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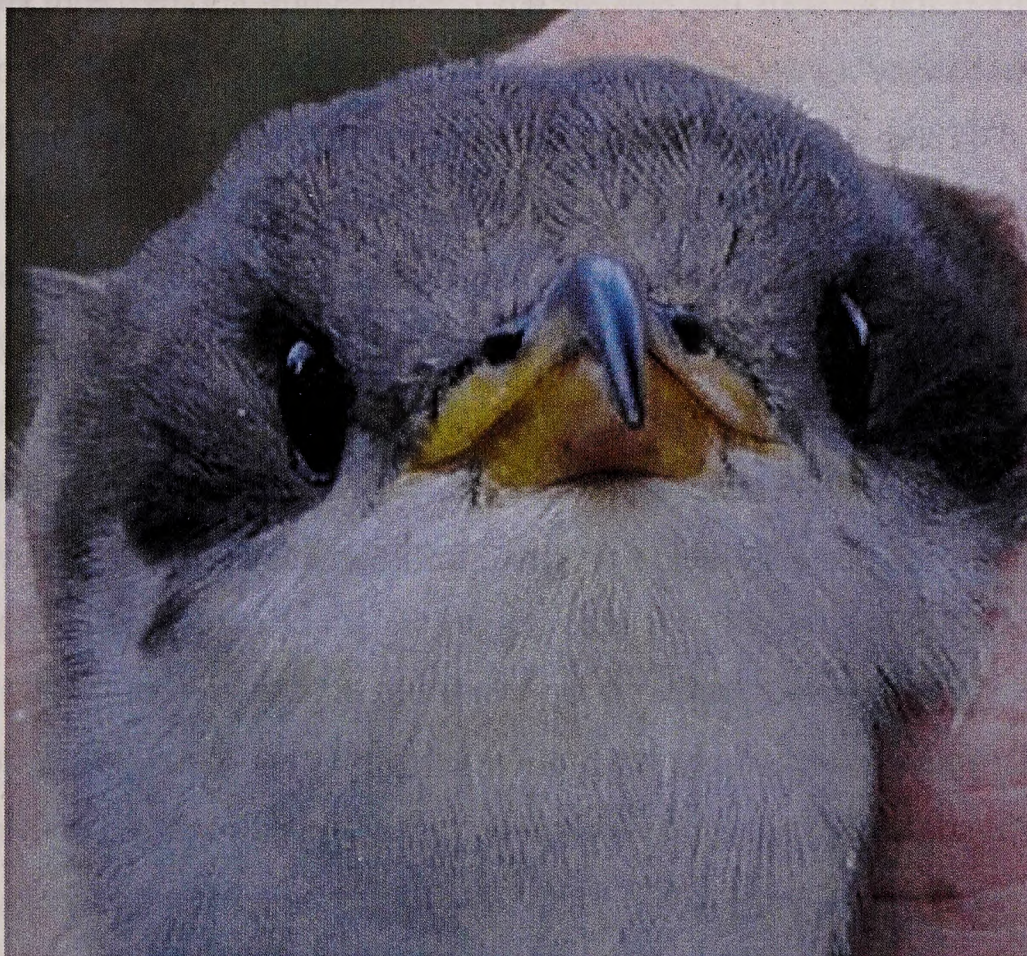
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# Final Report for the 2006-2007 Yellow-billed Cuckoo Project

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## ABSTRACT

In 2007 blood samples from 52 adult Yellow-billed Cuckoos were sexed by Avian Biotech International labs in Florida. They were able to assign sex for 49 of the 52 birds in the sample. Banding, telemetry and call-response data from 2001-2005 were analyzed for sexually-based differences in behavior and morphology. There is no evidence of sexual dimorphism in Yellow-billed Cuckoos in behavior, vocalizations, tail spots, or morphology. Response and detection rates for Yellow-billed Cuckoos are much lower than 100%. Males were more responsive than females (73% vs. 40%), and were detected more frequently than females (43% vs. 17%). Double observer counts had slightly higher probability of detection rate than did call response tests (42.5 vs. 32.4%). Conducting surveys with two observers increased the probability of detection to 80.7%. Cuckoos more than 300m from a surveyor are not detected. Three rounds of surveys were conducted on the San Pedro River NCA in 2006 and a single survey was conducted during the summer of 2007. The San Pedro population, which had been experiencing a marked decline from 2004 to 2006, has increased to one of the highest levels ever detected. This is a strong indication that cuckoos show metapopulations dynamics, and temporary local declines are symptomatic of temporarily poor local conditions, and not necessarily indicative of large-scale long-term decline. The results of this work are currently being prepared for publication in peer-reviewed ornithological journals.



## INTRODUCTION

Yellow-billed cuckoos (*Coccyzus americanus*) are a neotropical migrant found commonly throughout the eastern United States, and in extremely low numbers in the western United States (Hughes 1999). Cuckoo populations in the western US have declined dramatically over the last 100 years (Gaines and Laymon 1984, Halterman et al. 2001). This decline has resulted in great interest in monitoring western populations from local, state, and federal agencies, as well as private conservation organizations. Call playback is typically used to survey for this species, but the efficacy of this method is unknown. Knowing the sex of individuals is essential to interpretation of data on home range, mating system, parental care, detection probability, and site fidelity, yet this species is sexually monomorphic (Hughes 1999, Pyle and Howell 1997). Because cuckoos exhibit little territoriality, have large overlapping home ranges, and are quiet and secretive birds, it is difficult to obtain accurate population estimates (Hughes 1999, Laymon et al. 1997, Halterman 2002).

### Sexual Dimorphism

Recent advances in DNA sexing technology have made it possible to sex many avian species (Griffiths et al. 1998, Fridolfsson and Ellegren 1999), greatly enhancing studies of avian biology. Female birds are heterogametic, and Polymerase Chain Reaction (PCR) can be used to amplify CHD genes found on the sex chromosomes. The CHD-Z gene is found in both sexes, while CHD-W gene is found only in females. Gel electrophoresis of the PCR products results in two bands for females, and only a single band for males (Griffiths et al. 1998). Not only do these techniques allow accurate sexing, they allow the testing of less expensive non-genetic techniques.

Thousands of species of birds from a wide variety of taxa have been correctly sexed genetically (confirmed with known-sex samples). Avian blood is an excellent source of DNA for PCR. Feathers can also be used, although different primers are required. Genetic sexing using DNA is a widely-accepted method for determining the sex of birds. This method has not been used to sex for Yellow-billed Cuckoos in the past (Halterman 2005). Birds sexed by genetic methods can be used to determine if vocalizations and morphology are also reliable predictors of sex.



Many species of birds exhibit obvious dimorphism in plumage, such as the Birds of Paradise (family *Paradisaeidae*). There is also dimorphic variation in size (e.g. Sage Grouse (*Centrocercus urophasianus*) ♂ twice as large as ♀), vocalizations (e.g. most *Parulidae* warblers), and behavior (e.g. Buff-breasted Sandpiper (*Tryngites subruficollis*), males form loose leks). In some species it is difficult to distinguish the sexes (e.g. bill length in the Long-billed Curlew (*Numenius americanus*), eye color in Bushtits (*Psaltriparus minimus*)). In other species it is essentially impossible to determine sex in the field or in the hand, yet knowing the sex of birds is critical to interpreting many sorts of empirical data.

The ability to distinguish sexes in a non-dimorphic species is important to understand sexually dimorphic differences in behavior, vocalizations, and responsiveness. This knowledge also informs our understanding of mating systems, population dynamics, site fidelity, and parental investment. If a species experiences a sharp population decline, the small population may have a skewed sex ratio. There is a need to distinguish sex of species under study, and particularly in those species of conservation interest.

Information about the behavior and vocalizations of male and female cuckoos is common, primarily in grey literature (Laymon 1998, Bennett and Keinath 2001, Furtek and Thomlinson 2005). This information is occasionally found in peer-review literature (Laymon and Halterman 1985, Hughes 1999, Sibley 2001). Hughes (1999) in the Birds of North America Yellow-billed Cuckoo account states that males primarily give the "Kowlp call", described as a series of "kuk-kuk-kuk-kuk" notes followed by "kow-kow-kow" notes. The female is said to primarily give the "Knocker" call, a series of "kow-kow-kow" calls. There is also a fairly commonly heard "Coo" call, which is assumed by Hughes (1999) to be given primarily by unmated males. If this is true it would be possible to assign sex to birds detected on surveys, determine sex ratio in the population, and begin to answer other questions about the information conveyed by vocalizations. There has never been a study of banded, known-sex birds to confirm these assumptions, and there is no description of how sex was assigned.

There is anecdotal evidence, perpetuated in grey literature, that sexes of this species can be separated based on the color and extent of white in the tail (Suckerling and Greenwald 1998). This idea persists among biologists studying Yellow-billed Cuckoos. If



true, this would provide another means of accurately sexing cuckoos in the field. However, no test of sexually dimorphic tail coloration has been done with birds of verified sex.

Morphometrics has proven useful in sexing a number of apparently sexually monomorphic species. These studies used discriminant function analysis (DFA) to develop a function that can be used to sex individuals. DFA uses a combination of morphometric variables weighed against each other to determine the best combination to identify two classes of variables; in this case two sexes (Sharma 1996). This method has been successful with Dunlin (*Calidris alpina*) (Meissner 2005), Black-legged Kittiwake (*Rissa tridactyla*) (Jodice et al. 2000), Dovekie (*Alle alle*) (Jakubas and Wojczulanis 2007), and Pohnpei Micronesian Kingfishers (*Halcyon cinnamomina reichenbachii*) (Kesler et al. 2006). Because female Yellow-billed Cuckoos are slightly larger than males (Pyle and Howell 1997), it may be possible to use DFA to develop a function that will accurately sex individual cuckoos. However, the use of morphometric sexing with Yellow-billed Cuckoos must be verified with known-sex individuals.

### Population Estimation

Effective monitoring of most species requires a population estimate. The basic model for determining a population is given by:

$$E(n_i) = \beta_i N_i \quad (1)$$

Where  $(n_i)$  = count of animals detected at a point  $i$ ,  $\beta_i$  = probability of detection at point  $i$ ,  $N_i$  = population size at a point  $i$  (from Lancia et al. 1994). The assumptions of this model are: 1. There is a linear relationship between the count and population size, and 2. Detectability is constant over time (Bart et al 2004, Pollock et al 2002, Lancia et al. 1994). While it is difficult to determine the relationship between count and true population size, we can test detectability of known-location birds. Call-playback is a method used for rare or secretive birds such as Clapper Rails (*Rallus longirostris*) and Willow Flycatchers (*Empidonax traillii*) (Johnson et al. 1981, Sogge et al. 1997, Conway and Simon 2003). Its effectiveness must be tested with each species. Response to playback depends on a variety of factors, including time of day, weather, breeding stage, density, and observer bias (Kroodsma 1982, Kroodsma 1986, Slater 2004).

### Current Survey Methodology



A standardized survey methodology and data forms for yellow-billed cuckoos were developed in 1998 (Laymon 1998) and have been modified several times since (Haltermann 2007). The methodology and data forms were developed through collaboration of the Southern Sierra Research Station, Arizona Department of Game and Fish, and the United States Geological Survey - Colorado Plateau Field Station in Flagstaff, AZ. This standardized method requires four complete surveys of each site during the field season. Sequential surveys are spaced 12 to 20 days apart and take place between 0600 and 1200. Call-playback, described by Johnson et al. (1981) and Gaines and Laymon (1984), is used for all surveys. Surveyors wait at the survey point for a 1-minute listening period. This is followed by broadcasting the cuckoos' contact call (the "kowlp" call) once a minute for 5 minutes using a portable CD player with a handheld detached speaker. Five seconds of calling is followed by 55 seconds of listening. Playback stops as soon as a cuckoo is detected. The surveyor moves 300m from that detection point before resuming the survey.

Stops are made every 100 meters along the edge of, or within, riparian habitat, with the distances determined by a GPS unit or pacing. Each time a cuckoo is detected, the time of detection and type of vocalization is recorded. Locations of cuckoos are recorded using GPS and plotted as UTM coordinates on USGS quad maps. Birds are identified as either mated or mating status unknown based on observed behaviors. These behaviors include carrying nesting material, copulation, or the presence of a mate or nest.

### *Assumptions*

The current cuckoo survey method makes three major assumptions: 1. All cuckoos within 100m respond to a broadcast call; 2. Individuals do not move more than 300m in response to a broadcast call; 3. Surveyors detect all cuckoos that call within 100m. The efficacy of this widely used technique has not been tested previously. By determining probability of detection for yellow-billed cuckoos we can make more accurate estimates of local population size.

The first and second assumption about a cuckoo's responsiveness can be measured using call-response with known location individuals. When these birds are equipped with radio transmitters, the response rate can be compared to background call rates to determine effectiveness in eliciting a response to a call playback method.

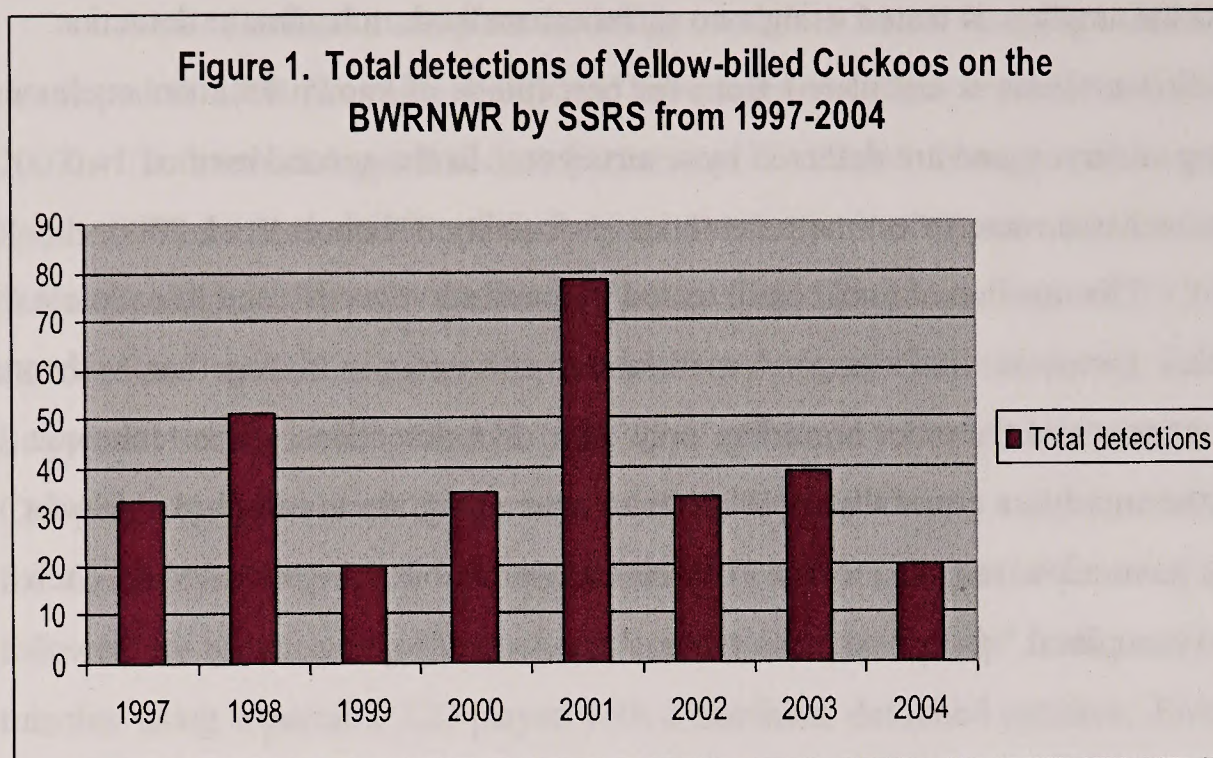


The third assumption is tested using two different methods to estimate detection probability. The first estimate is calculated from the percentage of known location cuckoos that respond during a survey and are detected by a surveyor. In the second method two observers survey the same route to estimate detection probability (Nichols et al. 2000, Forcey et al 2006). The number of cuckoos detected by each surveyor is used to estimate a detection probability for each. In the original method two surveyors work together, with one recording all detections, and the other recording only their own detections. This technique was modified so the surveyors cover the same route an hour apart, since call playback was used and cuckoos have a low response rate. The detection probabilities generated by each method were then compared.

### *Surveys*

Surveys for Yellow-billed Cuckoos were conducted on the San Pedro Riparian National Conservation Area during the summers of 2001-2006. The population was stable from 2001-2004, but showed a marked decline during 2005 and 2006 (Haltermann 2006). This is one of the largest populations in both Arizona and the western United States (Corman and Magill 2000). It undoubtedly contributes to the stability of the western population of Yellow-billed Cuckoos. A decline of this population would have far-reaching effects, and may be indicative of a general decline in western populations. It is, however, also possible that this population is merely experiencing a normal fluctuation. The population of cuckoos on the Bill Williams River NWR shows great annual fluctuation, possibly due to fluctuating prey base (Figure 1, Haltermann 2005). It is essential to continue to monitor this population to determine if it is declining or merely experiencing fluctuations similar to those detected on the Bill Williams River NWR.





**Objectives:**

1. Determine if current DNA-based genetic sexing techniques work with Yellow-billed Cuckoos;
2. Determine if Yellow-billed cuckoos exhibit sexual dimorphism in vocalizations;
3. Determine if tail-spot characteristics can be used to sex cuckoos;
4. Determine if morphological measurements and DFA can be used to develop a function to sex Yellow-billed Cuckoos.
5. Determine the responsiveness of marked cuckoos to determine the effectiveness of survey techniques;
6. Determine if cuckoos habituate to a broadcast vocalization;
7. Determine the responsiveness of marked cuckoos 300 m or more from a broadcast vocalization;
8. Determine detection probability with marked, known-location cuckoos;
9. Determine the detection probability of cuckoos using double-observer surveys.
10. Conduct partial surveys on the San Pedro RNCA in 2006 and 2007, and compare results to 2001-2005 surveys to determine long-term population trends



## Study Area

The study took place from June-September, 2001 to 2007 on the San Pedro River, southeast of Tucson, AZ. This site has one of the largest populations of yellow-billed cuckoos in the western United States (Corman and Magill 2000). The study site was on a 40 km stretch of the river within the BLM's San Pedro Riparian National Conservation Area (SPRNCA). The river channel is lined with a band of cottonwood (*Populus fremontii*) and willow (*Salix* sp.) that varies from 10 m to 500 m in width. Mesquite (*Prosopis* sp.) and netleaf hackberry (*Celtis laevigata*) are common, varying from small, scattered plants to dense stands of large trees (>10 m tall) more than 1 km in width.

## METHODS

### *Catching and Banding Adults*

Adult cuckoos were captured in 2001-2005 by going to areas where multiple cuckoos had been heard 1-2 days earlier. A total of four 60-mm mist nets were used, ranging from 6 to 12 meters in length, in a 'V' by a low mesquite or willow. The doubled nets were 6 m high. One person played a variety of cuckoo calls using two CD players connected by 15 m wires to two speakers placed 1 meter up in the mesquite or willow. Capture efforts typically began just after dawn. If no cuckoos displayed interest in calls after approximately 45 minutes, nets were moved to another site. The nets were typically relocated 2-3 times each morning, and attempts ceased when temperatures exceeded 30°C. This target netting technique is modified from methods currently used to capture Willow Flycatchers (*Empidonax traillii*) (Sogge et al. 2001).

After capture, each cuckoo was banded with a USFWS aluminum band and a unique color combination using 3 Darvic® color bands. A Holohill Ltd. BD-2 transmitter, weighing 1.95 gms (slightly less than 3% of the adult's body weight), was attached to the bird's central rectrices using dental floss (Bray and Corner 1972, Pitts 1995, Woolnough et al. 2004). The BD-2 has a 10-20 week life and an approximate ground range of 1 km.

### *Measurements and Genetic Material*

Measurements of wing chord and tail length were taken to the nearest mm using a stopped wing rule. Mass was measured to the nearest 0.5gms using a Pesola 100mg spring balance. Measurements of tarsus length, culmen length and depth were taken to the nearest



0.1mm using dial calipers. The majority of the measurements were taken by a single researcher.

Photos were taken of the underside of the tail of cuckoos in 2004 and 2005. These photos were examined to determine the size and extent of white spots. Because this is intended for field identification of sexes, precise measurement of the extent of white of was less interest than overall impression and quickly observed characteristics. Three variables were measured: 1. extent of white (>than 50%, less than 50%), 2. upper two tail spots touching, and 3. lower two tail spots touching.

Blood and feather samples were collected for genetic analysis. Blood was taken using either radial or femoral vein puncture technique, suspended in a lysis buffer, and frozen. All samples were sent to Avian Biotech International in Florida for genetic sexing.

#### *Radiotelemetry*

All marked birds were followed every two days until the transmitter failed or the bird left the area. When the bird was visible, its behaviors (sitting, flying, foraging, incubating, etc.), vocalizations, and prey captures were documented. Currently published accounts of cuckoo vocalizations describe four main calls: "kowlp", "knocker", "coo", and an alarm call (Hughes 1999). Contact calls were not readily categorized as a 'kowlp' or a 'knocker' call but fit a general category of contact calls comprised of 'kuks' and 'kows'. Contact calls were categorized based on the proportion of kuks and kows into the following categories: 1. kuk only, 2. more kuk, 3. equal numbers of kuks and kows, 4. more kows, 5. kow only, 6. mixed kuks and kows. Vocalizations of genetically sexed cuckoos were examined for patterns in call types given. It was assumed that the calls given during telemetry are representative of background calling rates, and are not influenced by observer presence.

#### *Call-response Testing*

Cuckoos equipped with radio transmitters were tested using call-response to determine response and detection rates in 2004 and 2005. Testing began 2-3 days after banding, and was repeated every 4 days until the transmitters failed or the bird left the area. Two people conducted this single blind test. One person ("observer") directed the surveyor to within 100m of the focal cuckoo, and watched this bird throughout the test. The second person ("surveyor") played the survey "kowlp" call 5 times, or until they detected the focal bird (following the standard survey methodology). The observer could not inform the



surveyor if the bird called or moved, but the surveyor could ask if a call heard was from the focal bird. Whether or not the focal bird was detected, the surveyor moved 300m and repeated the process. Vocal response and movement were recoded separately by each person. Tests were conducted between 0800 and 1000, and not conducted if it was raining or wind exceeded 15mph.

For each test, data collected included trial number and nesting phenology, which was categorized as: 1. unmated, 2. mated, 3. incubating, 4. feeding nestlings, 5. feeding fledglings, 6. subsequent nest. For each trial, responses are categorized as: 1. did not respond, 2. called, but did not fly towards researcher, 3. flew towards researcher, but did not call, 4. called and flew towards researcher, and 5. flew away from the researcher.

#### *Double Observer Trials*

Two observers surveyed the same route on the same day. The first surveyor began one hour before the second surveyor, and the two did not communicate about the position of any cuckoos detected. Any sightings within 300m on the same day are assumed to be the same bird. This is a basic assumption of the survey method, and is tentatively supported by telemetry data (Halterman 2006). This test gives an indication of the percentage of cuckoos that might be missed by a single surveyor. Detection probabilities for each observer and an overall detection probability were calculated. This method was used on 16 of 55 surveys conducted in 2005.

This method generated detection probabilities and population estimates based on available data. Calculation of detection probabilities are based on equations of Nichols et al. (2000) for double observer data, modified for two independent observers:

$$\hat{p}_1 = (x_{11}x_{22} - x_{12}x_{21}) / (x_{11}x_{22} + x_{22}x_{21}) \quad (2)$$

$$\hat{p}_2 = (x_{11}x_{22} - x_{12}x_{21}) / (x_{11}x_{22} + x_{11}x_{12}) \quad (3)$$

$$\hat{p} = 1 - (x_{12}x_{21} / x_{22}x_{11}) \quad (4)$$

where  $\hat{p}_1$  estimates detection probability by the first observer,  $\hat{p}_2$  estimates detection probability for the second observer, and  $\hat{p}$  is the overall probability of detection with two surveyors. The value of  $x_{ij}$  is the number of cuckoos detected by surveyor  $i$  ( $i = 1, 2$ ) when observer  $j$  was the first surveyor ( $j = 1, 2$ ). The number of cuckoos detected by the first surveyor is  $x_{11}$ , and  $x_{12}$  is the number of birds seen by surveyor one that were missed by observer two.



### *Yellow-billed Cuckoo Surveys*

Surveys were conducted following the standard survey methodology (see above; Halterman et al 2007). Due to funding priorities and timing issues, only three rounds of surveys were conducted in 2006, and only one round were conducted in 2007.

### *Data Analysis*

#### *Call-response testing*

Call-response data were analyzed using an information theoretic approach to rank candidate models (Burnham and Anderson 2002). Akaike Information Criterion weights (AICc), which estimate the probability that a specified model is the best of those considered, were used to address model selection uncertainty (Burnham and Anderson 2002). Model weights (AICc) were used to determine the model with the greatest ability to explain the responses and detections of marked yellow-billed cuckoos.

For the first analyses only data from the first call-playback test with known-sex birds ( $n = 18$  known-sex cuckoos) were used to avoid pseudoreplication (Hurlburt 1984, Kroodsma et al. 2001). The dependant variable was either responded (yes/no) or detected (yes/no). The independent variables were: sex, cuckoo responded (yes/no), Julian date, time, response type (1. did not respond, 2. called, 3. flew closer, 4. called and flew closer, 5. flew away), mating status, if the bird was on a nest at time of test, distance from surveyor, and play number (of the five times the recorded vocalization was played) were also included in the analysis. In the second analysis repeated measures analysis was used on known-sex birds with 4 tests ( $n=10$ ). The same independent variables were used with addition of test number (1-4).

Double observer survey data were analyzed using formulae from Nichols et al. (2000) and DOBSERV (Hines 2000). Equations 2, 3, and 4 were used to calculate detection probabilities. Models were compared using the Akaike Information Criterion with small-sample bias adjustment (AICc) computed by program DOBSERV. The model was selected with the lowest AICc. The only models considered were  $P(.,.)$ , which assumed constant detection probabilities for all species and observers, and  $P(.,i)$  which assumed constant detection probability for species but different probabilities for observers. The other models generated by DOBSERV assume that multiple species are being considered.



Finally, detection probabilities were used to estimate the minimum number of surveys required to determine with a given level of confidence that cuckoos absent from a site (Pellet and Schmidt 2005):

$$N_{\min} = \log (1-\text{desired confidence interval}) / \log (1-\text{detection probability}). \quad (5)$$

### *Sexual Dimorphism*

Two-sample T-tests (assuming unequal variances) were used to determine significant differences in morphological measurements and vocalizations of male and female cuckoos. Discriminant function analysis was used to determine if cuckoos could be sexed by morphological measurements. Logistic regression is used to fit a combination of morphological measurements to sex of birds in the sample. The model then was tested against the data to determine its ability to accurately sex individual cuckoos. Data were analyzed using SAS (SAS Institute 2002).

## RESULTS

A total of 259 calendar-days were spent on this project (Table 1).

Table 1. Effort for each task for agreement #06FG300044 in calendar-days.

Task	Surveys	Nest search	Nest Monitoring	Data entry	Preparation of samples	Data Analysis	Report Preparation	Meetings	Total
Days	48	45	4	3	9	80	60	10	259

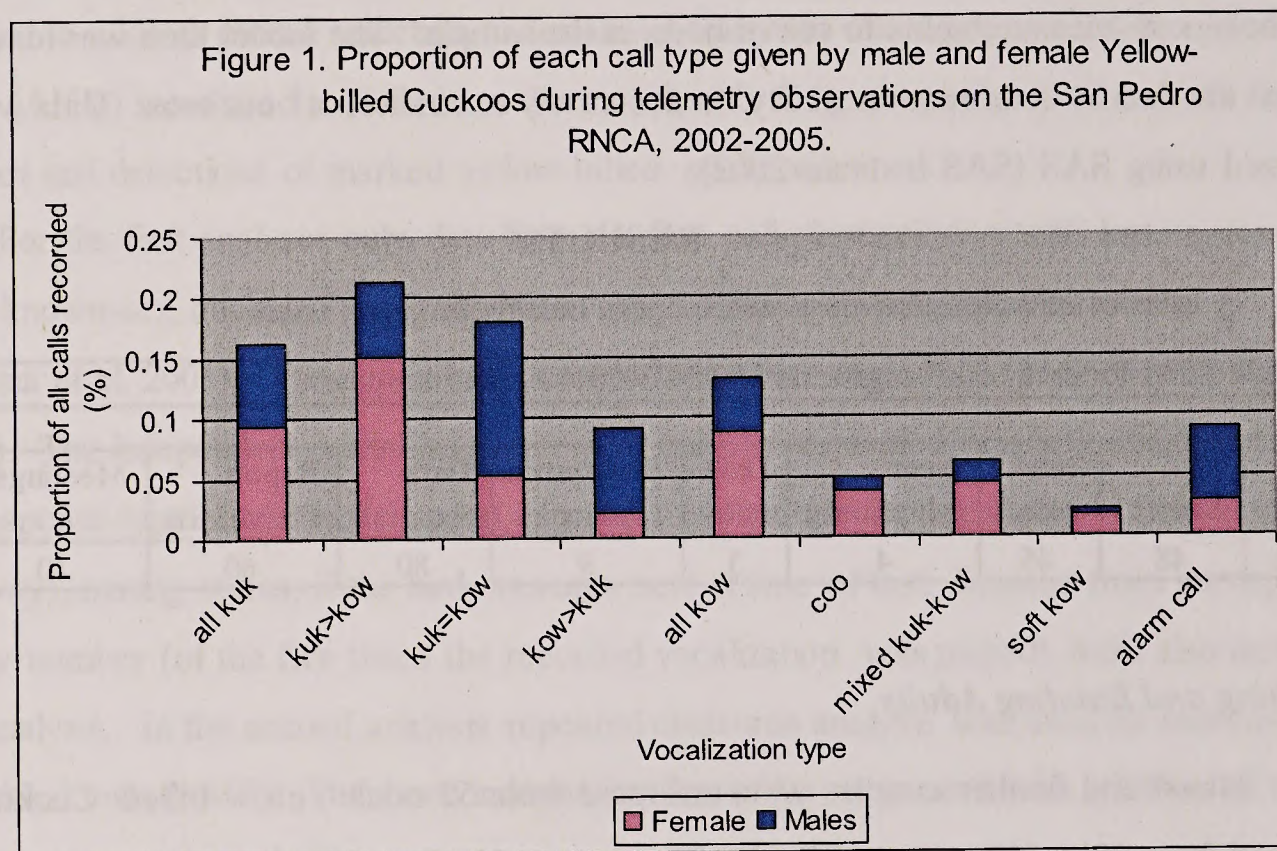
### *Catching and Banding Adults*

Blood and feather samples were collected from 52 adult Yellow-billed Cuckoos were captured from 2001-2005 (Appendix 1). The sexes of Yellow-billed Cuckoos have not previously been successfully determined using genetic material from blood samples (Halterman 2006). Blood samples from five birds were sent to Avian Biotech International labs in Florida using Permacode® blood collection cards. These samples resulted in clear separation of two bands for females and a single clean band for males. These results agreed with sex assigned on the basis of multiple observations of copulation for each individual. They subsequently assigned a sex for 49 of the 52 samples; their results agreed with observations for 11 birds tentatively sexed behaviorally. ABI identified 34 males, 15 females, and 3 unknowns in the sample.

### *Vocalizations*



Thirty-five of the 52 cuckoos captured from 2001-2005 (ten females, 25 males) were observed for a total of 338 days (853 hours). These birds gave a total of 1118 vocalizations during observation. There were no vocalizations given only by one sex, although some calls were given predominantly by one sex (Figure 2). Males gave call types three and four significantly more than females (call 3:  $t$ -test = 2.2,  $p < 0.05$ ,  $N = 32$ ; call 4:  $t$ -test = -2.64,  $p < 0.001$ ,  $N = 32$ ). Females gave call type six, the 'coo' call, more frequently than males ( $t$ -test = -5.31,  $p < 0.001$ ,  $N = 36$ ). This call has previously been reported as given primarily by unmated males (Hughes 1999, Suckerling and Greenwald. 1998), but these observations were not based on marked known-sex individuals.



Although some calls are given more by males than females, there is no single type of call that can be used to either identify the sex of cuckoos or determine their breeding status. The proportion of calls given by each sex could potentially be used to determine the sex ratio of responding cuckoos, if calls are given on surveys in the same proportions as during telemetry (Table 2). Two call types regularly given during telemetry were never detected during surveys – the soft kow and the alarm call. Both of these are soft calls usually given around a nest.



Table 2. Proportion of Yellow-billed Cuckoo calls given during telemetry (2003-2005), and proportion detected during surveys.

Vocalization type	Telemetry	2003	2004	2005
1	16.2%	21.5%	22.3%	14.7%
2	21.4%	25.9%	30.0%	40.5%
3	18.0%	23.5%	23.1%	21.8%
4	9.0%	15.1%	8.5%	9.1%
5	13.1%	4.1%	7.3%	7.1%
6	5.0%	6.4%	6.9%	2.8%
8	6.2%	2.3%	1.9%	2.4%
9	2.2%	1.2%		0.4%
13	8.9%			0.8%
Total calls	1118			

#### *Tail spot patterns*

I compared the tail spot patterns of eight females and twelve males. Females in this sample had less white on the tail than males (38% vs 67%), contrary to grey literature accounts that males have smaller and rounder tail spots than females (Suckerling and Greenwald 1998). There was no clear pattern to the extent and overlap of white spots on the underside of the tail which would make this a viable characteristic for sexing cuckoos in the field.

#### *Morphometric analyses*

Complete measurements and genetically-determined sex data were available for a total of 40 after second year (ASY) cuckoos – 13 females and 27 males. Six second-year birds were excluded from the analyses, since their mean measurements were smaller than the ASY birds (Table 2). This difference has not been noted in the literature (Pyle and Howell 1997), and sample sizes were too small to test for significant differences. There were no significant difference in any measurements between males and females (Table 3). The measurements for wing chord, weight, and tail length approached significance, but had a large degree of overlap.



Table 2. Mean measurements (mm) of male and female Yellow-billed Cuckoos captured on the San Pedro Riparian Conservation Area, 2002-2005. Means+ SD, Sample sizes (N) and ranges are given. *t*-test comparisons are between ASY males and ASY females\*.

Measurement	ASY Female	<i>t</i> -test*	ASY Male	SY female	SY male
Weight	66.6 ± 6.7 (13) 46 - 80	<i>t</i> = 1.46; <i>p</i> = 0.081	61.4 ± 5.2 (27) 50 - 73	55 ± 12.72 (2) 46-64	66.2 ± 10.5 (4) 54.5 - 75
Wing Chord	151.2 ± 5.0 (13) 145-164	<i>t</i> = 1.56; <i>p</i> = 0.067	145.7 ± 3.4 (29) 139-152	146.5 ± 2.1 (2) 145-148	143.5 ± 5.8 (4) 136-149
Bill Length	20.6 ± 1.5 (13) 18.1 - 23.1	<i>t</i> = 0.49; <i>p</i> = 0.32	20.2 ± 1.3 (28) 16.6 - 23.3	20.2 ± 0.98 (2) 19.5 - 20.9	19.9 ± 1.65 (4) 18.3 - 22.2
Bill Depth	9.1 ± 0.13 (13) 8.5 - 9.9	<i>t</i> = 0.41; <i>p</i> = 0.34	8.9 ± 0.5 (28) 7.9-10.1	8.95 ± 0.35 (2) 8.7-9.2	8.7 ± 0.48 (4) 8.3 - 9.4
Tarsus	31.64 ± 1.6 (13) 28.5 - 34.6	<i>t</i> = 0.62; <i>p</i> = 0.27	31.0 ± 1.8 (28) 27.9 - 36	30.45 ± 0.91 (2) 29.8 - 31.1	28.2 ± 4.9 (4) 21.3 - 32.6
Tail	152.9 ± 8.8 (13) 140 - 173	<i>t</i> = 1.58; <i>p</i> = 0.062	146.3 ± 9.9 (29) 126 - 168	143 ± 1.41 (2) 142-144	141.7 ± 5.0 (4) 135 - 147

\**t*-test assuming unequal variance

I used the data for 40 ASY cuckoos to build seven functions using SAS version 8 (SAS Institute 2002). These models used wing chord, bill length, bill depth, tarsus, tail, and weight measurements (Table 4). A number of other models were built, and those included here are representative of those with the highest probability of correctly assigning an individual to the correct sex. Models 4 and 5 were the poorest, misclassifying 39% of females as males. The best model for females, number seven (correctly identified 77%), was the poorest for males (correctly identified 74%). The rest of the models correctly identified between 81-89% of the males. None of the models performed well, and I would not recommend the use of any of them to identify the sex of cuckoos in the hand.



Table 4. Discriminant function models for Yellow-billed Cuckoos captured on the San Pedro RNCA, 2001-2005 ( $N_{\text{females}} = 13$ ,  $N_{\text{male}} = 27$ ).

Model	Discriminant Function	% Correctly					
		Classified	Wilk's Lambda	F	#variables	DF	P
1 - Female	$-1173+9.43012\text{Wing}+9.46178\text{Tars}+0.59845\text{Bill Length}+56.06043\text{Bill Depth}+0.48845\text{Tail}+0.32120\text{Weight}$	69%	0.60970201	3.52	6	6	0.0084
1 - Male	$-1103+9.15151\text{Wing}+9.36187\text{Tars}+0.74902\text{Bill Length}+54.80887\text{Bill Depth}+0.43271\text{Tail}+0.18966\text{Weight}$	78%					
2 - Female	$-1173+9.53043\text{Wing}+9.53043\text{Tars}+56.30564\text{Bill Depth}+0.49407\text{Tail}+0.31797\text{Weight}$	69%	0.61275953	4.3	5	5	0.004
2 - Male	$-1103+9.20688\text{Wing}+9.44779\text{Tars}+55.11578\text{Bill Depth}+0.49407\text{Tail}+0.18562\text{Weight}$	85%					
3 - Female	$-1032+9.57260\text{Wing}+52.40822\text{Bill Depth}+0.52983\text{Tail}+0.86655\text{Weight}$	69%	0.6145919	5.49	4	4	0.0015
3 - Male	$-965.20619+9.30426\text{Wing}+51.25215\text{Bill Depth}+0.47519\text{Tail}+0.72944\text{Weight}$	81%					
4 - Female	$-812.5080+8.09649\text{Wing}+7.83515\text{Tars}+0.91559\text{Tail}+0.19019\text{Weight}$	61%	0.64197434	4.88	4	4	0.0031
4 - Male	$-757.87821+7.85812\text{Wing}+1.18834\text{Tars}+0.85236\text{Tail}+0.06053\text{Weight}$	85%					
5 - Female	$-716.62115+8.25765\text{Wing}+0.92108\text{Tail}+0.65417\text{Weight}$	61%	0.64262652	6.67	3	3	0.0011
5 - Male	$-663.13393+8.01831\text{Wing}+0.85781\text{Tail}+0.52175\text{Weight}$	85%					
6 - Female	$-681.00450+8.89058\text{Wing}+0.26241\text{Weight}$	69%	0.67636183	8.85	2	2	0.0007
6 - Male	$-632.24231+8.60777\text{Wing}+0.15689\text{Weight}$	85%					
7 - Female	$-711.10711+8.55923\text{Wing}+0.83568\text{Tail}$	77%	0.68882067	8.36	2	2	0.001
7 - Male	$-659.62637+8.25885\text{Wing}+0.78970\text{Tail}$	74%					



### Radiotelemetry

A total of 25 adult cuckoos were captured in 66 days in 2004 and 2005, and tests of the current survey method were conducted with 18 of these birds. The remaining seven birds either left the area before the test could be conducted ( $n = 5$ ) or lost the transmitter ( $n = 2$ ). Tests took place between early July and late August. The median date of tests was August 8 in 2004, and 26 July in 2005. This test was repeated a total of 74 times, with an average of 4 times per bird.

### Call-response Testing

The background calling rate of marked cuckoos was 1.1 calls/hour ( $n = 18$ , from 115 hours of observation). Call playback increased this rate to 10 calls per hour. During playback testing cuckoos responded 59% of the time, but were only detected 32% of the time (Table 5). Males responded 70% of the time while females only responded 16% of the time (Table 5). Only males responded by both calling and flying closer; they were detected 85% of the time (Table 6). Of the 40 total responses, 42% were after the first play of the contact call.

Table 5. Results of call-playback with marked yellow-billed cuckoos on the San Pedro River AZ, 2004-2005.

Sex	Total captured		Total mated	Responded		Detected	
	2004	2005		Total	Percent	Total	Percent
Male	7	3	6	32	72.7%	19	43.2%
Female	4	4	6	12	40.0%	5	16.7%
Total	11	7	12	44	59.5%	24	32.4%

Table 6. Response type and detection of known-sex yellow-billed cuckoos during call-playback tests on the San Pedro River AZ, 2004-2005.

Type of response	Called	flew in	called&flew in	No Response	Average	Total (n=70)
Responded	19	11	10	26	57.1%	40
Detected	10	4	8	0	31.4%	22
Female response	6	6	0	16	42.9%	12
Male response	13	5	11	10	74.4%	29
Percent responded	27.1%	15.7%	14.3%	37.1%		57.1%
Percent detected	14.3%	5.7%	11.4%	0.0%		31.4%



I compared models using AICc (weighted Akaike's Information Criterion) scores (Burnham and Anderson 2002). For cuckoo responses during the first call-playback test, the model with sex best explained the data (Table 7). Other models with  $\Delta$ AICc less than two were time of survey, distance from surveyor, and mating status. The most parsimonious models for cuckoo detections had cuckoo response and time of survey (Table 8). All other models had  $\Delta$ AICc values greater than two.

Table 7. Model selection based on AICc scores for response rates of marked yellow-billed cuckoos during the first call-playback test on the San Pedro River AZ, 2004-05 (n=17).

Model	k	$\Delta$ AICc	AICc weights
Sex	2	0	0.2652
Time	2	0.713814619	0.1858
Distance	2	0.796602835	0.1782
Mated	2	1.729321194	0.1119
Nest	2	2.14095966	0.0911
Julian date	2	2.261542683	0.0858
no effect	2	2.354132439	0.0819

Table 8. Model selection based on AICc scores for detection rates of marked yellow-billed cuckoos during the the first call-playback test on the San Pedro River AZ, 2004-05 (n=17).

Model	k	$\Delta$ AICc	AICc weights
Responded+time	3	0	0.2425
responded	2	0.233358763	0.2158
Time	2	0.514585555	0.1876
No Effect	1	2.105358045	0.0848
Mated	2	2.152105406	0.0828
Distance	2	2.842205126	0.0587
Sex	2	2.906851018	0.0568
nest	2	4.062332577	0.0319
Julian Date	2	4.066556849	0.0319
Response Type	5	7.062376491	0.0071

The most parsimonious model for response with four call-response tests for each bird contained both mating status and sex (Table 9). The only other model with a  $\Delta$ AICc less than two was mating status, and its weight was only 0.141, as compared to the first model, with a weight of 0.368. The model with trial number had a  $\Delta$ AICc of 4.24, and a



model weight of 0.044, evidence that habituation to call playback was not an important factor in cuckoos responsiveness.

Table 9. Model selection based on AICc scores for response rates of marked yellow-billed cuckoos during call-playback tests 1-4 on the San Pedro River AZ, 2004-05 (n = 40).

Model	k	$\Delta$ AICc	AICc weights
Mated+sex	3	0	0.3682
Mated	2	1.9084	0.1421
Mated+nest	3	2.6678	0.0973
Mated+Jdate	3	3.124	0.0775
Nest	2	3.6814	0.0586
Sex	2	3.8706	0.0534
Jdate	2	3.971	0.0508
No Effect	1	4.0368	0.0491
Trial	4	4.2406	0.0444
Year	2	5.007	0.0303
Time	2	5.1266	0.0285

When models for detection with four tests per bird were compared, only the model with response type had a  $\Delta$ AICc of less than two, and a model weight of 0.8353 (Table 10). This is particularly impressive as there were 5 different levels of response.

Table 10. Model selection based on AICc scores for detection rates of marked yellow-billed cuckoos during call-playback tests 1-4 on the San Pedro River AZ, 2004-05 (n=40).

Model	k	$\Delta$ AICc	AICc weights
Response type	6	0	0.8353
Distance+Sex	3	4.5032	0.0886
Sex	2	6.2342	0.0374
Distance	2	8.5364	0.0118
No Effect	1	9.6534	0.0068
year	2	10.218	0.0051
Mated	2	10.6348	0.0042
Time	2	10.811	0.0038
JulianDate	2	10.8206	0.0038
Nest	2	10.8324	0.0038

### *Double Observer Trials*

I compared two models from program DOBSERV (Hines 2002) and found the P(.,.) model was the most parsimonious (Table 11). This had a model weight of 0.72, and suggested that there is no difference in detection probabilities between observers, though the power is limited due to the very small sample size. I calculated overall and individual



surveyor detection probabilities using formulae from Nichols et al. (2000) and compared these with probabilities from DOBSERV, raw call-response data and data used in the different models (Table 12). Detection probabilities were similar from raw data and data used in call-response models. Probabilities calculated using eqs 2 and 3 were higher for the first observer (57%) than for the second observer (36%). The overall probability of detection from equation 4 (80.7%) and from DOBSERV (89%) were much higher than those from the call response tests (32.4%).

Table 11.  $\Delta AICc$  values for DOBSERV models for independent-observer approach for Yellow-billed Cuckoos surveyed on the San Pedro River AZ, 2005.

Model	k	$\Delta AICc$	AICc weights
1 P(.,.)	3	0	0.728890256
P(.,I)	3	1.982	0.271109744

Table 12. Detection probabilities from different sources for yellow-billed cuckoos on the San Pedro River AZ, 2004-05

	Male	Female	overall
Raw data	42.5%	16.7%	32.4%
1-trial models	33.3%	25.0%	27.8%
4-trial models	33.3%	6.3%	22.5%
Double observer - hand calculation - surveyor 1			57.0%
Double observer - hand calculation - surveyor 2			36.0%
Double Observer - hand calculation - Overall			80.7%
Double observer - DOBSERV			89.5%

### Surveys

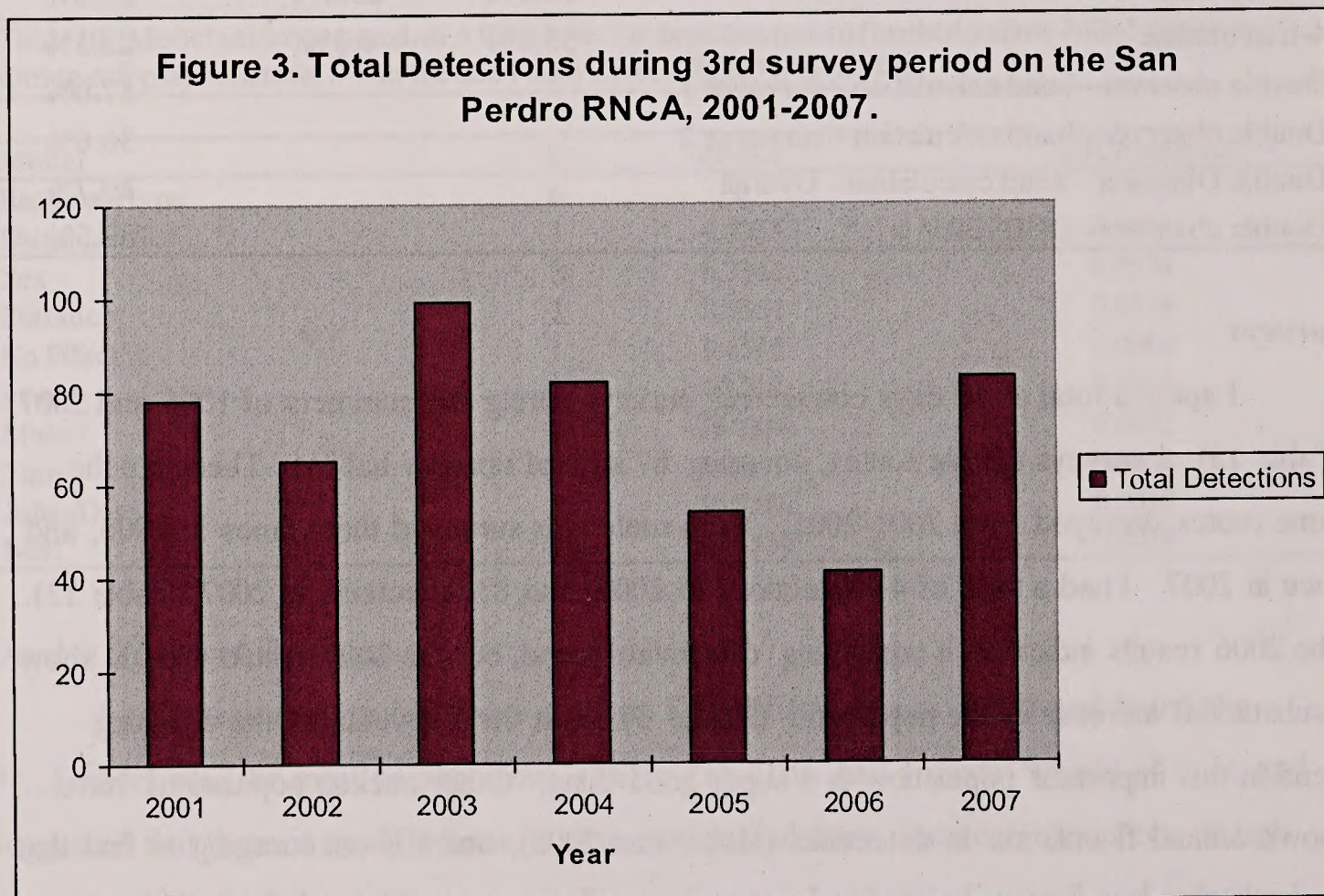
I spent a total of 48 days conducting surveys during the summers of 2006 and 2007 (Table 13). I surveyed twelve routes, covering 67 kms of riparian habitat. These are the same routes surveyed from 2001-2005. Each route was surveyed three times in 2006, and once in 2007. I had a total of 47 detections in 2006, and 83 detections in 2007 (Table 13). The 2006 results indicated a continuing downward trend, but the 2007 results clearly show a substantial increase in the population (Figure 3). This clear reversal of the declining trend in this important population is a super good thing. Other cuckoo populations have shown annual fluctuation in detections (Halterman 2005), and it is encouraging to find that



the decline observed on the San Pedro was apparently part of the normal fluctuation experienced by cuckoo populations.

Table 13. Survey dates and Yellow-billed Cuckoos detected on the SPRNCA in 2007, with comparison to 2001-2006.

Site name	Date	2007 Total	Km surveyed	2001 Det/km	2002 De/km	2003 Det/km	2004 Det/km	2005 Det/km	2006 Det/km	2007 Det/km
Charleston North	17-Jul	5	5.9	2.4	2.4	2.2	1.7	1.2	0.5	0.8
Charleston South	7-Jul	5	4.6	4.3	4.3	3.3	2.6	1.3	0.4	1.1
Fairbank North	15-Jul	2	6.6	3.0	3.0	1.4	0.9	0.9	0.5	0.3
Fairbank South 1	14-Jul	7	6.0	3.2	3.2	3.5	2.2	1.3	0.5	1.2
Gray Hawk Ranch	16-Jul	7	2.7	4.4	4.4	8.1	4.8	2.2	1.5	2.6
Hereford North 1	13-Jul	0	3.1	2.3	2.3	3.7	1.9	1	0.3	0.0
Hereford North 2	12-Jul	7	2.7	3.3	3.3	2.1	3.3	1.9	0.7	2.6
Hereford South	11-Jul	9	7.2	2.1	2.1	7.3	1.5	1.0	0.4	1.3
San Pedro North	8-Jul	7	6	3.8	3.8	1.0	1.3	0.8	0.7	1.2
San Pedro South 1	9-Jul	14	7	1.1	1.1	1.4	2.9	1.7	1.1	2.0
San Pedro South 2	9-Jul	10	7.0	1.1	1.1	2.3	2.1	2.1	1.0	1.4
San Pedro South 3	10-Jul	10	7.9	2.5	2.5	3.4	1.5	1.3	0.9	1.3
Total		83	67	152	65	99	82	54	47	83
Avg Det/km		1.24		2.5	1.5	1.5	2.0	1.3	0.7	1.2





## DISCUSSION

### *Sexual Dimorphism*

Genetically based sexing appears to not only be effective for sexing Yellow-billed Cuckoos, it is the only method for doing so. It is not possible to sex cuckoos based on vocalizations, tail spot characteristics, or morphology. Because there is no field method for sexing cuckoos, only population-level studies will benefit from the technique.

Both sexes gave all vocalizations, although there were statistically significant differences in proportion of several of the vocalizations given by males and females. These results cannot be used to differentiate the sexes in the field, however. It is intriguing that a vocalization long thought to be given by unmated males (the "coo" call) is in fact given predominantly by females, both mated and unmated. Additionally, the unbanded mates of four males cooed, while females' unbanded mates did not. There is extensive literature on the information being conveyed by avian vocalizations. This includes mating status, sex, and individual quality (Catchpole and Slater 1995, Kroodsma and Miller 1996, Penteriani 2003). The function of the coo call is unknown, but may convey information about mating status of females. The coo of mated females may be an advertisement for subsequent mates, since a number of females have second or third broods.

There were no significant difference in measurements between males and females (Table 3). The measurements for wing chord, weight, and tail length approached significance, but all measurements had a large degree of overlap.

Sexing based on copulation is nearly worthless primarily because it is rarely observed. Additionally, Yellow-billed Cuckoos practice reverse copulation (Halterman 2006). This is when the female mounts the male, and can result in misidentification of sex of an individual. Reverse copulation is also documented in another *cuculidae*, Groove-billed Anis (*Crotophaga sulcirostris*) (Bowen 2002). This behavior may be common in other cuckoos, but most species are poorly studied.

It was worthwhile to explore discriminant function analysis as an alternative to molecular sexing, since not all researchers or banders take blood samples. If effective, it would be possible to sex birds captured and monitored in the past. Unfortunately, it is too unreliable to use to sex cuckoos. Although the accuracy with males was acceptable (85%), the best model misclassifies 31% of females as males, and therefore is not useful as a



sexing tool. There is no standard rule as to what is an acceptable level of error for DFA; it is generally a matter of what is considered acceptable. Most researchers accept levels over 85% as sufficiently accurate to be biologically meaningful. The low reliability may be due in part to the high variance in measurements, and in part to errors in precision of measurements (Yezerinac et al. 1992).

It is difficult to capture Yellow-billed Cuckoos. During 2002-2005 an experienced crew typically averaged 2 days to capture each cuckoo. This usually entailed setting up the nets and speakers at least 4 times, and required a minimum of three people. Due to the effort required to capture cuckoos, genetic sexing will truly only be of value during population level studies, where banded birds are followed, and behavioral observations take place which can be linked to the sex of individuals.

The majority of *Cuculiformes* species for which there is information show sexual dimorphism in body size (87%) (Payne 2005). Plumage dimorphism is found only in old-world parasitic cuckoos (Payne 2005, Payne *in* Del Hoyo 1997). The majority of cuckoo species exhibit female-biased body size dimorphism (57%), with 30% of species male-biased, and 13% of equal size. The variation is unrelated to parasitism, but may be related to New vs. Old World subfamily representatives. In the old world nesting *cuculinae*, for example, 67% of species show male-biased size dimorphism ( $N = 9$ ), while in New World representatives 76% of species ( $N = 13$ ) show female-biased size dimorphism. The most uniform group is the Coucals (*Centropodinae*), with all but one species showing strong female-biased dimorphism in mass (89%,  $N = 18$ ). This is a poorly studied group in which at least one species, the African Black Coucal (*Centropus grillii*) exhibits classical polyandry (Goyman et al. 2004).

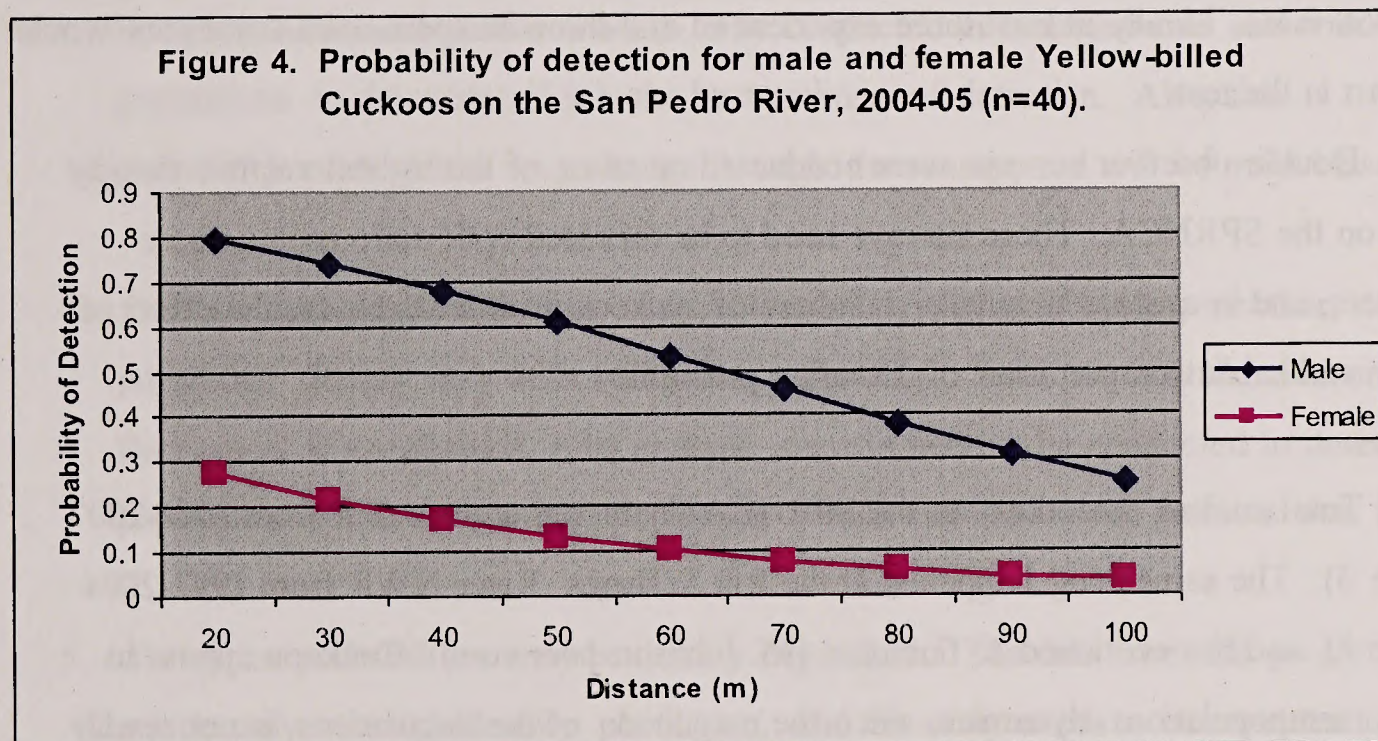
#### *Detection Probability*

Cuckoos have a low response rate during surveys, based on call-response tests. Call playback resulted in a 10X increase in the probability that a cuckoo would vocalize during a six-minute survey period. There was a large difference in the detection probabilities generated from the program DOBSERV ( $\hat{p} = 89.5\%$ ), from Nichols et al. (2000) formula ( $\hat{p} = 80.7\%$ ) and those from the call-response data set ( $\hat{p}$  between 22.5% and 32.4%) (Table 12). The probabilities from the double observer method assume that two surveyors are covering the area. The probabilities for single observers were close to



those generated by the call-response data. This method does not work well with species with low detection probabilities, those that occur in low numbers, or both (Nichols et al. 2000, Thompson 2002). Because cuckoos meet both criteria, the detection probabilities generated by this method must be considered with caution. Between 2001 and 2006 I did intensive nest searching on the San Pedro River. This work meant spending many hours in a small area, and typically two to three times more cuckoos were detected during these long observation periods than during surveys. This anecdotal evidence supports the lower detection probability of the call-playback tests and single observer.

Response rates and type of response of males and females were different, with males calling more frequently as well as flying closer to the surveyor and calling (Table 6). Females either called or flew in, but did not do both. The response of males appears to be responsible for their increased probability of detection, since probability of detection is higher when a cuckoo is closer (Figure 4).



Sex and mating status were important parameters in response of cuckoos when both first test and four tests were considered (Tables 7 and 8). Males and mated birds both appear to be more responsive than females and unmated birds. These are responses that are typical of many territorial birds, but puzzling in this species, because cuckoos appear to exhibit little territoriality. This observation is based on 20 years of cuckoo research, during which I have seen only three incidences of apparently aggressive cuckoo interactions. Additionally, I had limited response to the variety of vocalizations used in the



capture attempts. A highly territorial species should show a stronger response to an invasion of its territory. These results nevertheless provide added importance to surveys conducted later in the season when more cuckoos will be mated and more responsive.

Cuckoos at 300m distance had a very low response rate (<5%) and an even lower detection rate (1%). The assumption that cuckoos won't respond from a distance of 300m appears to be functionally valid, and I believe this distance can be used with confidence to separate individuals. One caveat is that occasionally in sparse habitat a cuckoo will follow the surveyor over the 300m distance (M Halterman, unpublished data). It is typically obvious when this happens, and a new detection should not be recorded.

There is a need to test effective detection distance. This should take place in at least three habitats where cuckoos are commonly surveyed: Cottonwood/Willow; Mesquite/Hackberry; and Salt Cedar dominated. This test could be conducted using two people, speakers placed at set intervals, and calls played at random times to determine detection rates. Ideally at least three experienced and three inexperienced surveyors would take part in the test.

Double observer surveys were conducted on some of the highest relative density routes on the SPRNCA. These surveys need to be repeated with more routes, more observers, and in areas with smaller numbers of cuckoos in order to assess the effect of density and habitat on precision of detection probability.

### *Surveys*

Total cuckoo detections on the SPRNCA fluctuated a great deal from 2001-2007 (Figure 3). The same thing happened at the Bill Williams River NWR from 1997-2004 (Figure 1), and has continued to fluctuate (M. Johnson pers com). Cuckoos appear to exhibit metapopulations dynamics, since the magnitude of the fluctuations is not readily explained by reproductive success.



## MANAGEMENT RECOMMENDATIONS

1. Blood or feather samples should be collected from all cuckoos banded for genetic sexing.
2. Collect measurement data from museum specimens for comparison to the discriminant function analysis used in this study. A greater sample size may yield a useful DFA tool, but only measurements taken when specimens were fresh should be used, since significant shrinkage occurs after preparation.
3. Population estimates can be conditionally calculated in habitats similar to the San Pedro RNCA for single surveys using the average 25% detection probability. Before this detection probability is applied to other habitats and populations, however, additional research needs to be conducted in other habitats and with other populations to determine if it is the best estimate of detection. Also, this probability of detection may change through the season, and will be male-biased.
4. Intrinsic and extrinsic factors, such as time of day, nesting cycles, weather, and population density may affect detection probability. Since there is also seasonal fluctuation in vocalization rate, multiple surveys need to be conducted to determine population levels and monitor population trends and habitat use patterns over time.
5. Detection probabilities may be density-dependant. The data from this project relates only to a high-density population, and the study needs to be repeated in areas of medium and low density populations.
6. Test effective survey distance using a number of speakers and "surveyors" at a variety of distances from the speakers.



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