge in the Mediterranean region and has relied on extensive literature review and personal contacts elsewhere.

A comprehensive review is presented of all the major worldwide fisheries involving use of artificial light including species, gear, and techniques. One chapter is devoted to the physics of light including units of measurement, wavelengths and penetration into water. Practical considerations for selection of artificial lights for fishing and the types in use are also presented.

A detailed enumeration is made of all the major species caught using lights including typical behavior patterns to light, seasonality, and fishing gear used. Fishing methods, tactics, and the factors affecting them are described. A final section presents details of all major fishing gears used in association with use of lights in fishing. These include roundhaul nets, liftnets, gillnets, and hook and line gear.

This book provides valuable background to anyone considering capture of fish by light attraction whether it be for commercial or scientific purposes. Under similar conditions, fish of the same or related species generally have similar behavior patterns, even if geographically remote. The methods described for the major groups in this book could serve as a guideline for developing means of capture for a particular species.—*Ken Mais*.

















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