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Behavioral ecology of reproduction in the pomacanthid angelfish, *Centropyge potteri*

Lutnesky, Marvin Michael Francis, Ph.D.

University of Hawai'i, 1992

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BEHAVIORAL ECOLOGY OF REPRODUCTION IN THE POMACANTHID ANGELFISH, CENTROPYGE POTTERI

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

AUGUST 1992

BY

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This work is dedicated to all those who have taught me the value of exploration, especially my parents, Patricia R. Lutnesky and the late Marvin L. Lutnesky, and my wife Kimberly D. K. Lutnesky.
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ABSTRACT

The Hawaiian angelfish, *Centropyge potteri*, was used as a model system to develop and test hypotheses regarding the integration of stimuli from both physical and social environments into reproductive strategies; specifically the social control of sex change, and temporal patterns of mating.

Prerequisite to these topics are descriptions of sexual dimorphism and dichromatism, and protogynous sex change. These descriptions include differences between the sexes in meristics, morphometrics, and color pattern; a field-test of identification of the sexes; and histological descriptions of the gonads.

Encounter-rate models are developed for territorial-haremic fishes. They show how a combination of social-group composition and density may form predictable patterns of contact between social-group members. The patterns of contact are asserted as cues for sex change. The models are tested with *C. potteri* by demonstrating that encounters with smaller fish are needed for sex change, encounters with larger fish prevent sex change, and changing the density of a social group alters encounter rates and can induce sex change. Sex change is associated with conditions that yield high smaller-fish encounter, and low larger-fish encounter, for the sex-change candidate.

A temporal-threshold model of polygynous mating (TMPM) is developed. This model shows how females that mate with polygynous males may offset polygyny costs by mating during less advantageous times for which fewer females compete. The model's potential for broad applicability is shown to be worthy of study with examples from African elephants and *C. potteri*.

The TMPM is empirically tested using *C. potteri*. Females compete for mating order. Competition results in a larger variance in daily time of mating,
independent of the time required for mating, when females are in a larger group. Females probably disperse mating in time to avoid polygyny costs, including interference by competing females. These results were a priori predictions of the TMPM.

The conclusion is that information from both physical and social environments is integrated into *C. potteri* reproductive strategies. In this way females may minimize potential costs associated with changing sex, and reproducing in a polygynous mating system.
# TABLE OF CONTENTS

**ACKNOWLEDGMENTS** ........................................................................... v

**ABSTRACT** ....................................................................................... vi

**LIST OF TABLES** ................................................................................ x

**LIST OF FIGURES** .............................................................................. xi

I. **GENERAL INTRODUCTION** ......................................................... 1

   Organization of the dissertation ....................................................... 3

II. **SEXUAL DIMORPHISM, PROTOGYNOUS HERMAPHRODITISM, AND SOCIAL BEHAVIOR OF THE POMACANTHID ANGELFISH, CENTROPYGE POTTERI** .... 6

   Introduction .................................................................................. 6

   Methods ...................................................................................... 8

   Collections ................................................................................. 8

   Identification of sex ................................................................. 9

   Sexual dimorphism and dichromatism .................................... 10

   Histology .................................................................................. 13

   Sex change demonstration ...................................................... 14

   Behavior .................................................................................... 16

   Reproductive behavior ............................................................. 16

   Results and Discussion ............................................................. 17

   Identification of sex ..................................................................... 17

   Sexual dimorphism and dichromatism .................................... 18

   Histology .................................................................................. 26

   Sex change demonstration ...................................................... 31

   Behavior .................................................................................... 34

   Reproductive behavior ............................................................. 37

   Conclusions .............................................................................. 44

III. **DENSITY-DEPENDENT PROTOGYNOUS SEX CHANGE IN TERRITORIAL-HAREMIC FISHES: MODELS AND EVIDENCE** .............................................. 45

   Introduction .............................................................................. 45

   Proximate control theory .......................................................... 46

   Encounter-rate models .............................................................. 50

   Assumptions .............................................................................. 50

   Absolute encounter-rate threshold hypothesis ....................... 53

   Differential encounter-rate threshold hypothesis .................. 54

   Inhibition-only hypothesis ........................................................ 57

   Testing the models .................................................................... 57

   Methods ..................................................................................... 58
LIST OF TABLES

Table                                                                                           page
2.1 Regression equations for sexually dimorphic characters of *Centropyge potteri* ................................................................. 24
2.2 Spawning of *Centropyge potteri* during all weeks of the lunar month ................................................................. 43
3.1 Sex-change experiments using the pomacanthid angelfish, *Centropyge potteri* ........................................................................ 70
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Schematic drawing of <em>Centropyge potteri</em> showing line segments used in calculation of the color-pattern index</td>
<td>12</td>
</tr>
<tr>
<td>2.2</td>
<td>Color pattern of typical male and female <em>Centropyge potteri</em></td>
<td>20</td>
</tr>
<tr>
<td>2.3</td>
<td>Plots of the relationships between sexually dimorphic characters and standard length for <em>Centropyge potteri</em></td>
<td>22</td>
</tr>
<tr>
<td>2.4</td>
<td>Histology of the gonads of <em>Centropyge potteri</em></td>
<td>28</td>
</tr>
<tr>
<td>2.5</td>
<td>Plots of the relationships between proportion male score and standard length, and proportion male score and female size differences, for <em>Centropyge potteri</em></td>
<td>33</td>
</tr>
<tr>
<td>2.6</td>
<td>Reproductive behavior in <em>Centropyge potteri</em></td>
<td>40</td>
</tr>
<tr>
<td>3.1</td>
<td>Encounter-rate relationships expected between the alpha female and other members of her social group, as functions of fish density; the encounter-rate threshold models</td>
<td>56</td>
</tr>
<tr>
<td>3.2</td>
<td>Plots of the relationships of harem size, and territory size, over fish density for <em>Centropyge potteri</em></td>
<td>66</td>
</tr>
<tr>
<td>3.3</td>
<td>Simple linear regressions of alpha-female encounter rates over fish density for <em>Centropyge potteri</em></td>
<td>69</td>
</tr>
<tr>
<td>3.4</td>
<td>Encounter rates in the enclosure experiments using <em>Centropyge potteri</em></td>
<td>74</td>
</tr>
<tr>
<td>4.1</td>
<td>The polygyny-threshold model of Orians (1969)</td>
<td>85</td>
</tr>
<tr>
<td>4.2</td>
<td>The temporal-threshold model of polygynous mating</td>
<td>91</td>
</tr>
<tr>
<td>4.3</td>
<td>Temporal distribution of births by African elephants, <em>Loxodonta africana</em>, as related to rainfall</td>
<td>97</td>
</tr>
<tr>
<td>4.4</td>
<td>Spawning by haremic females of <em>Centropyge potteri</em> during April, 1989</td>
<td>102</td>
</tr>
<tr>
<td>5.1</td>
<td>Mean adjusted time of mating for <em>Centropyge potteri</em> on a daily time scale</td>
<td>118</td>
</tr>
<tr>
<td>5.2</td>
<td>Frequency of daily time of mating in ten-minute time intervals for <em>Centropyge potteri</em></td>
<td>120</td>
</tr>
</tbody>
</table>
5.3 Interference behavior by haremic *Centropyge potteri* females: enclosure ................................................................. 123

5.4 Interference behavior by haremic *Centropyge potteri* females: field ............................................................................. 125

5.5 Spawning order as related to size rank in haremic *Centropyge potteri* females ................................................................ 128

5.6 Frequency of mating for *Centropyge potteri* during April, 1990 .................................................................................. 131

5.7 Average number of spawns for individual *Centropyge potteri* females during April, 1990 ........................................... 134
CHAPTER I
GENERAL INTRODUCTION

This dissertation is about problems in the behavioral ecology of reproduction in the pomacanthid angelfish, Centropyge potteri. Where possible, I have tried to make the study more useful for general application by using C. potteri as a "test species" to examine concepts, rather than making this work a specific study only pertaining to C. potteri.

The two major topics covered in the dissertation, protogynous or female-to-male sex change, and temporal patterns of mating, at first glance appear to have little in common. This study brings them together to examine how factors from both physical and social environments may be integrated into the reproductive strategies of female animals. For both topics, individual females must make behavioral "decisions." They must choose whether to change sex, or when to spawn, in various environments that differ in their physical and social characteristics depending on ecological circumstance. I examine how the combination of both factors may influence behavior.

It should be noted that throughout the dissertation I use words such as "choice," "decision," and "strategy" regarding the behavioral options of C. potteri and other species. The use of such terms is not intended to imply volition on the part of the animals. For example, the impact of social stimulation on the physiology of a C. potteri female may decide for her that it is time to change sex or time to spawn. It is assumed that natural selection results in the differential success of the options and that animals are selected to follow different "strategies" depending on ecological circumstance.
When I began working with *C. potteri* in 1985, only one major paper by Lobel (1978a) described spawning behavior, and female-biased sex ratios in high-density populations. I was intrigued by the sex-ratio pattern, and how ecological factors might influence social organization. The problem became even more interesting, however, because protogynous sex change is common in pomacanths, and may be universal in the genus *Centropyge* (Moyer and Zaiser 1984, Thresher 1984). Sex change potentially leads to changes in sex ratio, thus it has the potential to change social organization. It appeared to me that the first step in understanding the social organization of *C. potteri* is to study the proximate control of sex change.

Previous work (e.g. Robertson 1972, Shapiro and Lubbock 1981, Ross et al. 1983) had concentrated on how social-group composition influences sex change. My work adds to the subject by focusing on how the amount of space the fish live in, a physical factor, influences the delivery of social stimuli and thus the potential for sex change.

Spawning in *C. potteri* is readily observable in both the field and semi-natural environments (Lobel 1978a, Lutnesky Chapters II, IV, and V). This led to a study of how polygynous females might optimize their reproductive efforts by choosing times to mate that are most suited to their competitive ability. The basic idea is that they may balance costs in fitness due to cyclical changes in their environment against polygyny costs (loss of fitness) associated with female-female competition that is highest during optimal environmental conditions for mating (Lutnesky Chapters IV and V). This topic is thus another example of how reproductive strategies may be influenced by a combination of factors from physical and social environments. Additionally, this study gives

The behavioral ecology of these reproductive processes in *C. potteri* is intrinsically an interesting subject that helps us understand its social organization and possibly that of similar territorial-harem fishes, species in which several females share a general-purpose territory with a male (Lutnesky Chapter III).

**ORGANIZATION OF THE DISSERTATION**

Since sex change is not documented in *C. potteri* (but see Lutnesky 1988), the first part of my study was descriptive. In Chapter II sexual dimorphism, protogynous hermaphroditism, and social behavior including reproduction are described. These descriptions set a foundation for the dissertation, and for future studies involving *C. potteri*. For example, Chapter II includes a demonstration that the sexes can be reliably identified in the field. This will likely be important to anyone doing field work on this species. Similarly, the partial ethogram of social behavior that is provided sets the foundation for future behavioral studies.

The development and initial tests of the encounter rate threshold (ERT) models, models that predict different rates of sex change in different-density populations, are presented in Chapter III. The models were developed for *C. potteri* and are potentially useful for other territorial-harem fishes. The ERT models are largely based on the relationships of increasing harem size, and decreasing territory size, with increasing population density. These patterns are common among territorial-harem fishes, and may lead to systematic changes
in encounter rates among social-group members, and thus systematic changes in the stimuli for sex change.

A temporal-threshold model of polygynous mating (TMPM) is developed in Chapter IV. It is a general model, and asserts that females can offset polygyny costs by mating during nonoptimal environmental conditions for which fewer females compete. The TMPM includes specific predictions regarding when females are expected to mate if they are balancing costs of reproduction due to physical and social factors. The model is developed using C. potteri as an example, but to show the model's potential for broad applicability, African elephants (Loxodonta africana) are also used to illustrate a mating system in which the model may be tested.

Physical factors of periodic light, tide, and current patterns are thought to influence the survival and dispersal of larval reef fishes (Johannes 1978, Lobel 1978a, 1989, Barlow 1981) and thus influence spawning periodicity. Periodic food abundance may also influence when fishes spawn (Tyler 1992). The TMPM adds female-female competition, a social factor, as a potential source of variation in temporal patterns of spawning.

C. potteri can be used to test the TMPM because it is a small species, lives in small social groups that can be manipulated, and it spawns in enclosures. A test of the TMPM in a daily time scale is presented in Chapter V.

Concluding remarks and directions for future research are presented in Chapter VI. The significance of the project is summarized, and new directions for further research in the behavioral ecology of reproduction in sex-changing fishes, and other polygynous animals, is suggested.

Finally, there is some redundancy in Chapters II - V. Each chapter was originally written as an independent manuscript for publication. Some chapters
contain similar background material in order to introduce *C. potteri* and its mating system, or summarize the findings of other chapters when extending a study begun in another chapter. This small overlap allows each chapter to retain its integrity when it is read independently of the other chapters.
CHAPTER II
SEXUAL DIMORPHISM, PROTOGYNOUS HERMAPHRODITISM, AND
SOCIAL BEHAVIOR OF THE POMACANTHID ANGELFISH,
CENTROPYGE POTTERI

INTRODUCTION

Centropyge potteri was first described by Jordan and Metz (1912). It is often one of the ten most abundant fishes in the coral-rich habitat of Hawaiian reefs (Hobson 1974), and is endemic to the Hawaiian Islands and Johnston Atoll (Randall et al. 1985). It is also the third most commercially important fish of the aquarium-fish trade in Hawaii (Randall 1985), yet little about its basic biology has been documented.

Hobson (1974) found C. potteri to be primarily herbivorous, and Lobel (1978a) described spawning by pairs and harems of C. potteri, and recorded sex ratios on the reef. He also described diel, lunar, and seasonal periodicity in reproduction (Lobel 1978a, 1989).

I became interested in the behavioral and ecological processes of possible sex change in C. potteri (Lutnesky Chapter III) because many pomacanthids are known to change sex (Shen and Liu 1976, Moyer and Nakazono 1978, Bruce 1980, Aldenhoven 1984, Hourigan and Kelley 1985), and protogynous sex change may be universal in the genus Centropyge (Moyer and Zaiser 1984, Thresher 1984).

In order to describe the social organization of C. potteri (Lutnesky Chapter III), it was important to be able to reliably identify the sex of individuals in the field. Although it was reported that C. potteri are not sexually
dichromatic except temporarily with blanching during spawning at dusk (Thresher 1982, 1984, Moyer 1987), my preliminary observations suggested a subtle, but clear, permanent dichromatism: larger fish appeared to have more of their bodies covered with a dark blue pigment than did smaller fish. A figure caption in a popular article by Lobel (1978b) indicates he made a similar observation. I thought it was likely that the larger fish with more blue pigment were males because sexual dimorphism in size is usual for pomacanthids (Moyer et al. 1983, Thresher 1984, Moyer 1987, 1990).

The purpose of this article is to provide descriptions of sexual dimorphism, sexual dichromatism, protogynous hermaphroditism, and social behavior of C. potteri. The fact that this fish is small (see below) and lives in relatively small territories (Lutnesky Chapter III), make it potentially useful in the study of problems involving the behavior and ecology of coral-reef fishes. This work is intended to document the basic morphology and behavior of C. potteri to provide a foundation for future studies.

I first present the results of a field test designed to demonstrate the accuracy of identifying the sexes in the field. Next, sexual dimorphism in size and morphometrics are described. Males seem to have fewer preopercular spines than females (Lobel 1978b), so this character is also examined for quantitative evidence of sexual dimorphism. A "color pattern index" is provided for a quantitative description of sexual dichromatism. Additionally, all sexually dimorphic traits are related to body size to examine the extent to which differences between the sexes are due to allometry or gender.

Gonads are histologically examined to describe their structure, and also to look for evidence of protogynous sex change. Additionally, a sex-change
demonstration provides evidence of the phenomenon of sex change in *C. potteri*.

Finally, social behavior, including reproductive behavior, is qualitatively described. The intent of this section of the chapter is to provide a partial ethogram of behavior relevant to sex change and spawning in *C. potteri*. It includes undescribed behavior patterns of female *C. potteri* during spawning that may be involved with female-female competition for breeding order (Lutnesky Chapters IV and V), and reports some differences with the original description of spawning (Lobel 1978a).

**METHODS**

**Collections**

A total of 341 *C. potteri* were collected between February, 1986, and April, 1989. The collections were made from three locations in the waters (3 - 35 m depth) surrounding the island of Oahu, Hawaii. One collection was made offshore of Portlock, another offshore of Kaneohe Bay (OKB), and the third inside Kaneohe Bay (IKB).

The Portlock collection consisted of 52 fish that were collected by miniature spear or handnet. The IKB collection contained only 3 fish collected by miniature spear, but included one interesting specimen (see below). The fish were haphazardly caught without regard to sex in these two collections.

The OKB collection consisted of 286 fish that were collected alive by handnet and anesthetic (10:1, 95% ethanol: quinaldine). They were used in the sex change demonstration of this study and experiments beyond the scope of this paper (Lutnesky Chapter III). Females were preferentially collected on the basis of their color pattern and size (see below) in the OKB collection, yet haphazardly chosen among the fish that appeared female. Males in this
collection were taken in a similar manner, thus rare transitional individuals most likely were missed in the OKB collection.

Identification of Sex

The accuracy of sexing fish in the field was tested with the Portlock collection. As each fish was caught, it was identified as a male or female based on the assumptions that males had more dark blue pigment than females, and were also larger. After capture, each fish was placed into a bag marked male or female, and placed on ice. The sex of each fish was identified again, but this time in the laboratory, by itself, completely out of social context. The sequence for sexing isolated fish was chosen haphazardly by a disinterested volunteer.

The gonads were then removed and fixed in 10% buffered formalin (Humason 1979). Finally, the sex of each fish was identified histologically, and the results of the three methods of identifying sex were compared using a chi-square contingency table (Zar 1984).

Fish were identified as male or female; no transitional category was used. This did not present a problem because only one fish in the Portlock collection was transitional at the time of capture. It appeared to be female, and this was corroborated histologically. The ovary was full of healthy previtellogenic oocytes, yet it also contained some larger concentrations of gonial cells, possibly the earliest stages in the development of spermatogenic tissue.

Although the collection methods of the OKB collection potentially caused some bias (probably a very small bias, given the rarity of transitional individuals) in testing the overall accuracy of sexing fish by color pattern and size, it was still useful to show if males and females could be accurately selected in the field. Freshly captured, living fish were sexed. The sex of the fish was
then verified by a combination of cannulation and histology (Lutnesky Chapter III)(See Ross [1984] for a description of cannulation). A comparison was made between the results of visual sexing, and the combination of cannulation and histology. Again, a transitional category was not used. Transitional individuals were not expected and did not occur because of the way the fish were collected, and given the results of the Portlock collection, transitional fish are rare.

**Sexual Dimorphism and Dichromatism**

Because of the potential bias of the OKB collection, and the small size of the IKB collection, only the Portlock collection was used to quantitatively describe sexual dimorphism and dichromatism in *C. potteri*.

A color-pattern index (CPI) was developed to test for differences in color pattern between males and females. Transect line measurements were taken over the fish body from the base of the first dorsal spine to the vent, and from the base of the sixth dorsal spine to the base of the first ventral fin spine. Each transect consisted of three line segments; the middle segment covered the dark blue area (Fig. 2.1), while the first and last segments covered lighter pigmented areas. The index was given by the equation:

\[
\text{CPI} = \frac{X_2 + X_5}{\sum_{i=1}^{6} X_i}, \quad i = 1, 2, ..., 6
\]

where \(X_i\) are line segments and segments \(X_2\) and \(X_5\) covered the dark blue area. The index gave possible values between one and zero, and allowed a quantitative comparison between males and females.

Other characters examined for sexual dimorphism were standard length (SL), head length (HL), proportional head length (HL/SL), length of the longest preopercular spine (PSL), preopercular spine length proportional to head length (PSL/HL), preopercular spine length proportional to body length...
Figure 2.1. A schematic drawing of *Centropyge potteri*. The line segments $X_2$ and $X_5$ cross the area of dark blue pigment. These measurements allow a quantitative comparison between males and females. Scale equals one cm.
(PSL/SL), and number of spines along the margin of the preopercular bone (PS#). Body measurements were in mm and follow the definitions in Lagler et al. (1977). Measurements and counts were taken from the right side of specimens.

A methodological problem arose because _C. potteri_ is a monandric species in which all males are derived from females. Because males are larger than females, it was necessary to determine if sexually dimorphic characters were due to size or gender differences. Male and female _C. potteri_ overlap very little in size range. For this reason, where possible, I regressed the characters over SL separately for males and females, and then tested for significant differences in the slopes from zero by analysis of variance (Zar 1984). Following this, t-tests were used to test for significant differences between the slopes (Zar 1984). Based on these results, reasonable conclusions can be drawn for many of the characters.

All data sets, or their transformations, were found not to differ significantly (P > 0.05) from normality (D'Agostino's tests, [Zar 1984]). Additionally, where appropriate, tests of equality of variance were performed and variances were found to be statistically homogeneous (P > 0.05) by variance ratio tests (Zar 1984) before parametric analyses were utilized. If these conditions were not satisfied, a nonparametric analysis was utilized, where possible. Data transformations and statistical analyses followed the procedures given by Zar (1984).

**Histology**

Formalin-fixed tissue was routinely embedded in paraffin, but positioned haphazardly in the block. Seven to nine micron sections were made, and stained with hematoxylin and eosin, or with Mallory's trichrome (Humason
1979). At least 10 sections were taken from the first part of the gonad to meet the microtome knife, "peripheral sections," and again from the center of the gonad "central sections." Since the gonads were only about 2 - 17 mm in diameter, sections were cut through the entire gonad.

Gonads from the IKB and Portlock collections, and also from the fish used in the sex change demonstration, were examined to describe their structure, and to look for evidence of protogynous hermaphroditism. The criteria used in the diagnosis of hermaphroditism in this study are those defined by Sadovy and Shapiro (1987). Histological evidence in testes include 1) a lamellar organization, when females of the species have a lamellar organization in the ovary; 2) membrane-lined central cavities; 3) sperm sinuses in the gonadal wall; and 4) atretic oocytes that had once been vitellogenic. The most conclusive evidence, however, is the induction of transitional individuals which show proliferation of spermatogenic tissue, and degeneration of oocytes.

**Sex Change Demonstration**

Thirty females from the OKB collection were haphazardly divided into fifteen "pairs." After cannulation, each pair was housed in a 1 m(l) x 1.2 m(w) x 1 m(h) floating enclosure. The enclosures were constructed of wire mesh (12.7 mm² mesh size), and placed in the lagoon at the Hawaii Institute of Marine Biology, Kaneohe Bay, Oahu, Hawaii. This demonstration also provided a basis for comparison with treatments designed to tease out the proximate stimuli that are important for sex change in this species (Lutnesky Chapter III).

The enclosures provided a seminatural environment as they were exposed to natural physical patterns (tidal currents, photoperiod, etc...) in the lagoon. On average, the replicates lasted 35.1 ± 2.8 (SD) days. The experiments took
place between May, 1987, and July, 1988. The fish fed on algae that grew naturally within the enclosures.

At the end of each replicate the fish were killed with an overdose of anesthetic, and the gonads removed and fixed in 10% buffered formalin. The gonads were examined for histological evidence of sex change. Former females that contained sperm were considered sex changed.

Linear transects were taken at randomly chosen angles through the center of peripheral (n = 15) and central sections (n = 15) of the gonad for the larger member of each experimental pair. One section from each location was chosen randomly from the sections representing the area. A point on the transect was sampled every 95 microns. On average, 101.6 ± 36.3 (SD) points were sampled per transect. The proportion of transect points falling on male (M) and female (F) germ cells for the transect was examined. All gonial cells were considered male because spermatogonia and oogonia could not be distinguished. Transects were scored as "proportion male" [ M / (M+F) ]. The peripheral and central sections of each gonad were compared by paired t-test (Zar 1984) to see if there was a polarity to the sex-change process within the gonad.

Because a female did not change sex in every replicate, and when sex change occurred it was always the largest female of the pair, the influence that female size has on the sex-change process was examined. For the larger fish of each experimental pair, the proportion male score from a randomly selected central section was regressed over standard length. Additionally, to examine if the size difference between females of an experimental pair was influential, the proportion male score of the larger fish was regressed over the size difference between the paired females.
Behavior

In this section, the modal action patterns (Barlow, 1977) of *C. potteri* social behavior, including reproductive behavior, are qualitatively described. The description provides a partial ethogram for *C. potteri*.

Social behavior was observed using scuba at all times between 0900 h and sunset, at a depth of 3 - 23 m, inside and offshore of Kaneohe Bay, Oahu, Hawaii. Territory sizes and rates of behavior (Lutnesky Chapter III) were measured and recorded during 159 dives (184.1 h), between April, 1985 and July, 1990. Notes on behavior were recorded by pencil on Nalgene plastic sheets. Observations of social behavior were made on 20 different harems ranging in size from one male and one female to one male and five females.

In addition to field observations, social behavior of captive *C. potteri* was observed in large enclosures (102 and 12 m², planar area) that are described elsewhere (Lutnesky Chapter III). From September 1987, to April 1989, I made 142 observations, on average 30.5 ± 10.8 (SD) minutes in duration. A total of 72.1 h of observation were made at all times between 0900 h and 1800 h. Observations were taken from 20 harems, ranging in size from one male and two females to one male and fifteen females. Behavior was recorded on Nalgene plastic sheets or described on audio tape.

Reproductive Behavior

In this section, the reproductive behavior of *C. potteri* is qualitatively described. Observations of *C. potteri* spawning in the wild were conducted during May 1989, and May to July, 1990, on scuba at 15 - 23 m depth, offshore of Kaneohe Bay, Oahu, Hawaii. Fourteen dives were made and 21 spawns were recorded during 13.5 h of observation. Observation usually began about 45
minutes before sunset, and continued until five minutes after all fish had sheltered for the night, usually 20 to 25 minutes after sunset. Observations were made of 19 different harems ranging in size between one male and two females to one male and five females. Single harems were usually observed, but on occasion it was possible to monitor two harems because both of their small, contiguous, territories could be observed from a single vantage point.

Further observations were made of nine captive harems living in semi-natural environments (Lutnesky Chapters III, IV, and V). Harems ranged in size from one male and one female to one male and fifteen females, and were part of an experimental paradigm to examine proximate mechanisms in sex change and temporal patterns of spawning. During April - May 1989, two harems, eight and 15 females large, were watched almost nightly for 33 days (Lutnesky Chapter IV). During April - May 1990, seven harems, six having one female and one having six females, were watched nightly for 26 days (Lutnesky Chapter V). In total, 82 observations were made, on average 93.2 ± 29.0 (SD) minutes in duration, giving 127.3 h of observation during which 225 spawns were recorded.

Observations began as early as two h before sunset, and lasted until the fish had sheltered for the night, usually about 20 to 25 minutes after sunset. Notes were recorded by pencil on Nalgene plastic sheets, or the behavior was recorded on video tape and subsequently analyzed.

RESULTS AND DISCUSSION

Identification of Sex

The Portlock collection consisted of 41 females and 11 males. There was no difference in identification of the sexes between the field, the laboratory, and
the histological methods of identifying sex, and thus no significant difference between methods in identifying sex ($P > 0.999$, chi-square analysis for contingency tables).

The OKB collection consisted of 262 females, 23 males, and 1 fish of unconfirmed sex. The sex of fish was correctly identified in over 99% of the visual assessments as verified by cannulation and histology. Cannulation revealed that one female was misidentified as a male; eggs could not be cannulated from 3 apparent females that were never histologically examined. Sex was verified by gross dissection of the gonads in two cases, but the third fish escaped before a verification could be made. All fish identified as male were verified histologically.

Typical male and female color patterns are shown in Figure 2.2. As the Portlock data and Figure 2.2 indicate, it is easy to distinguish the sexes using the color pattern difference, and the sex of \textit{C. potteri} can be reliably identified in the field. Additionally, the large OKB collection indicates that fish of a particular sex can be reliably chosen in the field for use in experiments.

\textbf{Sexual Dimorphism and Dichromatism}

\textit{C. potteri} was found to be sexually dimorphic for all of the characters examined (Fig. 2.3). Except where stated otherwise, probability values in the following paragraph refer to the results of t-tests.

Males are typically longer than females in SL ($P < 0.0005$). Scores on the CPI indicate that a greater proportion of the male body is covered by dark blue pigment than the female body (arcsin transformed, $P < 0.0005$). The heads of males are also larger than those of females on an absolute scale (square transformed, $P < 0.0005$), but significantly smaller as a proportion of SL
Figure 2.2. Color patterns of A) a typical male, and B) a typical female *Centropyge potteri*. Note the male has a larger area of dark blue pigment. Photographs courtesy of R. Pyle.
Figure 2.3. *Centropyge potteri* are sexually dimorphic in: A) color-pattern index (CPI), B) head length (HL), C) head length proportional to standard length (HL/SL), D) number of preopercular spines (PS#), E) length of longest preopercular spine (PSL), F) length of preopercular spine proportional to head length (PSL/HL) and G) length of preopercular spine proportional to standard length (PSL/SL). With the exception of CPI, all characters are significantly related to standard length in females (see Table 2.1). Circles and solid lines = females; triangles and dashed lines = males. Lines indicate significant relationships (P < 0.05) between the character and standard length by simple linear regression.
(reciprocal transformed, \( P < 0.0005 \)). The length of the longest preopercular spine is longer for males than females on an absolute scale (\( P < 0.0005 \)), as a proportion of HL (\( P < 0.0005 \)), and as a proportion of SL (arcsin transformed, \( P < 0.05 \)). Males also have fewer preopercular spines than do females (\( P < 0.05 \), Mann-Whitney test).

To determine if the sexually dimorphic characters are due to differences in size or gender, the characters as functions of SL are shown in Figure 2.3. Regression equations are given in Table 2.1.

The slope of the color-pattern index (arcsin transformed), as a function of SL, is not significantly different from zero in either females (Fig. 2.3A, \( r^2 = 0.003, P > 0.25 \)), or males (Fig. 2.3A, \( r^2 = 0.055, P > 0.25 \)). A greater proportion of the body is covered by dark blue pigment in males due to their gender, not their larger body size.

Square transformed head length significantly increases as a function of SL for females (Fig. 2.3B, \( r^2 = 0.953, P < 0.0005 \)), but not males (Fig. 2.3B, \( r^2 = 0.173, P > .10 \)), and thus the slope of the line for males is significantly less than for females (\( P < 0.025 \)). Males have larger heads simply because they are larger fish. If anything, the apparent effect of male gender is to retard this relationship, but the trend is still positive. Reciprocal transformed proportional head length significantly increases, i.e. proportional head size decreases, as a function of SL for both females (Fig. 2.3C, \( r^2 = 0.456, P < 0.0005 \)) and males (Fig. 2.3C, \( r^2 = 0.436, P < 0.05 \)), and the slope of the line for males is significantly steeper than that for females (\( P < 0.025 \)). It thus appears that males also have proportionally smaller heads simply because they are larger fish. The apparent effect of male gender in this case, however, is to enhance this relationship.

23
Table 2.1. Regression equations for sexually dimorphic characters of *Centropyge potteri* (Portlock collection: females, n = 41; males, n = 11), as functions of standard length (SL). CPI = color pattern index; HL = head length; and PSL = length of the longest preopercular spine. HL/SL = HL as a proportion of SL; PSL/HL = PSL as a proportion of HL; and PSL/SL = PSL as a proportion of SL. Asterisks indicate a slope that is significantly different from zero by analysis of variance (P < 0.05 and < 0.0005, for * and **, respectively). P indicates the probability that the slope of the character for males is greater or lesser than the slope of the character for females. Where slopes were not significantly different (P > 0.05), a common slope was calculated ($b_c$). NA = analysis or calculation not appropriate.

<table>
<thead>
<tr>
<th>CHARACTER</th>
<th>FEMALE</th>
<th>$r^2$</th>
<th>MALE</th>
<th>$r^2$</th>
<th>P</th>
<th>$b_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcsin $\sqrt{\text{CPI}}$</td>
<td>$-0.0205\ (\text{SL}) + 37.6293$</td>
<td>0.003</td>
<td>$-0.2345\ (\text{SL}) + 75.3502$</td>
<td>0.055</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>$\text{HL}^2$</td>
<td>$9.5276\ (\text{SL}) - 231.3031^{**}$</td>
<td>0.953</td>
<td>$4.9399\ (\text{SL}) + 190.6159$</td>
<td>0.173</td>
<td>$&lt;0.025$</td>
<td>NA</td>
</tr>
<tr>
<td>$1/(\text{HL/SL})$</td>
<td>$0.0091\ (\text{SL}) + 2.7247^{**}$</td>
<td>0.456</td>
<td>$0.0263\ (\text{SL}) + 1.2123^{*}$</td>
<td>0.436</td>
<td>$&lt;0.025$</td>
<td>NA</td>
</tr>
<tr>
<td>PSL</td>
<td>$0.1728\ (\text{SL}) - 3.8386^{**}$</td>
<td>0.872</td>
<td>$0.1610\ (\text{SL}) - 2.3270^{*}$</td>
<td>0.416</td>
<td>$&gt;0.25$</td>
<td>0.1722</td>
</tr>
<tr>
<td>PSL/HL</td>
<td>$0.0042\ (\text{SL}) + 0.1012^{**}$</td>
<td>0.599</td>
<td>$0.0047\ (\text{SL}) + 0.0626^{*}$</td>
<td>0.420</td>
<td>$&gt;0.25$</td>
<td>0.0042</td>
</tr>
<tr>
<td>Arcsin $\sqrt{\text{PSL/SL}}$</td>
<td>$0.0860\ (\text{SL}) + 13.9145^{**}$</td>
<td>0.461</td>
<td>$0.0280\ (\text{SL}) + 19.0437$</td>
<td>0.022</td>
<td>$&gt;0.10$</td>
<td>0.0832</td>
</tr>
</tbody>
</table>
There is a significant negative correlation between the number of preopercular spines, and SL, for females (Fig. 2.3D, \( r_s = -0.423, P < 0.005 \), Spearman rank correlation), but no significant correlation between the number of preopercular spines and SL for males (Fig. 2.3D, \( r_s = 0.349, P > 0.10 \), Spearman rank correlation). Males probably have fewer spines than females because the number of spines decreases with SL in females, regardless of whether or not spine number continues to decrease once a certain size is reached.

The longest preopercular spine was the second most ventral spine in all but a single specimen when it was the third most ventral spine. As a function of SL, it is an increasing function for both females (Fig. 2.3E, \( r^2 = 0.872, P < 0.0005 \)) and males (Fig. 2.3E, \( r^2 = 0.416, P < 0.05 \)), and the slope of the line for males is not significantly different from that for females (\( P > 0.25 \)). This spine, as a proportion of head length, also increases as a function of SL for both females (Fig. 2.3F, \( r^2 = 0.599, P < 0.0005 \)) and males (Fig. 2.3F, \( r^2 = 0.420, P < 0.05 \)), but as a proportion of standard length (arcsin transformed), it significantly increases for females (Fig. 2.3G, \( r^2 = 0.461, P < 0.0005 \)) but not males (Fig. 2.3G, \( r^2 = 0.022, P > 0.25 \)). In either case, as a proportion of head or standard length, respectively, the lines from males are not significantly different in slope from the lines from females (\( P > 0.25, P > 0.10 \)). This suggests that males have larger preopercular spines, in either absolute or proportional scales, simply because they are larger fish.

In summary, male and female *C. potteri* are highly dimorphic. With the exception of the color-pattern index, however, the sexual dimorphism is largely size related, not gender related.
Although *C. potteri* is sexually dichromatic, the difference between the sexes is one of pigment pattern, not pigment color, and thus the difference may appear subtle. This is probably why *C. potteri* has been previously reported as having no permanent sexual dichromatism (Thresher 1982, 1984, Moyer 1987).

Sexual dichromatism in pomacanthids in general is variable and often subtle (Thresher 1982, 1984, Hourigan and Kelley 1985, Moyer 1987, 1990), but sexual dimorphism in size appears to be a general characteristic of pomacanthids (Thresher 1982, 1984, Moyer et al. 1983, Moyer 1987, 1990). To my knowledge, this is the first study to quantitatively relate sexually dimorphic characters to body size in a pomacanthid fish. Because so many characters are related to body size in this study, similar studies are needed for other pomacanthids to examine if presumed sexually dimorphic characters are actually gender related.

**Histology**

Ovaries are lamellar in structure (Fig. 2.4A). During the breeding season oocytes of all developmental stages can be found mixed within the ovary (Fig. 2.4B). Rather than having an asynchronous ovary, however, the ovary is group synchronous (Wallace and Selman 1981) in that individual females appear to hydrate eggs daily based on abdominal swelling, and have been observed to spawn for up to 21 consecutive days (Lutnesky Chapter V).

Histological examination of wild-caught individuals revealed evidence of protogynous hermaphroditism. Down to at least the size of 45 mm SL (the smallest female in the Portlock collection), females exhibit a precursive "male" structure of empty ducts on the periphery of the gonad (Fig. 2.4A). These ducts fill with sperm in males (Fig. 2.4C). This appears to be the same "labyrinth" tissue as was found in *C. interruptus* (Moyer and Nakazono 1978). Presumptive
Figure 2.4. Histology of the gonads of *Centropyge potteri*. A) Ovary; B) ovary showing all stages of oocyte development; C) testis; D) gonial cells within an ovary; E) and F) oocytes within testes. Except for the specimen in E which came from the sex-change demonstration, all other specimens were directly from the field (A - D, Portlock collection; F, IKB collection). ao = atretic oocytes; b = blood vessel; d = ducts; g = gonial cells; h = hydrated oocyte; l = ovarian lumen in ovaries or old ovarian lumen in testes; lm = lamella; p = previtellogenic oocyte; s = sperm; st = spermatogenic tissue; v = vitellogenic oocyte. Scale = 0.01 mm in D; 0.1 mm in all other panels. All photographs by the author.
"male" structures are also known to occur in other protogynous fishes (e.g. Gobiidae, Cole and Robertson 1988).

Testes may exhibit a lamellar structure and a membrane-lined central cavity (Fig. 2.4C), i.e. the old ovarian lumen. The testis is an unrestricted spermatogonial type (Grier, 1981), although at the beginning of sex change spermatogenesis appears to spread from the long edges of the old ovarian lamellae. This is also the case in the wrasse Thalassoma duperrey (Nakamura et al. 1989).

Gonial cells occur in nests (Fig. 2.4D). The appearance gonial cell nests in females is the same as the appearance of nests of spermatogonia in males. Whether the nests in females are oogonia or spermatogonia is unknown. Oogonia in fishes are known to occur singly, but are usually in small nests (Tokarz 1978). In the protogynous wrasse Thalassoma duperrey, single gonium cells are found in females, but it is unknown whether they are oogonia or spermatogonia (Nakamura et al. 1989). Nests of presumptive dormant male tissue, however, are thought to occur in the ovaries of the protogynous damselfish, Dascyllus marginatus (Shpigel and Fishelson 1986).

If gonial cells in females had the potential to be oogonia or spermatogonia, sex change would be a simpler process. It is interesting that early transplantation experiments on anurans have shown that sex is not determined by the gender of the donor of gonial cells; the gonad develops according to the sex of the donor of the tissue surrounding these cells (Humphrey 1933). Similar studies are needed in fishes because this is a current area of interest (Reinboth 1988, Nakamura et al. 1989, Francis 1992).

Males resulting from the sex-change demonstration described always produced testes with a lamellar organization, and some fields of previtellogenic
oocytes often remained (Fig. 2.4E). Yet a lamellar structure is not apparent in all males (Fig. 2.4F). The lamellae appear to coalesce, and in some cases the old ovarian lumen is completely occluded. This is probably due to the ontogeny of the gonad, or its state of ripeness, and is similar to the situation in some other pomacanthids (Aldenhoven 1984, Hourigan and Kelley 1985). Additionally, the peripheral ducts appear to grow into the lamellae and become nearly continuous with them.

The testis of a wild-caught male that exhibited atretic oocytes that had once been vitellogenic, with stages 2 and 3 of oocyte atresia (Saidapur, 1978), is shown in Figure 2.4F.

The sexes of the fish from every collection, a total of 341 specimens, were identified by histological examination or cannulation. Some small, immature females were collected, usually less than 47 mm SL, but females can mature as small as 42 mm SL based on the presence of vitellogenic oocytes.

All wild-caught males (n = 36) were histologically examined. They were mature, and showed some evidence of sex change. In addition to this, except for a small overlap with the largest females, males do not occur in smaller size classes as do females. Barring a sex change in progress, only one male is found per social group (Lutnesky Chapter III), and he is the largest fish in the group. Based on this evidence, it is reasonable to conclude that *C. potteri* is a monandric species, meaning all males are derived from females. Other workers have concluded that congeners are also monandric (Moyer and Nakazono 1978, Aldenhoven 1984).
Sex Change Demonstration

A sex change occurred in eight of the 15 replicates of the sex change demonstration. Some previtellogenic oocytes often remained in the sex-changed gonads; this is common in the testes of newly sex-changed fishes (Reinboth 1988). Those remaining, however, were often atretic as indicated by the loss of the nuclear membrane and the presence of a flocculent ooplasm (Tokarz 1978).

Within the gonad, there was no significant difference in the degree of sex change between peripheral and central sections ($P > 0.50$, paired t-test); sex change was uniform throughout the gonad. There was, however, a process apparent within lamellae. Spermatogenic tissue first began to develop along the long edges of lamellae. It filled in from the edges of lamellae and often left a small field of stromal tissue where oocytes had disintegrated in the center of lamellae. Qualitatively, the process is similar to that described by Nakamura et al. (1989).

There was a significant relationship between the size of the female undergoing sex change, and the degree to which she had changed sex. Larger females progressed further (Fig. 2.5A, $r^2 = 0.42$, $P < 0.01$). This result is interesting because the size hypothesis, that states that females are capable of sex change at a certain size, is currently discounted (Shapiro 1979, Ross et al. 1983, Lutnesky Chapter III). These data suggest that although small females are capable of changing sex, they are less competent at doing so. Ultimately, this may be to their advantage as they would likely require a longer time to commit to sex change. A small female that changed sex could easily lose a harem given the appearance of a larger male. Smaller females may thus require more time to reach a "point of no return" and thereby take longer to assess the
Figure 2.5. Plots of the relationships between: A) proportion male score \((M / (M+F))\), see text, and standard length, B) proportion male score and size differences between paired females in the sex-change demonstration. Solid circles represent newly sex-changed males; open circles are females that did not change sex. Line indicates a significant relationship \((P < 0.01)\) using simple linear regression.
potential of their environment for sex change (e.g. Shapiro and Lubbock 1980, Ross et al. 1983, Lutnesky Chapter III).

A longer time in treatment may have yielded a higher percentage of sex change among the replicates of this demonstration.

There is no significant relationship between the difference in size between the females in each pair, and the degree to which they changed sex (Fig. 2.5B, $r^2 = 0.001, P > 0.25$). The presence of a smaller female is required to initiate sex change in C. pottersi (Lutnesky Chapter III), but the relative size of the smaller female appears to be unimportant to the time course of sex change.

Behavior

C. pottersi live in harems consisting of one male and from one to eight females in an all-purpose territory that ranges in size from 19 - 297 m$^2$ (Lutnesky 1988, Lutnesky Chapter III). Harem and territory sizes are correlated with fish density (Lutnesky Chapter III). Aspects of behavior are treated quantitatively in Chapters III and V. The following list of Modal Action Patterns, sensu Barlow (1977), gives qualitative descriptions of C. pottersi social behavior. Behavior is listed roughly from least to most aggressive.

Circuit-swimming: the subject swims around the whole territory, and continues the behavior after completing the circuit. The path chosen by the subject varies, but high points, tunnels formed by corals, and borders shared with neighbors are consistently used. The subject usually swims only about 10 cm or less above the substrate. This behavior is most prominent in males, but is also performed by females.

Follow: after the subject encounters a circuit-swimming fish, it swims after the fish for a few meters. The behavior occurs at normal
swimming speed. The subject matches the maneuvers of the circuit-swimming fish, but stays about 0.5 m behind it. Any fish may follow another, but it is common for females to follow the male after he encounters them on his circuit-swim, particularly after being rush/chased or bitten.

Flee: the subject rapidly swims away when approached by another fish. The subject may flee in a near-linear way, or spiral up into the water column. Smaller fish usually flee from larger fish.

Fin-erection: the subject extends its fins, and swims slowly, or holds its position in the water column. The median fins are prominent during this behavior. The subject is usually oriented laterally to another fish that is also performing fin-erection. The fish often rise above the substrate higher than is usual for circuit-swimming (i.e. > 10 cm).

Preopercular Flare: without sigmoid behavior, a gill cover is raised exposing the large preopercular spine. The position is held in the water column. This behavior is uncommon, but I have seen larger females exhibit this behavior towards smaller females when the male is courting several females in a harem.

Sigmoid: the subject flexes the body into an ess shape, and flares the outward facing gill cover. This exposes the large preopercular spine, and the position is held in the water column. The behavior usually occurs during the approach of a larger fish, and is discontinued when the larger fish swims away.
Rush/Chase: the subject rapidly approaches or pursues a fish that is stationary or fleeing.

Bite: the subject pecks or clamps another fish with its mouth. Biting often occurs at the termination of a rush/chase, but may occur independently.

Preopercular Stab: after a rush/chase, bite, sigmoid or preopercular flare, with a quick flexion of the body, the subject strikes another fish with the large preopercular spine. This behavior is uncommon, and usually occurs between members of different harems.

None of the behavior listed above is specific to one sex. As was the case with many morphological characters, however, there appear to be differences associated with body size. Circuit-swimming, for example, is exhibited by all but the smallest members of the social group. Different size fish appear to perform the behavior at a different absolute speed. The male, the largest fish, patrols the territory regularly, and quickly. Females share the entire territory with the male, but circuit-swim through it more slowly.

Juveniles (< 40 mm SL) do not circuit swim, at least not in a time scale that can be observed with scuba equipment. They restrict their activity to a few coral heads. On subsequent observation, however, they will often have moved to a new set of coral heads within a territory, or even to a different territory.

Rush/chasing and biting are more common during observation of large fish, while fleeing and sigmoid behavior occur more often during observation of medium to small-size fish. Mutual fin-erection often occurs between neighboring males at a common border. Pairs of similar-sized neighboring females also exhibit fin-erection to each other.
Although preopercular stab is a socially uncommon behavior, I have observed both males and large females use it against conspecifics that intrude into the territory, or as the result of mutual fin-erection that escalated at a border site. The behavior is quite common, however, when a fish defends itself against capture. For example, it is used almost without exception against human hands when trying to remove a fish from a net or bucket. It may be important behavior in defense against predators.

Reproductive Behavior

This section of the chapter is organized differently from the previous section, where each behavior was given a discrete description, because much of the reproductive behavior of \textit{C. potteri} has been previously described (Lobel 1978a), or is quite similar to the behavior of congeners (Moyer and Nakazono 1978, Bauer and Bauer 1981, Thresher 1984).

Courting behavior by male \textit{C. potteri} begins as early as 1.5 h before sunset (Lutnesky Chapter V). I observed both males and females to blanch, as did Lobel (1978a). Light blue lines in the dark blue area on the side of the body become wider and more prominent. When a male sees a female he at rushes her. Blanching in a male often appears to suddenly intensify as he approaches a female(s). No sudden intensification of blanching was noticed in females.

When the male is 5 to 50 cm away, he begins soaring (Moyer and Nakazono 1978) above and away from the female (Fig. 2.6A). The median fins are extended and the pectoral fins are held straight out. The male either holds this position motionless in the water column for about one or two seconds, or glides by the female in this position. My observations of soaring by males are mostly consistent with the description of spawning by Lobel (1978a), however, I
never observed fluttering of the pectoral fins during soaring, as he described. Most of the time, females are unresponsive to soaring by the male.

If a female is responsive to a soaring male, she initiates spawning by swimming past him, and rises above him in the water column. The time of day this occurs depends on social-group composition (Lutnesky Chapters IV and V).

When a female rises in the water column, the male follows her. She adopts a tail-up behavior (Fig. 2.6B). In tail-up behavior, the female orients her body at about a 30 - 45 degree angle with the snout pointing to the substrate. The pectoral fins are extended and motionless. Tail-up behavior is not the same as "mutual soaring" in C. interruptus (Moyer and Nakazono 1978) as both fish have snouts pointed up and fins extended during mutual soaring. While the female is in the tail-up posture, the male swims up from below her and places his snout beside or directly under her vent (Fig. 2.6B). Moyer and Nakazono (1978) called this behavior nuzzling in C. interruptus.

In the field, C. potteri shows nuzzling from 10 cm to about 1 m above the substrate, but nuzzles high in the water column were associated with prominent coral heads of about the same height. The pair swims about 10 - 50 cm away from the coral head, and nuzzles. Nuzzling did not always lead to spawning. If the nuzzle was followed by spawning, the pair would hold the nuzzle about 2 to 10 s before the spawn. Many times, however, a pair would break from the nuzzle and either swim away from each other, or the female would swim forward and again adopt the tail-up posture and the male would reposition himself. Nuzzling of the same female occurred in many different parts of the territory.

If a spawn occurs, nuzzling is followed by a quick swimming movement in which both the male and female roll into a ventral-to-ventral orientation,
Figure 2.6. A) Male *Centropyge potteri* soaring above a female during courting. Note blanching of the male. B) Male nuzzling a female before spawning. Note female is exhibiting tail-up behavior. Photographs by the author (A) and courtesy of R. Pyle (B).
release gametes, and then swim toward the bottom with a quick burst of speed. Spawning eggs were collected from captive fish. When the fish swam toward the bottom after spawning, a water plume hit the surface. A dip net on a pole was used to collect eggs by sweeping the plume. Eggs were fixed in 10% buffered formalin. I measured 100 eggs taken about equally from the spawns of three different females. Eggs were spherical, contained a single oil droplet, and were on average $0.72 \pm 0.03$ (SD) mm. Thus egg size is similar to the range of egg sizes known for congeners, 0.60 - 0.70 mm (Bauer and Bauer 1981, Hioki and Suzuki 1987, Hioki et al. 1990). I did not distinguish between fertilized and unfertilized eggs when taking measurements.

In the field, I observed males spawning with different females in different locations within their territory. High points were common spawning sites, or "rendezvous sites" (Moyer and Zaiser 1981, Thresher 1982), but spawns were not restricted to these sites as described by Lobel (1978a). The same pattern was seen in captive fish. Spawns were more prevalent near bricks which provided shelter, but occurred in many locations including places away from bricks.

Although there are subtle differences, spawning behavior is much the same for many pomacanthids (Lobel 1978a, Moyer and Nakazono 1978, Bauer and Bauer 1981, Thresher 1982, Moyer et al. 1983, Moyer 1984, 1990, Gronel and Colin 1985). Indeed, congeners are known to spawn with each other (Moyer 1981).

I also observed an alternate sequence of mating behavior. Instead of being courted by the male (rushed and soared), a female swam by herself up into the water column and performed tail-up behavior. The male, courting another female close by or up to 2-3 m away left this female, quickly swam to the posing
female, and then nuzzled and spawned. When this behavior occurred it was quite noticeable because a few meters were traveled either by an uncourted female to pose in tail-up by the male that was courting another female, or by the male to swim to a female who was posing in tail-up alone in midwater.

In addition to this behavior that sometimes caused an indirect interference with the spawning of another female in the harem, females would sometimes directly interfere. They would place themselves between a male and a female he was courting, or even chase or bite a female that was in the tail-up posture. I have called this interference behavior (Lutnesky Chapter V). This direct interference was usually exhibited by the largest female, the alpha female, but was not exclusive to her (Lutnesky Chapter V). Bauer and Bauer (1981) and Moyer (1981, 1987) also noted female-female agonistic behavior associated with spawning in Centropyge.

Lobel (1978a) described C. potteri as having a lunar spawning periodicity. He noted that spawning occurred only during the week before full moon, starting eight days prior to full moon and continuing daily until full moon. Spawning periodicity in Centropyge is controversial, however, and a re-examination of the temporal pattern of spawning is suggested (Bauer and Bauer 1981, Thresher 1982, 1984, Moyer et al. 1983).

My observations of C. potteri spawning indicate that spawning occurs during all weeks of the lunar month (Table 2.2). Furthermore, spawning did not occur each day for every female in a harem (Lutnesky Chapters IV and V) as is suggested by the strategy outlined by Bauer and Bauer (1981) for angelfishes of the genus Centropyge.
Table 2.2. *Centropyge potteri* can spawn during all weeks of the lunar month. T - N = day after three quarter to new moon; N - Q = day after new to quarter moon; Q - F = day after quarter to full moon; and F - T = day after full to three quarter moon. Number before comma indicates number of days sampled in a time period, number after comma indicates number of days at least one female was observed to spawn. SN = observations in a semi-natural environment (see text); field = field observations. NA = not applicable.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>T - N</th>
<th>N - Q</th>
<th>Q - F</th>
<th>F - T</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>April-May, 1989 (SN)</td>
<td>9,9</td>
<td>6,5</td>
<td>8,8</td>
<td>7,6</td>
<td>30,28</td>
</tr>
<tr>
<td>May, 1989 (Field)</td>
<td>1,1</td>
<td>2,2</td>
<td>1,0</td>
<td>1,1</td>
<td>5,4</td>
</tr>
<tr>
<td>April-May, 1990 (SN)</td>
<td>7,7</td>
<td>6,5</td>
<td>5,5</td>
<td>8,8</td>
<td>26,25</td>
</tr>
<tr>
<td>May-July, 1990 (Field)</td>
<td>0,NA</td>
<td>1,1</td>
<td>3,3</td>
<td>1,1</td>
<td>5,5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>17,17</td>
<td>15,13</td>
<td>17,16</td>
<td>17,16</td>
<td>66,62</td>
</tr>
</tbody>
</table>
Temporal patterns of spawning in *C. potteri* are not, however, unaffected by lunar cycles. The timing of spawning in *C. potteri* has been studied more thoroughly (Lutnesky Chapters IV and V). It appears that a variety of temporal patterns of spawning may occur, and that individual harems are influenced by a combination of habitat characteristics and female-female competition as influenced by social-group size.

CONCLUSIONS

The pomacanthid angelfish, *Centropyge potteri*, exhibits sexual dimorphism in size, and is sexually dichromatic. The sexes can be reliably identified in the field.

*C. potteri* is a monandric, protogynous hermaphrodite. Sex change is uniform within the gonad, and larger females can change sex faster than smaller females.

The partial ethogram of social behavior, including some new descriptions of reproductive behavior, sets a foundation for future behavioral studies that use *C. potteri*.

Spawning by *C. potteri* can occur during all weeks of the lunar month, but spawning does not occur everyday for all females.
CHAPTER III
DENSITY-DEPENDENT PROTOGYNOUS SEX CHANGE IN TERRITORIAL-HAREMIC FISHES: MODELS AND EVIDENCE

INTRODUCTION

Protogynous hermaphroditism, or the sequential possession of first female and then male reproductive systems in an individual during its adult life, is an important life-history strategy in many families of teleost fishes (Smith 1975, Policansky 1982, Shapiro 1984, Warner 1984, Sadovy and Shapiro 1987). It is the most common form of hermaphroditism in coral-reef fishes (Warner 1984). Although there now exists a large body of theory for the evolution of protogynous sex change (e.g. Ghiselin 1969, Warner 1975, 1988, Warner et al. 1975, Charnov 1982, Moyer and Zaiser 1984, Aldenhoven 1986, Iwasa 1991), only a few mechanisms for the proximate control of this phenomenon have been proposed. The subject has been recently reviewed by Ross (1990). The purpose of this paper is to add to this theoretical base by presenting a priori graphical models that suggest how the density of territorial-haremic fishes may influence their potential for sex change, and to provide empirical evidence of the importance of this factor.

Territorial-haremic fishes are species in which individual males hold all-purpose territories defended against other males, and more than one female lives and mates with the male in his territory. In some species females form separate territories or home ranges inside the male’s territory (e.g. Labridae: Robertson 1972, 1974, Kuwamura 1984, Moyer 1991; Pomacanthidae: Hourigan and Kelley 1985, Hourigan 1986; Cirrhitidae: Donaldson 1987, 1990), 45
while in others females are herded by the male, or share his territory as a common home range (e.g. Pomacanthidae: Moyer and Nakazono 1978, Aldenhoven 1984; Labridae: Moyer 1991). Sometimes a newly sex-changed, nonterritorial, bachelor male (e.g. Aldenhoven 1986) also lives with a male and his harem. Throughout this paper "territory size" will refer to the male's territory which may impose a spatial constraint on the entire social group (the male, his harem, and any bachelors).

Wittenberger (1981a) classifies similar mating systems in mammals as territorial-harem polygyny. That the polygyny results primarily from resource defense, rather than female (harem) defense (Emlen and Oring 1977), is not crucial to the validity of the models developed in this chapter. When access to the same females is consistently guarded through territorial defense, the mating system is de facto harem polygyny, even though little or no direct herding or guarding of females occurs (Wittenberger 1981a).

I start with a brief review of proximate control theory because the hypotheses are few, and many of the ideas are synthesized into the models presented here. Next, I present encounter-rate threshold (ERT) models that predict the influence that fish density will have on the potential for sex change in territorial-haremic species. Finally, a territorial-haremic fish, the pomacanthid angelfish Centropyge potteri, is used to empirically test current theory and the assumptions of the ERT models.

Proximate Control Theory

The size hypothesis asserts that females change sex when they reach a critical size (e.g. Bullough 1947). This hypothesis was tested with captive fish, and evidence from the field showed it to be unlikely. Ross (1981) and Ross et al. (1983) showed that both small and large females of the protogynous wrasse,
_Thalassoma duperrey_, changed sex when held captive with a smaller female. Similarly, Warner (1982) showed that female _Thalassoma lucasanum_ could be induced to change sex even when they were much smaller than the minimum of the size range for naturally occurring males.

Shapiro (1981) studied the protogynous fish _Anthias squamipinnis_ from two ecologically distinct populations. Gene flow was not restricted between the populations, but females changed sex at different sizes in the two populations. These studies suggested that some social or environmental factors are involved in the sex-change process.

The inhibition hypothesis (Robertson 1972) asserted that an environmental factor, the presence of a male in a social group, suppresses the natural tendency of females to change sex. Fishelson (1970), working with _Anthias squamipinnis_, and Robertson (1972) working with the cleaner wrasse, _Labroides dimidiatus_, concluded that sex change was controlled by male removal.

This mechanism was criticized by Shapiro (1979) because, barring simultaneous sex changes, the number of males in a growing population can not increase in monandric species (in which all males are derived from sex change) if the production of a new male is only stimulated by the loss of a male. Robertson (1972) pointed out, however, that occasionally sex change occurred without male disappearance in social situations where interactions of a male with peripheral females were reduced. Additionally, simultaneous sex changes did sometimes occur after male disappearance (Robertson 1974). This hypothesis needs further investigation.
At present, the prevailing hypotheses are the sex-ratio threshold (Shapiro and Lubbock 1980) and size-ratio threshold (Ross et al. 1983) hypotheses. The sex-ratio threshold hypothesis asserts that a female will change sex when social-group composition reaches a threshold sex ratio. When the threshold is reached, the dominant female becomes a male. Similarly, the size-ratio threshold hypothesis asserts that a sex change occurs when social-group composition reaches a threshold level of smaller-to-larger individuals, for a given female.

All of the hypotheses above are mutually exclusive, and will be addressed in this paper. Finally, there is the priming hypothesis (Shapiro 1979). It asserts that a behavioral profile (i.e. a certain pattern of interaction with both males and females) "primes" a limited number of females to change sex. Such a mechanism could be incorporated into the sex or size ratio threshold hypotheses, or even some of the ERT models presented here. It is only addressed here in this context.

The sex and size ratio threshold hypotheses are general enough to explain protogynous sex change in the presence of a male, and the number of males in a population could increase if controlled by one of these mechanisms. Females of many protogynous species change sex in the presence of a male, including the wrasses Labroides dimidiatus (Robertson 1972), and Thalassoma duperrey (Ross et al. 1983), the fairy basslet Anthias squamipinnis (Shapiro 1981), the angelfishes Centropyge interruptus, C. tibicen, (Moyer and Zaiser 1984), C. bicolor (Aldenhoven 1986), and C. potteri (this study), and the goby Coryphopterus nicholsi (Cole 1983).
Moyer and Zaiser (1984) used the term "early sex change" to indicate sex change in the presence of a male as opposed to sex change associated with male disappearance. Although not intended by the authors, "early" conveys that the event was premature, and thus maladaptive. Throughout this paper, I use the term "facultative sex change" instead of early sex change.

None of the above hypotheses address how fish density may alter the potential for facultative sex change in a given environment. How does a fish assess social-group composition or density? Regardless of the cue that is triggering sex change, if the cue is social, the female must be assessing her social environment through sensory contact with other individuals. Shapiro and Lubbock (1980) suggest behavioral interactions as an obvious candidate for the assessment of sex ratio. Because harem and territory sizes may vary predictably with population density for territorial-haremic fishes (see below), so may patterns of contact and therefore rates of facultative sex change.

I present three testable models that predict different rates of facultative sex change in different-density populations. The models were developed considering the social systems of monandric, territorial-haremic fishes (usually single-male, multiple-female social groups). They are general, and intended to provide a foundation that can be altered for different species when data become available.

It should be noted that this paper examines how harem and territory sizes may together influence the sex-change process. The problem is dynamic, however, in that sex change in turn may alter harem and territory sizes. This second problem is not addressed here.
ENCOUNTER-RATE MODELS

Assumptions

Assume the suitable habitat available to territorial-haremic fishes occurs in discrete patches on the reef. These patches form "finite worlds" available for territories. Increases in population density in a patch are primarily from recruitment of juveniles to the reef. Assume also that most males are territorial, and that they use all available space until they encounter another male's territory, or unsuitable habitat.

Given these conditions, it follows that as the number of males in the patch increases (from sex-change in monadric species), territory size will decrease as the males carve up the finite world. This is the classical elastic disk concept of territoriality (see Wilson 1975). Additionally, as long as recruitment rate of juveniles (all female in monandric species) is greater than the rate of sex change, it follows that harem size will increase with population density as juveniles join the patch.

In this simple example it is assumed that the environmental potential for polygamy (Emlen and Oring 1977) causes insignificant variation in social-group organization within a patch. Thus the density of a social group, the specific density sensu Odum (1959), is correlated with the overall population density of the patch. Population density may have some predictive value, but it is the density of the social group that is of primary interest because it may influence a sex-change candidate's immediate cues for sex change.

Thus territorial-haremic species that are monandric, and whose social groups live contiguously, may be characterized by these relationships: 1) harem size increases with the density of a social group, and 2) size of the male's territory decreases with the density of a social group. A social group is defined
as the fish that share a territory, harem size as the number of females that live and mate with the male in his territory, and the size of the male's territory as the area (m²) he defends against other males.

Many monandric, protogynous hermaphrodites are stated to show these general relationships between harem and territory sizes and fish density. Examples include the cleaner wrasse *Labroides dimidiatus* (Robertson 1974), the angelfishes *Centropyge interruptus* (Moyer and Nakazono 1978; Moyer and Zaiser 1984) and *C. potteri* (this study), the hawkfishes *Cirrhitichthys falco* and *C. aprinus* (Donaldson T. J., personal communication), and the grouper *Cephalopholis argus* (Fishelson L., personal communication).

The ERT models were developed for the general situation of contiguous territories. They may be less appropriate for uncommon, isolated social groups because of exceptions to the assumed relationships. Isolated social groups living in only several m² of suitable habitat may have large or small harems (e.g. *Centropyge potteri*, personal observation). Given the space limitation, both situations may be considered "high density," yet those with few females do not fit a "high density profile" that would be found among contiguous territories. The comparison is invalid, however, because the male's territory size is totally constrained by unsuitable habitat. Under these conditions, territory size is not a dynamic variable of social organization as it may be when territories are contiguous.

In some species, such as the cleaner wrasse *Labroides dimidiatus* (Robertson 1972) and the angelfish *Holacanthus tricolor* (Hourigan and Kelley 1985), large females hold territories with a subgroup of smaller females within the male's territory. For the purposes of the following ERT models the
subgroups within these social systems are thought of as harems; furthermore, males in these systems have multiple harems.

An "encounter" is defined as a social interaction where a fish is close enough to another individual so that its neuro-endocrine system receives stimulation from that contact (see Testing the Models for operational definitions). The sensory modality is unimportant to the models. Encounters may vary in intensity. They may range from simply being in the presence of another individual to agonistic behavior. Even if an overt behavior is responsible for stimulating or inhibiting sex change, encounters in which no overt behavior occurs may acquire importance through conditioning of the neuro-endocrine system to the potential for the overt behavior.

Encounters involving no overt behavior may constitute a large proportion of social interaction. For example, Kuwamura (1984) found that no overt behavior was exhibited in 33 - 55% of intraspecific Labroides dimidiatus encounters. In the ERT models, an encounter with a larger individual is assumed to inhibit sex change, while an encounter with a smaller individual is assumed to stimulate sex change.

In monandric, territorial-haremic fishes it is usually the largest female of the harem that changes sex (e.g. Moyer and Nakazono 1978). For this reason, and for simplicity, the models will take the encounter-rate "perspective" of this largest or alpha female in a single-male, multiple-female social group. The models assume negligible encounters between members of different social groups, so the male is the only larger individual the alpha female encounters. The models also assume that social-group members utilize the territory independently, and that behavioral modifications (e.g. changes in swimming speed) are insufficient to change the general relationships described below.
The predicted relationships between alpha-female encounter rates with the male (larger) and female (smaller) members of her social group, and fish density, are given in Figure 3.1. Both are increasing functions.

The alpha female encounters the male at some rate under low-density conditions, but as his territory size becomes smaller under higher-density conditions, she encounters the male more frequently. This is because she is also constrained by his territory size (or a fraction of it for species in which the large females are also territorial). I used a linear relationship to illustrate the concept because it is the simplest first approximation, but linearity is not requisite to the construction of the models.

Given random association of social-group members, the alpha female's encounter rate with females, EF, is given by EF = EM(h - 1), where EM is her encounter rate with the male, and h is harem size. The EF-function is nonlinear because of the interaction of shrinking territory size, and increasing harem size, with increasing density, i.e. females are encountered more frequently because of shrinking territory size, but there are also more females to encounter under higher-density conditions. Thus, the slope of the EF-function is steeper than the slope of the EM-function (Fig. 3.1), and EF > EM whenever h ≥ 3.

What is important to the models on a gross level is that both functions are increasing, and the EF-function has the steeper slope. Note also that the EF-function remains at zero until with some increase in density, h = 2, and EF = EM.

**Absolute Encounter-Rate Threshold Hypothesis**

This model asserts that facultative sex change will occur when the alpha female receives a threshold level of stimulation from encountering smaller
females (S, Fig. 3.1A) and is below a threshold level of inhibition from encountering the larger male (I, Fig. 3.1A). The model predicts that facultative sex change would not occur under very low-density conditions because the alpha female would not reach the threshold level of stimulation from encountering smaller females. Similarly, the model predicts that facultative sex change would not occur under very high-density conditions because the alpha female would not be below the threshold level of encounters that release her from inhibition by the male. Thus the major prediction of the model is that facultative sex change will occur under intermediate-density conditions, between S and I on Figure 3.1A.

Differential Encounter-Rate Threshold Hypothesis

This model asserts that facultative sex change will occur when the alpha female receives stimulation from smaller-female encounter at some threshold level greater than inhibition from larger-male encounter (D, Fig. 3.1B). Thus the major prediction of the model is that facultative sex change will occur where females are encountered much more often than the male, i.e. under higher-density conditions.

It is interesting that the differential encounter rate ([female encounter - male encounter] / time) could increase with density even if only harem size, or only territory size, changed with density as stated in the assumptions section. For example, it would increase if harem size increased with density and territory size was held constant (this could work the same way as a sex-ratio threshold). Alternatively, it would also increase with density if harem size (for harems ≥ 3) was held constant, and territory size decreased with density. The general prediction that high density stimulates facultative sex change, however, remains the same.
Figure 3.1. General encounter-rate relationships expected between the alpha female and other members of her social group, as functions of fish density. Fish density = density of a social group (see text). Dashed line = encounter-male function; solid line = encounter-females function. A) Absolute Encounter-Rate Threshold Hypothesis. Facultative sex change (FSC) will not occur until the female receives a threshold rate of stimulation (S) from female encounter, and is below a threshold rate of inhibition (I) from male encounter. FSC will occur under intermediate-density conditions, between S and I. B) Differential Encounter-Rate Threshold Hypothesis. FSC will not occur until the female receives a threshold rate (D) of stimulation by female encounter greater than inhibition by male encounter. FSC will occur above D under higher-density conditions. C) Inhibition-Only Hypothesis. FSC will not occur when a female receives a threshold rate of inhibition (I) from male encounter. Stimulation by females is not required. FSC will occur where male encounter is least frequent; under lower-density conditions.
A) Absolute Encounter-Rate Threshold Hypothesis

B) Differential Encounter-Rate Threshold Hypothesis

C) Inhibition-Only Encounter-Rate Threshold Hypothesis

Fish Density →

Encounter / Time

S I D
Inhibition-Only Hypothesis

This model is the inhibition hypothesis of Robertson (1972) revisited with an encounter-rate interpretation. The model assumes that only inhibition through male (larger) encounter is important to the sex-change process. Stimulation by females (smaller) is not needed for sex change. If the alpha female receives less than a threshold level of male encounters (I, Fig. 3.1C), she is disinhibited, and sex change occurs. Loss of the male from the social group is not required. Since the alpha female encounters the male least frequently under lower-density conditions, the major prediction of this model is that facultative sex change would most likely occur under lower-density conditions.

TESTING THE MODELS

The first step in evaluating the models is to test their assumptions. Field work can determine if harem and territory sizes, and encounter rates, agree with the assumptions of the models.

Controlled enclosure studies such as those by Ross et al. (1983) can determine the influence of smaller female and larger male conspecifics on a sex-change candidate. This will give insight into current theory and the ERT models. For example, the inhibition-only hypothesis is untenable if stimulation by smaller conspecifics is required for sex change. Additionally, the other ERT models are inappropriate if stimulation by smaller conspecifics is not required for sex change.

Next, the hypotheses involving social-group composition thresholds, the sex-ratio and size-ratio threshold hypotheses, can be tested by an experiment using one social-group composition in two treatments that alter fish density with different size enclosures. If there is a different result regarding sex change in
the different size enclosures, it suggests that there is a greater complexity to the sex-change process than a social-group composition threshold, and that fish density is an important factor. For the experiment to be valid, facultative sex change must occur in at least one treatment to demonstrate that it can be induced, and all fish should interact at some level before sex change occurs.

Methods

*Centropyge* potteri, a pomacanthid angelfish endemic to Hawaii, is a monandric, protogynous, hermaphrodite (Lutnesky 1988, Chapter II) as are its congeners (Moyer and Nakazono 1978, Thresher 1982, Aldenhoven 1984, Moyer and Zaiser 1984).

Field

Data were collected between July, 1986, and December, 1989. Only reefs with several contiguous territories were used for data collection. Reefs were located both inside and offshore of Kaneohe Bay, Oahu, Hawaii. They ranged in depth between 3 and 24 m. Measurement of territory size followed the observation-area curve method of Odum and Kuenzler (1955) by using the male as a focal animal for 20 or 30 five-minute observation periods. Social groups used for data collection were chosen haphazardly. Harem sizes, territory sizes, and number of fish (males and females) in the social groups were measured and recorded (n = 14 social groups). Fish density (specific density) was calculated as fish in the social group (those that share a territory) divided by territory size. Simple linear correlation was used to examine the relationship between harem and territory sizes, and density.

After harem and territory size measurement, encounter rates were recorded for eight alpha females. (Encounter-rate data were also collected for males and some smaller females, but that data will not be reported here).
average, 5.5 ± 1.2 (SE) five-minute observation periods were used to record the alpha female’s encounters with the male and other females.

An operational "encounter distance" must be defined by the observer for each species studied. The distance will most likely be in error because man and fish do not share the same perception. As long as the error is constant, however, the data will be useful for comparisons of relative differences in encounter rates between different-density locations in the field, or different experimental treatments. For this field work, an encounter was defined as another fish being within 0.5 m of the alpha female. I carried a 0.5 m scale for reference.

Mean EM and EF data were regressed over density, analyzed for significantly increasing slopes using analysis of variance, and for significant differences in their slopes by t-test (Zar 1984).

Enclosures

Between June 1987 and April 1989, 270 mature C. potteri (42 - 93 mm, standard length; 3.7 - 42.2 g wet weight) were captured using handnets and anesthetic (10 : 1, 95% ethanol : quinaldine) at a depth of 15 - 35 m offshore of Kaneohe Bay, Oahu, Hawaii. Experiments were usually started on the same day of capture. Prior to experimental placement, females were held at high density with at least one larger male while weights (nearest .1 g) and measurements (nearest mm of standard length) were obtained. Color pattern is a reliable indicator of sex in C. potteri (Lutnesky 1988, Chapter II), but sex was also verified by cannulation (Ross 1984) before experimental placement. Fish were haphazardly chosen for experimental treatments.
The first experiment consisted of three treatments designed to test the assumptions of the ERT models that interactions with a smaller female stimulates sex change, and that interactions with a larger male can prevent sex change. The first treatment, SC-standard (15 replicates), demonstrated if sex change (SC) could be socially induced in *C. potteri*, and became the standard with which to compare other treatments in this experiment. Two females of different sizes were placed in 1 m wide x 1.2 m long x 1 m high wire mesh (12.7 mm² mesh size) pens. The pens were supported in water by styrofoam floats, and attached to floating docks. Pens had visual barriers between them, but shared a common body of water, a lagoon at the Hawaii Institute of Marine Biology. *C. potteri* does not naturally occur in the lagoon.

The second treatment, isolated-female (11 replicates), was used to test if stimulation by smaller (female) conspecifics is important to the sex-change process. Single females were placed in 1 m³ wire mesh pens. Given that all other things are similar, if stimulation by a smaller female is not important to the sex-change process, the first (SC-standard) and second (isolated-female) treatments should show similar sex-change results.

The third treatment, larger-male (7 replicates), was used to test if the continued presence of male (larger) conspecifics is important to the sex-change process. Using the same size pens as in the first treatment, a larger male was included with a group of two females. Again, given that all other things are similar, if larger male presence is not important to the sex-change process, the first (SC-standard) and third (larger-male) treatments should show similar sex-change results.

Statistical analyses followed Sokal and Rohlf (1981) or Zar (1984). G-tests of independence, with Williams' correction, were used to make
comparisons between the frequency of sex change (the number of replicates in which a sex change occurred) in the SC-standard and each treatment within experiment 1. It should be noted that the comparison between the SC-standard and isolated-female treatments was not biased even though there were more females in the SC-standard treatment. Given the clear results of previous studies (e.g. Ross et al. 1983) and that any multiple sex changes would result in homosexual (male) pairs, the \textit{a priori} design of experiment 1 included only one sex-change candidate (the largest female) per treatment. All females would later be histologically examined for evidence of sex change, however, as a check on the soundness of this design.

The second experiment consisted of two treatments designed to induce facultative sex change (FSC) in \textit{C. potteri}, and test the importance of fish density in this process. The first treatment, FSC-standard (7 replicates), was used to demonstrate that facultative sex change could be induced in \textit{C. potteri}. One male (the largest fish in the group) and 15 females were placed into a 3 x 34 x 2 m plastic mesh (6.4 mm\textsuperscript{2} mesh size) enclosure.

The enclosure sat on the bottom of a tidal pond. Water height in the enclosure was usually about 1 m. \textit{C. potteri} does not naturally occur in the tidal pond. The planar surface area of the enclosure (102 m\textsuperscript{2}) was about five times the area of the smallest natural territory I have measured, but only about a third of the area of the largest (see below). The harem size (15 females) was almost twice the size of the largest natural harem I have observed (8 females).

The second treatment, space-reduction (6 replicates), tested if fish density is important to the sex-change process. This treatment used the same social-group composition as the first treatment, i.e. one male and 15 females. The fish
were placed into a 3 x 4 x 2 m enclosure. The planar surface area of this enclosure (12 m²) was less than that of a small, natural territory (see below).

The construction of the enclosures was the same in both treatments. The fish in the two treatments were visually isolated from each other, but the two enclosures shared the same tidal pond. All other things being similar, if fish density is not important to facultative sex change and social-group composition is the crucial factor, the first (FSC-standard) and second (space-reduction) treatments should show similar sex-change results.

It should be mentioned that a few of the smallest fish disappeared (presumed dead) from the enclosures in the tidal pond. About the same loss was experienced in both enclosures per replicate (1.1 ± 0.7 and 0.7 ± 0.2, mean ± standard error, FSC-standard and space-reduction enclosures, respectively). Usually the smallest fish disappeared. Males and large females were never lost; fish were identified by individual marks and fin clips. Small fish may have been caught and eaten by crabs that lived within the enclosures. On one occasion I observed a crab eating the remains of a small *C. potteri* within an enclosure. No attempt was made to replace lost fish.

A G-test of independence, with Williams’ correction, was again used to make a comparison of the frequency of sex change (the number of replicates in which a sex change occurred) between the FSC-standard and the space-reduction treatments in experiment 2. Both treatments had the same number of females, but the design did not include an a priori number of sex change candidates. All females were histologically examined for evidence of sex change.

The fish fed on algae that grew abundantly within the enclosures. After 36.3 ± 3.0 (mean ± standard deviation) days, all fish were killed by overdose of
anesthetic. Gonads were fixed in 10% buffered formalin, and routinely embedded in paraffin. They were sectioned at seven to nine microns, stained with hematoxylin and eosin, and examined by light microscopy. The criteria used to determine if a female had changed sex followed Sadovy and Shapiro (1987). Criteria included a testis with lamellar organization, a membrane-bound lumen, oocytes in atresia stages 2 and 3 (Saidapur 1978), and sperm sinuses in the gonadal wall. Former females that contained sperm were considered sex changed.

Encounter-rate data were also recorded from the FSC-standard and space-reduction enclosures. Encounter distance was defined as 0.2 m to make data collection manageable, and behavioral data such as "chased" and "chases" were also recorded. PVC pipe scales (0.2 m) were placed in the enclosures for reference, and data were recorded on audio tape while making surface observations of the fish from an edge of the enclosure.

An average of 3.7 ± 1.1 (SE) five-minute observations were made for each alpha female and beta female for six of each of the two treatments during one day early in the experiment, on average, day 12 ± 1 (SE). This time was chosen for sampling because it should avoid any biases associated with experimental set-up, yet still be early enough to record interactions before completion of group fission that occurs with sex change.

The average values of these observations were used for data analysis. Using a 2-factor, nonparametric ANOVA (Zar 1984) the influence of pen size, female size rank, and their interactions on differential encounter-rate ([encounter smaller - encounter larger] / 5 minute observation period) were examined. I used the same analysis, and factors, to examine the absolute level
of inhibitory stimuli (encounter-larger / 5 minute observation period). The purpose in using these variables was to distinguish between the differential and absolute ERT models. For example, if sex change occurs in the FSC-standard but not in the space-reduction treatment, and the differential encounter rate is higher in the latter treatment, the differential ERT can not account for sex change. Likewise, for the absolute ERT to be responsible for sex change in the FSC-standard, the level of inhibitory stimuli there should be significantly lower than in the space-reduction treatment.

Finally, the alpha female's ratios of chases / encounters, and chased / encounters were examined for significant differences between the FSC-standard and space-reduction enclosures by Mann-Whitney tests (Zar, 1984). These tests would serve to check if density also alters the relationship between agonistic behavior and encounters. Such a change would make interpretation the results more difficult.

**Results and Discussion**

**Field**

Harem size increases with fish density (Fig. 3.2A, \( r = 0.78, P < 0.0005 \)), and log territory size decreases with fish density (Fig. 3.2B, \( r = -0.95, P < 0.0005 \)). Additionally, harem size and log territory size are negatively correlated (\( r = -0.78, P < 0.0005 \)). The trajectory of the relationship through all three variables is shown in Figure 3.2C.

In addition to the data on *C. potteri* presented here, Lobel (1978a) found nearly even sex ratios in low-density populations, and female biased sex ratios in higher-density populations, of *C. potteri*. These data are consistent with the relationships found here.
Figure 3.2. A) Harem size significantly increases and B) log territory size significantly decreases, with increasing fish density (Fish / m²) for the Hawaiian angelfish, *Centropyge potteri*. Fish density = density of a social group (see text). C) 3-dimensional relationship between harem size, log territory size, and fish density.
These sociodemographic patterns do lead to encounter-rate functions that increase with density as assumed by the ERT models (EM-function, $r^2 = 0.85$, $P < 0.0025$; EF-function, $r^2 = 0.90$, $P < 0.0005$; Fig. 3.3). Additionally, the EF-function is significantly steeper than the EM-function ($|t| = 3.2$, $P < 0.005$). Although it is not clear whether the EF-function rises as steeply as one might expect based on the assumptions of the models, the basic relationships hold.

Enclosures

The results of the experiments are summarized in Table 3.1. In the SC-standard treatment, experiment 1, a sex change occurred in eight of 15 replicates. Analyses beyond the scope of this paper showed that larger females changed sex faster (Lutnesky Chapter II). Thus, had the paradigm included a longer time in treatment, it is likely that sex change would have occurred in more replicates. These data do not support the size hypothesis, however, because some females (56-63 mm SL) changed sex at a much smaller size than the smallest males I have observed in the field (about 75 mm SL).

No sex changes occurred in the 11 replicates of the isolated-female treatment, experiment 1. This result is significantly different from the SC-standard treatment (G-test of independence, $P < 0.005$), and indicates that stimulation by a smaller (female) conspecific is important to the sex change process. In addition to this evidence, three more females were allowed to stay in treatment for > 100 days as a check on the experimental design. Sex change did not occur.

The gonads of isolated females were regressed; they contained only previtellogenic oocytes, and sometimes a small proliferation of stromal and gonial tissues. The gonads of non sex-changed females in all other treatments (both experiments) ranged from regressed to containing hydrated oocytes. It is
Figure 3.3. Simple linear regressions of alpha-female encounter rates in *Centropyge potteri*. Both functions significantly increase with density, and the encounter-female function (solid line, circles) is significantly steeper than the encounter-male function (dashed line, triangles). Error bars show standard error of the mean.
Table 3.1. Sex-change experiments using the pomacanthid angelfish, *Centropyge potteri*. Experiment 1 = tests of the importance smaller female and larger male presence in the sex-change (SC) process; Experiment 2 = tests of the importance of density in the induction of facultative sex change (FSC). Treatments within each experiment are compared with the standard for that experiment (treatment 1). n = number of replicates; sex change = number of replicates showing a sex change; percent change = percentage of replicates showing a sex change. Single asterisk indicates a significant difference with \( P < 0.01 \); double asterisk \( P < 0.005 \); and triple asterisk \( P < 0.001 \) (G-tests of independence).

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Treatment</th>
<th>Description</th>
<th>n</th>
<th>Sex Change</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. SC-standard</td>
<td>2 females</td>
<td>15</td>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>2. Isolated-female</td>
<td>1 female</td>
<td>11</td>
<td>0</td>
<td>0 **</td>
</tr>
<tr>
<td></td>
<td>3. Larger-male</td>
<td>1 male + 2 females</td>
<td>7</td>
<td>0</td>
<td>0 *</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2</th>
<th>Treatment</th>
<th>Description</th>
<th>n</th>
<th>Sex Change</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1. FSC-standard</td>
<td>1 male + 15 females</td>
<td>7</td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3 x 34 m pen)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Space-reduction</td>
<td>1 male + 15 females</td>
<td>6</td>
<td>0</td>
<td>0 ***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3 x 4 m pen)</td>
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</tr>
</tbody>
</table>
suggestive that reproductively "active" females were found in all but the isolated-female treatment, i.e. that stimulation of some kind is needed to be "female" as well as to change into a male. An analysis to determine significant differences in female reproductive state between treatments, however, should control for lunar patterns of reproductive activity (Lobel 1978a, Lutnesky Chapters IV and V). That scale of control was not used in this study. It should be noted, however, that sex changes occurred whether or not females were initially vitellogenic, and during all seasons.

Sex change was expected in the isolated-female treatment if inhibition-only was the operating mechanism. Thus, inhibition-only is an unlikely mechanism in C. potteri. Most sex-changing fishes likely need stimulation by conspecifics to change sex (Shapiro 1984, Moyer 1987, Ross 1990), thus inhibition-only is generally an unlikely mechanism. There may be exceptions, however, as Robertson (1974) showed that L. dimidiatus underwent a slow sex change in isolation. Yet, even in L. dimidiatus the presence of smaller females stimulates the process (Robertson 1974).

No sex changes occurred in the seven replicates of the larger-male treatment, experiment 1. This result is significantly different from the SC-standard treatment (G-test of independence, P < 0.01), and demonstrates the importance of larger-male presence in the sex-change process. Although the smaller female was present to stimulate sex change in the larger female, the larger male prevented sex change. The presence of the male either inhibited sex change, or the sex change candidate was not stimulated to change sex because of his presence (see General Discussion). It is clear that size is important. It is significant (binomial test, P < 0.004) that it was always the larger of the two females in the SC-standard treatment to change sex, when sex
changes occurred. At this time it is unclear, however, if the influence of the larger male was enhanced due to his sex.

Facultative sex change was induced in six of seven replicates of the FSC-standard treatment, experiment 2. In all cases, when a facultative sex change occurred, only one female changed sex. Usually within two weeks, the largest female (in one case, the second largest female) changed to male color pattern, started defending a territory, and the harem divided. No sex changes, male color patterns, or territorial defense occurred for females in the space-reduction treatment, experiment 2. This result is significantly different from the FSC-standard treatment (G-test of independence, \( P < 0.001 \)).

Since the same social-group composition was used in both the FSC-standard and space-reduction treatments, the different results suggest that there is more to the sex-change process than a sex or size ratio threshold for \( C. \) potteri. Fish density is an important factor in the induction of facultative sex change.

Of the hypotheses described, this leaves only the absolute and differential ERT models as viable possibilities for \( C. \) potteri. Encounter-rate data reveal, however, that the differential encounter rate (Fig. 3.4A) was significantly different between enclosures (\( H = 10.1, P < 0.005 \)), but not between female size ranks (\( H = 2.1, P > 0.1 \)) nor the interaction (\( H = 0.003, P > 0.95 \)). The larger differential encounter rate in the space-reduction enclosure, yet lack of sex change, indicates that differential encounter rate does not stimulate sex change in \( C. \) potteri.

Encounter-larger data (Fig. 3.4B), i.e. the level of inhibitory stimuli of the absolute ERT model, was significantly different between enclosures (\( H = 5.5, P \)
Figure 3.4.  A) Differential encounter rate ([encounter smaller - encounter larger] / 5 minute observation period). There was a significant difference caused by enclosure treatment, but not by female size rank, nor the interaction. B) Absolute level of inhibitory stimuli (encounter larger / 5 minute observation period). There was a significant difference caused by enclosure treatment, and female size rank, but not by the interaction. Error bars show standard error of the mean.
< 0.025), and female size ranks (H = 4.4, P < 0.05), but not the interaction (H = 1.3, P > 0.1). The lower level of inhibitory stimuli in the FSC-standard treatment, and for alpha females, potentially explains why alpha females usually changed sex in the FSC-standard treatment.

The results of the experiments and the encounter-rate data leave only the absolute ERT model as a viable hypothesis in explaining sex change in C. potteri.

Looking for alternative explanations, I examined the ratio of agonistic behavior to encounters. Neither the times the alpha female chases/encounter nor was chased/encounter was significantly different between enclosures (P > 0.2 for both tests, Mann-Whitney tests).

Additionally, one might think it is possible that the fish in the space-reduction treatment did not change sex because they were food limited in their smaller enclosure. This was unlikely. For example, condition factor (e.g. Pauly, 1984) is given by weight x (100/ standard length^3). A comparison of condition factor between males or beta females (excluding one beta female that changed sex) from the two treatments showed no significant differences. At the end of treatment, average condition factor for males was 5.2 ± 0.1 SE, and 5.1 ± 0.4 SE (P = 0.20, Mann-Whitney test), and for beta females 5.5 ± 0.1 SE, and 5.3 ± 0.1 SE (P > 0.20, Mann-Whitney test), in the FSC-standard and space-reduction enclosures, respectfully.

Excess food resources, primarily algae, had to be periodically removed from inside both enclosures to keep it from occluding the plastic mesh. The angelfish could not keep up with its production in either of the shallow enclosures.
Although data on fat deposits and spawning were not systematically collected as part of this experiment, what little data I have is also evidence against food limitation in the space-reduction enclosure. Five of six (83%) alpha and beta females from the space-reduction enclosure had visible fat when dissected. Fish in the space-reduction enclosure were also observed to spawn (Lutnesky Chapter IV). Energy reserves (fat) and spawning are not indicative of a system too energy poor for reproductive function (in this case sex change).

Finally, the density in the space-reduction enclosure should not be considered pathological, and thus too high for sex change. Sex change occurred in the SC-standard treatment, experiment 1, when there was only 0.60 m$^3$ / fish. There was 0.75 m$^3$ / fish in the space-reduction treatment.

**GENERAL DISCUSSION**

The ERT models are a synthesis of the hypotheses involving social-group composition thresholds as triggers for sex change (Robertson 1972, Shapiro and Lubbock 1980, Ross et al. 1983), and expected patterns of contact based on harem and territory sizes. Without variation in population density or type of habitat, an encounter-rate threshold could be similar to a sex-ratio or size-ratio threshold. An encounter-rate threshold is essentially different from these mechanisms, however, because it incorporates fish density and its affect on overt behavioral acts and simple proximity that could be as important as overt behavior because of conditioned responses. At least in some territorial-harem fishes, variation in fish density or type of habitat may alter encounter rates in a systematic way. Fish density may be as integral to the sex-change process as social-group composition.
With an encounter-rate interpretation, it should be apparent that the distinction between facultative sex change and sex change associated with male disappearance is only semantic. When encounter rate with the male falls below the threshold that inhibits sex change in a female, he has "disappeared." Mechanistically, the processes are the same.

The conceptual framework behind the absolute and relative ERT models involves a sex-change cue that comes from two sources: a stimulatory signal from smaller females and an inhibitory signal from the larger male. Sex change is dependent on integrating information from both signals, and in one case their strength relative to each other. This two-signal system follows that described by Ross et al. (1983).

Shapiro (1988) distinguished between disinhibition and stimulation by male removal (or separation of the sexes) as proximate control mechanisms. In the former case a female is disinhibited when a male is removed and her tendency to change sex, given stimulation by the presence of smaller females, is no longer blocked by inhibitory stimuli. In the latter case, the act of separating the sexes triggers a previously inactive sex change mechanism.

Based on this distinction, Shapiro (1988) described an alternative to the two-signal system. The presence or absence of males and other females alters a sex-change candidate’s profile of "behaviors-received" from other females. It is the change in this signal, upon male removal, that is proposed to stimulate sex change rather than some process of disinhibition (Shapiro 1988). Behavioral stimulation of sex change may be important in some hermaphroditic fishes, especially those that form all-female groups as does Anthias squamipinnis (e.g. Shapiro and Boulon 1982, Shapiro 1988). Since all-female groups are maintained without males by definition, disinhibition processes can not control
sex change in such species. To my knowledge, territorial-haremic fishes do not form such all-female groups.

In the present study, all fish experienced the act of separation of the sexes due to the methods of capture and handling. That different treatments yielded different results suggests that the act of separation is not crucial to the sex-change process of *C. potteri*. This is the same argument Ross (1990) used to distinguish between disinhibition and stimulation of sex change through the act of separation of the sexes. Additionally, in *C. potteri* sex change occurs in the presence of the male, so the act of separation could not be crucial to its sex-change mechanism.

However, viewing separation of the sexes as an encounter-rate threshold, and not as a unitary event (an act), again makes stimulation of sex change by separation of the sexes a viable possibility. From this perspective, my experimental paradigm did not distinguish between disinhibition and stimulation through separation. The distinction (i.e. how the male influences sex change) would not alter the mechanics of the ERT models, but is important to the interpretation of experiments (Shapiro 1988).

Encounter-rate data are needed from a diversity of protogynous fishes. Sex change has arisen independently many times in fishes (Smith 1975, Warner 1984), thus studies of several species are needed to uncover the possible diversity in proximate control mechanisms. Even within *Centropyge*, Aldenhoven (1986) suggests that higher density induces sex change in *C. bicolor*, and Moyer (1987) suggests that lower density induces sex change in *C. interruptus* and *C. tibicen*. Obviously, controlled experiments are needed to determine the proximate control mechanism for each species.
The ERT models appear to be most suited to monandric, territorial-haremic fishes. Data from diandric (males are initially derived or come from sex-changed females), home-ranging fishes suggest that the sex-ratio (harem size) assumption of the models may not be met for these species (e.g. Warner and Robertson 1978, Cowen 1990). Additionally, the relationship between density and home-range size is generally unknown.

These species typically have initial-phase males that group spawn with females, and terminal-phase males that pair-spawn and individually defend spawning-territories. The influence density may have on their social systems is unclear. For example, Lejeune (1987) showed that spawning-territory size is decreased in a high-density population of Coris julis, but similar rates of agonistic and courting interactions between initial-phase males or females, respectively, and terminal-phase males are maintained. This result is unlike that found for Thalassoma bifasciatum. In this system agonistic interactions increase, and number of spawns for terminal-phase males decreases, with an increase in population density (Warner and Hoffman, 1980a,b). Warner and Hoffman (1980a,b) predicted that rates of sex change would be reduced in high-density populations because sex change would be a less successful strategy under these conditions.

Until we know how population density influences home ranges and the "sampling" by females of their social groups, we can not know how it influences the social stimuli that induce sex change.

Home-ranging fishes may also be less influenced by the presence of dominant individuals. It is interesting that large, high-density groups of a home-ranging species, Thalassoma duperrey induced multiple, simultaneous sex changes (Ross et al. 1990). No males were present in the study, but Ross et al.
(1983) showed that sex change is influenced by size, not sex, in this species. Only one sex change would be expected if the largest individual could control the rest of the group. By contrast, sex change did not occur when large, high-density groups of a territorial-harem species, *Centropyge potteri*, were used in this study.

Patterns of sensory contact in protogynous fishes are likely to vary considerably depending on the species' intragroup structure. Probably no species has completely independent movement of social-group members. The models should, however, withstand considerable deviation from this ideal condition, and remain useful for making predictions. If one worked on a species whose social groups behaved as schools or aggregations within their territory or home range, however, the models likely do not apply, yet patterns of contact may even vary within aggregations. Shapiro (1986) showed *Anthias squamipinnis* females had larger home ranges than males and may thus increase the probability of changing sex by monitoring more than one male's home range.

The ERT models took the encounter-rate perspective of the alpha female. To examine the perspective of any other female in a social group, simply remove any encounters with larger females from the stimulation (encounter-females) function, and add them to the inhibition (encounter-male) function at the appropriate density. It should be apparent that the likelihood of a low-ranking female changing sex is small. Most of her encounters would inhibit sex change, and she would receive only a small amount of stimulation to induce sex change; none if she is the smallest female.
CONCLUSIONS

Density is important in the induction of sex change in Centropyge potteri. It systematically alters patterns of social contact, i.e. encounter rates, and thus the cues for sex change. Social-group composition is also important in the determination of encounter rates, but social-group composition alone does not trigger facultative sex change.

The absolute encounter-rate threshold hypothesis is the most probable model of the social control of sex change in C. potteri. A diversity of proximate control mechanisms may occur in fishes, however, because sex change has independently arisen several times in their evolution.
CHAPTER IV
A TEMPORAL-THRESHOLD MODEL OF POLYGYNOUS MATING
IN CYCLICAL ENVIRONMENTS

INTRODUCTION

Most animals have temporal patterns of reproduction. They breed at certain times of the day, month, or year depending on the scale examined. Additionally, the physical conditions under which they breed are usually cyclical (e.g. diel, lunar, or annual cycles), and relatively predictable. The purpose of this paper is to present a temporal-threshold model of polygynous mating (TMPM). It shows how cyclical environments and polygyny costs may together influence temporal patterns of reproduction in polygynous animals.

Within social groups of females that breed with polygynous males, how may a cost of sharing a male influence when each female chooses to breed? How may the number of concurrently reproductive females in the group change through time? This paper proposes a hypothesis that females assess breeding-situation quality (e.g. Wittenberger 1981a), as influenced by polygyny, within the context of their social group. The result of this assessment determines the time when each female chooses to breed with a male who has reproductive access to the group.

The TMPM is an extension of the polygyny-threshold model (PTM, Verner 1964, Verner and Willson 1966, Orians 1969) into a temporal dimension. Wittenberger (1981b) explored time as a hidden dimension in the PTM. He focused primarily on how predictability of habitat quality through time influences with whom (or where) females choose to mate. The TMPM focuses on when females choose to mate during an environmental cycle, given
that they share their mate with other females. The choice in the former case is
between groups, in the latter case it is a within-group reproductive decision.

Before exploring this temporal aspect of polygyny, the PTM should be
clearly understood (Fig. 4.1). It postulates that females choose between males
or breeding sites of different quality, collectively referred to as breeding-
situation quality, to maximize their fitness. Although there are now a suite of
models, the original model and most of its derivatives assume a fitness cost
when a female mates with an already-mated male. PTM theory, including
alternatives to polygyny-cost models, has been recently reviewed by Searcy and
Yasukawa (1989).

Fitness costs to females in a polygynous mating system may be numerous.
Examples include the potential loss of fitness when females share available food
and shelter, the male’s parental care or fertility, or combinations of the above
(e.g. Orians 1969, Dewsbury 1982, Nakatsuru and Kramer 1982). They may also
arise when attempts to reproduce fail due to aggressive interactions between
females within a social group (e.g. Dunbar 1980, Smuts 1987).

The polygyny threshold is reached when the cost of mating with an
already-mated male is equal to the fitness of mating with an unmated male of
lesser breeding-situation quality (Fig. 4.1).

When considering the TMPM, it should be noted that the PTM and
TMPM are hierarchical. Breeding-situation quality likely varies between males,
and may vary differently as a function of time for different males (Wittenberger
1981b). Anytime a female can increase her fitness by making between-group
reproductive decisions she is expected to do so, i.e. the PTM is invoked. The
Figure 4.1. The polygyny-threshold model (Orians 1969). $F_1$ = fitness function for a primary female; $F_2$ = fitness function for a secondary female. At the polygyny threshold (PT), a secondary female mating under the best breeding-situation quality (BSQ₁) achieves the same fitness as a primary female mating under a lesser breeding-situation quality (BSQ₂).
within-group reproductive decisions of the TMPM are relevant only after between-group reproductive decisions are made.

Given that groups of females choose to mate with polygynous males, and there are optimal times to mate due to cyclical environmental conditions, how may polygyny costs influence when these females reproduce?

A TEMPORAL-THRESHOLD MODEL OF POLYGYNOUS MATING

Assumptions

The first assumption is that continuous breeding yields a fitness function that varies over time because of cyclical environmental conditions (Fig. 4.2). This is the environmental period of the fitness function. The crest of the function is the best time to breed, and the trough the worst. The function is symmetrical about the crest and trough because it illustrates the concept, and is the simplest first approximation.

The environmental period is divided into time intervals, or opportunities to breed. For example, many coral-reef fishes that spawn pelagic eggs at dusk show a lunar periodicity in spawning (Johannes 1978). The lunar month would be the environmental period, and different days represent the time intervals of the period.

As in the original PTM, polygyny has a fitness cost. The average fitness of females mated by the male, during a given time interval, decreases as a function of polygyny. Average fitness functions for numbers of successively mated females are parallel because they are subjected to the same environmental conditions, but drop as the number of females mated increases (Fig. 4.2). The successive drops in fitness are linear, again, because they illustrate the concept, and are the simplest first approximations.
To construct the simplest model, it is assumed that the cost of polygyny is recovered between time intervals. The first female to mate within a time interval does not incur a polygyny cost, regardless of the male’s activity in previous time intervals. Time intervals should thus be long enough for mating of all females that choose that time interval, and for male recovery before the next time interval.

Choice of time interval by females is assumed to form an ideal-free distribution (Fretwell and Lucas 1969, Fretwell 1972). Each time interval in the TMPM forms a "temporal habitat" for breeding. The distribution of habitat choice by females is ideal in that females are assumed to discern time-interval quality over the entire environmental period. Time-interval quality is based on both the environmental quality of the time interval and the number and attributes of females choosing that time interval. Females choose the time interval most suited to themselves. Once they choose a time interval, it is free in that individual females compete on an equal basis for breeding order within any time interval during the environmental period. An individual female breeds only once during the period.

Based on equity of competitive ability within a time interval, a female expects average fitness in that time interval. Average fitness drops as more females choose a given time interval. As more females choose the "best" temporal habitat, average fitness will approach that accruing to a female who chooses a time interval of lesser quality that fewer females choose. The result is that females choose between habitats so that on the average they have equal fitness in any chosen habitat (Fretwell and Lucas 1969, Fretwell 1972). Thus,
although all chosen habitats yield the same average fitness, the "good" habitats are chosen by more females (Fretwell and Lucas 1969, Fretwell 1972).

Because many animals live through several environmental cycles before breeding, the ideal condition of the model could be approached through experience for these animals. It is less likely that several females have equal competitive ability.

In mating systems where dominant females can exclude some subordinate females from time intervals, time interval choice is assumed to form an ideal-despotic distribution (Fretwell 1972). The basic mechanism is similar to an ideal-free distribution, but subordinates are not free because they have lower fitness (on the average) than dominants in any given habitat. Subordinates will have additional polygyny costs due to their potential exclusion by dominants. Given the additional costs, subordinates will more readily disperse from optimal time intervals. Thus dominant females should compete during optimal time intervals and subordinate females during less environmentally advantageous time intervals.

Finally, a choice of when to mate is not assumed to be a cognitive process. For instance, the impact of a female's social environment on her physiological condition could decide for her that it is time to breed during a certain time interval.

Predictions

Where $F_i, i = 1,2,...,n$, is the average fitness function for i mated females in Figure 4.2, and $t_0$ is the best time interval to breed because of optimal environmental conditions, a female that breeds at $F_i t_0$ accrues the highest fitness. The time $t_j, j = 1,2,...,w$, (or $t_{j'}, j' = 1',2',...,w'$) represents less advantageous time intervals before (or after) optimal environmental conditions.
Females lose fitness when not mating at $t_0$. For females unable to breed at $F_1t_0$, however, there is no loss of fitness breeding at $F_1t_1$ (or $F_1t_j$) relative to $F_1t_0$ when the loss of fitness due to polygyny is equal to the loss of fitness due to changes in the environment, that is, when

$$F_1t_0 - F_1t_0 = F_1t_0 - F_1t_j,$$

or simply

$$F_1t_0 = F_1t_j.$$

This is the temporal-threshold for polygynous mating, and is represented by $F_2t_0 = F_1t_1$ in Figure 4.2.

Because of the cyclical environment, temporal-thresholds occur in pairs, before and after optimal environmental times. The same argument made for $F_1t_0 = F_1t_j$ can be made for $F_1t_0 = F_1t_j$. For instance, $F_2t_0$ is also equal to $F_1t_1$, in Figure 4.2.

The major predictions of the model are that females will compete for breeding order and that proportionally more females will compete for breeding order on environmentally advantageous time intervals. However, at the temporal-threshold, less environmentally advantageous time intervals will also be chosen. If dominant females can exclude subordinate females, dominants will choose optimal (peak) time intervals and subordinates will choose less advantageous time intervals.

Additionally, the proportion of the environmental period utilized for breeding will increase with the number of females in the social group. With more females in the group, synchrony in mating will decline. A greater number of time intervals will be utilized for breeding (symmetrically around $F_1t_0$ in this simple model).
Figure 4.2. A temporal-threshold model of polygynous mating. $F_1, \ldots, F_n = $ average fitness functions for numbers of successively mated females with a single male. The time $t_0$ is the best environmental time to breed, $t_1$ and $t_1'$ are equally less advantageous times, while $t_w$ and $t_w'$ are equally the worst environmental times to breed. Females compete for $F_1 t_0$. Unable to obtain it, they do equally well at $t_1$ (or $t_1'$) relative to $t_0$ at a temporal threshold (A, $F_2 t_0 = F_1 t_1$ [or $A', F_2 t_0 = F_1 t_1'$]). Mating may become aperiodic at a continuous-mating threshold (B, $F_5 t_0 = F_1 t_w$ [or $B', F_5 t_0 = F_1 t_w'$]).
AVERAGE FEMALE FITNESS

TIME

$A$, $A'$

$B$, $B'$

$F$

1, 2, 3, 4, 5, n

$t_w$, $t_1$, $t_0$, $t_1'$, $t_w'$
The last prediction is independent of time required for mating, i.e., handling time. It is obvious that more time is required to mate with more females due to handling time. The model predicts that more time will be used in larger groups because females disperse their choices to avoid polygyny costs.

The prediction is that variance in time of mating will be greater in larger groups of females, even when the calculation is adjusted for handling time. An adjusted time of mating for the \(i^{th}\) female is given by \(g_i\), \(i = 1, 2, \ldots, n\), where

\[
g_i = A_i - \sum_{j=1}^{i} H_j.
\]

\(A_i\) is the actual time of mating for the \(i^{th}\) female, and \(H_j\) is the handling time for the \(i^{th}\) female. Thus the prediction is that

\[
\sigma_{g_n}^2 < \sigma_{g_n}^2 + m
\]

where \(\sigma_{g_n}^2\) is the variance in adjusted time of mating for a group of \(n\) females and \(m\) is the number of females needed to fill the tier of breeding positions (\(F_{i:t}, F_{i:t0},\) or \(F_{i:t0}^{'}\) with equal average fitness) of the tier chosen by the \(n^{th}\) female, and the following tier. On a gross level this simply means that larger groups of females will have greater variance in adjusted time of mating.

Another prediction is relevant to larger groups of females. Mating becomes continuous when polygyny costs are equal to the amplitude of variation in fitness due to environmental cyclicity, i.e., \(F_{i:t0} = F_{1:t_w}\) or \(F_{i:t0} = F_{1:t_{w'}}\), where \(t_w\) and \(t_{w'}\) are the time intervals when average fitness is lowest due to environmental cost. Peak breeding activity remains symmetrical about \(F_{1:t0}\). This is the continuous-mating threshold, and is represented by \(F_{5:t0} = F_{1:t_w}\) or by \(F_{5:t0} = F_{1:t_{w'}}\) (Fig. 4.2). In this example, a female has the same fitness on
the average being the only female mating during the worst time interval as she
does as one of five females that compete during the best time interval.

Amplitude of variation in fitness due to cyclical environmental conditions
likely ranges from nothing to very high. For example, where fitness is relative to
periodic weather or tidal phenomena, some environments may experience
extreme fluctuations while others may be relatively static. It should be apparent
that \( F_{i}t_{0} = F_{1}t_{w} \) could be reached by smaller groups of females in low-
amplitude environments. Aperiodic, continuous mating would thus be more
likely in low-amplitude environments.

The time scale of the model has been left open to interpretation. The
model can be used to predict temporal patterns of mating on daily, monthly, or
seasonal time scales. Symmetry of the fitness function about \( F_{1}t_{0} \) is not
requisite to application of the model. An asymmetrical model works in the
same way, but mating activity is skewed to one side of the peak. Additionally, if
the amplitude of a fitness function varies over time, not all \( t_{w} \) time intervals
would be paired. The basic mechanism remains the same.

**DISCUSSION**

The TMPM may have predictive value in any mating system where
environmental cycles create an optimal time for breeding, females share males,
and polygyny costs occur. To illustrate I discuss two examples where the model
may be tested. By using examples of such divergent animals as elephants and
angelfish, and different time scales as seasonal and lunar cycles, I point out that
the model has potential for broad applicability. Finally, I discuss some
limitations of the TMPM, and describe how it may be tested.
Elephants

The TMPM may be applicable to a mating system such as that found in the African elephant, *Loxodonta africana*. Mature female African elephants form stable all-female social groups (Kingdon 1979, Moss 1988). Their young also live in the group, but young males leave the group when they mature (Poole 1987, Poole et al. 1988). Most females within a group are relatives and the family is led by a dominant matriarch (Buss 1961, Dublin 1983, Moss 1988).

Mature males live alone or travel in bachelor herds, and associate with groups of females only for breeding (Poole 1987). Except for males in younger age classes, mature male African elephants come into musth (Poole and Moss 1981, Poole 1987). Musth is comparable to rutting behavior in other ungulates (Moss 1983, Poole 1987).

Both musth and nonmusth males will attempt to mate with estrous females (Moss 1983, Poole 1989b). Competition between males over estrous females can be intense, but fights are rarely escalated (Poole 1989a). Regardless of the dominance hierarchy between males outside of musth, musth males are usually dominant to nonmusth males in competition over females (Moss 1988, Poole 1989a). Fights between musth males are rare, and can result in serious injury or death (Poole 1989a).

Few males are in musth simultaneously. In a population that contained 52 matriarchal groups, and 37 males that came into musth periodically, on the average only 3 males were in musth per month over a 10-yr period (Poole 1989a). Musth is sporadic and may last only a few weeks in smaller males, but is continuous and lasts for several months in larger males (Poole 1987, Poole 1989a). These large musth males obtain most matings, especially when females
are most likely to conceive (Poole 1989b). This is due not only to male-male competition, but also because females prefer large musth males (Moss 1983, Poole et al. 1988, Poole 1989b).

Wild female African elephants can come into estrous anytime of year (Moss 1988), but peaks in breeding are associated with seasonal rains (Laws et al. 1975). In areas with bimodal peaks in rains, there are also bimodal peaks in breeding activity (Laws et al. 1975, Dublin 1983). Peak breeding occurs 1 - 2 months after the onset of the rains, and after about 22 months of gestation most young are born during the onset of the rains (Kingdon 1979, Dublin 1983)(Fig. 4.3).

Young born before the onset of the rains, or after the rains, may suffer higher mortality due to the need to trek about 20 miles per day between food and water (Laws et al. 1975, Kingdon 1979). Young born during the peak of the rains may be threatened by exposure (Dublin 1983). Thus, one may expect female (and male) African elephants to compete for breeding opportunities during optimal times (Dublin 1983). The pattern of rains determine the environmental fitness function for African elephants.

The ability of a musth male to monopolize the matings of a group of female African elephants likely varies with local circumstance. Where several males compete to breed with a female, copulations can occur while the musth male is occupied with competitors (Moss 1988). At other times and locations, matings are uncontested or males are successful in defending females (Kingdon 1979, Moss 1988). It is at these times and locations that the TMPM may be applicable.

For a Tsavo West National Park population, Dublin (1983) suggested that female African elephants have limited access to males, and must compete for
Figure 4.3. Temporal distribution of births by African elephants, *Loxodonta africana*, in Tsavo West National Park, Kenya, as related to rainfall. Shaded bars, dominant females; open bars, subordinate females; dashed line, average rainfall. (Redrawn from Dublin 1983.)
breeding opportunities. Competition for breeding opportunities by female African elephants may take the form of induced infertility through social aggression (Dublin 1983), and also the solicitation of copulations from a male even when he is engaged in sexual activity with another female (e.g. Moss 1988).

In Tsavo West, subordinate females must contend with monopolization of the bull by the dominant female during the optimal time, temporary losses of his libido after copulation, and possibly reduced fertility following multiple copulations (Dublin 1983). Although subordinate females are free to contest the dominant female, rather than incur the polygyny costs associated with this, most choose to mate at less optimal times (Fig. 4.3).

This breeding behavior, and the resultant temporal patterns of reproduction in Tsavo West, are consistent with the predictions of the TMPM. The data are insufficient for conclusions, but they do suggest that the TMPM is worthy of study in this and similar polygynous mating systems. It may explain asynchronous mating patterns, regardless of cyclical optima due to environmental conditions.

A corollary worth mentioning is found in the temporal pattern of reproduction of male African elephants. Their seasonal peak time of musth coincides with the seasonal peak time of female receptivity (Poole 1987, Moss 1988). Younger males come into musth during less advantageous (off peak) times (Poole 1987, 1989a, Moss 1988). This pattern allows many younger males to avoid the costs associated with competing with large musth males (Poole 1989a, Moss 1988). This is potentially another type of temporal-threshold where costs of male-male competition are avoided by mating at less advantageous times.
Angelfish

Lunar or semilunar spawning periodicity is thought to be advantageous for coral-reef fishes that produce pelagic eggs or larvae. Major advantages are thought to be increased survival of eggs and larvae (Johannes 1978, Lobel 1978a), or increased dispersal (Barlow 1981, Ross 1983), both associated with cyclical light and tidal-current patterns. Several other hypotheses of reproductive advantage to lunar or semilunar spawning are reviewed by Robertson et al. (1990). Regardless of the identity of the advantage, the breeding period appears to track the environmental period of lunar or semilunar cycles for many coral-reef fishes (Johannes 1978, Robertson et al. 1990). Any cyclical advantage may yield a fitness function that varies periodically through time.

For coral-reef fishes that produce pelagic eggs, individual males of certain species may pair-spawn with tens of females per day (Ross 1986, Warner 1987). Costs in male fertility, likely due to sperm depletion or viability, have been demonstrated from multiple pair-spawning of a polygynous freshwater fish (Nakatsuru and Kramer 1982). It seems plausible that many coral-reef fishes may also incur this polygyny cost. In coral-reef fishes that lay eggs in nests but still produce pelagic larvae, polygyny costs may involve a higher probability of cannibalism by parental males on more recent clutches of eggs (Rohwer 1978, Pressley 1980). These are examples of polygyny costs that may be incurred by female coral-reef fishes that mate with an already-mated male.

Given these conditions, the TMPM ought to be applicable whenever a group of females mate primarily with a single male. For example, pygmy angelfishes of the genus Centropyge live in stable harems consisting of one male.
and several females, and the harem females breed almost exclusively with their male (Moyer and Nakazono 1978, Moyer and Zaiser 1984, Lutnesky and Kosaki, unpublished data).

Potter's angelfish, *Centropyge potteri*, is known to occur only around the Hawaiian islands and Johnston Atoll (Randall et al. 1985). Its mating behavior is pertinent to the predictions of the TMPM. Spawning by *C. potteri* was originally described by Lobel (1978a). Males pair-spawn at dusk with females (producing pelagic eggs) with whom they share a territory. Spawning may occur daily starting eight days prior to full moon, and continue until full moon (Lobel 1978a). If a female spawns, she spawns only once per night. Natural harems consist of one male and from one to eight females (Lobel 1978a, Lutnesky 1989).

I observed captive *C. potteri* spawning in a tidal-pond environment that is described elsewhere (Lutnesky Chapter III). Spawning was observed in two harems during a single lunar month (Fig. 4.4). The harems of eight and fifteen females were comparatively large (Lutnesky Chapter III). The spawning data are consistent with the predictions of the TMPM (Fig. 4.4). For example, spawning in these large harems was almost continuous over the entire lunar month, but peak spawning activity occurred during the 8-day period preceding the full moon.

Female *C. potteri* also appear to compete for access to the male. Females may follow the male as he courts other females, drive other females away from the male, or place themselves (often in a spawning posture) between the male and a female he is courting (Lutnesky and Kosaki, unpublished data).

The spawning data are different from the simple TMPM in that females spawned more than once during the environmental period. Under certain
Figure 4.4. Percentage of females spawned in large harems of the angelfish *Centropyge potteri* during April 1989. Data before arrow are observations of a harem of one male and eight females; data after the arrow are observations of a harem of one male and 15 females. Number of females spawned per day ranged from zero to seven. Peak spawning occurred during the time for which the species is described as being a periodic spawner (thickened abscissa; Lobel 1978a), but was nearly continuous. F, full moon; N, new moon.
conditions, this does not influence the predictions of the TMPM. The effect of
number of single-spawning females for the simple model, and total number of
spawns for multiple-spawning females, are interchangeable if refractory periods
(the time needed for females to become ready to mate again) are less than a
single time interval. A female with several decisions is expected to place her
matings around $F_{140}$. She maximizes her fitness as might an equivalent group
of single-spawning females. The basic predictions of the TMPM hold.

If refractory periods are one or more time intervals, however; predicting
optimal mating patterns for individuals is not intuitive. For example, a female
that mates twice during an environmental period, but must wait several intervals
before mating again, could achieve the same fitness choosing different temporal
patterns. Two off-peak matings may be the equivalent of a peak mating
combined with one at a poor-quality time. This problem is avoided in the $C.\ potteri$ example because an individual female can mate daily for up to 21 days
(Lutnesky and Kosaki, unpublished data). They should choose every high-
quality time interval to which they are suited.

These data do not constitute a crucial test of the TMPM, but they suggest
the possibility of a temporal-threshold mechanism during polygynous mating.
Tests of the TMPM appear to be worthy of pursuit in this and similar mating
systems.

Spawning periodicity in $Centropyge$ is controversial (Lobel 1978a, Bauer
provide insight. It predicts that studies in various habitats that differ in harem
sizes, and in amplitude of variation in environmental conditions, would yield
variable results. A generalization can be made from these studies, however; at
least one female in a harem will spawn each day (Bauer and Bauer 1981,
Thresher 1982) except during presumed environmentally disadvantageous times (e.g. dusk slack tide, Thresher 1982).

Limitations and Tests of the TMPM

The TMPM is an unlikely mechanism in polygynous mating systems where females have easy access to unmated males. The within-group reproductive decisions in the TMPM would be irrelevant in such systems. Females could simply avoid costs by choosing unmated males at the optimal time. The TMPM relates better to mating systems where a female's access to males is limited. Haremic mating systems are prime candidates for temporal-thresholds because the movement of females between social groups may be difficult.

Another limitation of the simple model presented here is that it is only appropriate in systems where the cost of polygyny is recovered between time intervals, i.e. opportunities to breed. In systems where the cost of polygyny is carried to subsequent time intervals, the appropriate strategy would be much more complex. In general, time intervals early in the environmental period would be preferred as the polygyny cost accrued in later intervals. The best time to mate would depend on the rate at which costs accrue, the number of females, and their behavior.

As described for the C. potteri example, the TMPM may be applicable in mating systems where females breed more than once during an environmental period. Yet the model would not apply if all females in a group bred during every time interval, and had a set breeding order. Subordinate females could never recover the cost of polygyny in such systems, and the TMPM would have no application.
An additional limitation may be found in habitats with very large amplitude of the fitness function. If large enough, i.e. fitness falls to zero, or an "unacceptable" level, continuous-mating thresholds may not be reached. Rather than incurring polygyny or environmental costs at unacceptable levels, a female may make the best of a bad situation by skipping reproduction during that cycle. She may save her resources for future investment, or in the case of relatives she may help with the young of others (e.g. African elephants, Dublin 1983).

The TMPM has the potential for rigorous testing. A test of the TMPM should involve some demonstration of a polygyny cost, and manipulation of female group size in a single habitat. Testing in a single habitat ensures that different groups are experiencing the same environmental cues. If there is a temporal-threshold during polygynous mating, groups of different sizes should have different temporal patterns of mating, in the manner predicted above.

Small, haremic animals seem the best candidates for manipulation. For example, harems of pair-spawning coral-reef fishes can be experimentally decreased or augmented to investigate the effect of group size on temporal patterns of mating. *C. potteri* is presently being studied for such effects. Studies of many different animals are needed to determine if females within social groups offset polygyny costs by mating at less advantageous times.

**CONCLUSIONS**

The temporal-threshold model of polygynous mating is a general model that shows how patterns of mating through time may be structured by female-female competition in some species in polygynous mating systems.

Data from animals as diverse as African elephants and Hawaiian angelfish show the model has potential for broad applicability, and that studies which
examine polygynous mating systems for evidence of temporal thresholds are worthy of pursuit.
CHAPTER V
A TEST OF THE TEMPORAL-THRESHOLD MODEL
OF POLYGYNOUS MATING

INTRODUCTION

Male-male competition and female choice are primary focal points in the study of sexual selection. Female-female competition and male choice are much less thoroughly studied (Smuts 1987). A good reason for this is that the first two are usually the primary components of sexual selection due to the differential investment in young by the sexes (Darwin 1871, Trivers 1972, 1985, Smuts 1987).

Males are usually expected to compete for mating opportunities because their reproductive success is limited by the number of females whose eggs they can fertilize, whereas passivity is usually expected in females because they are usually not considered to be limited by male sperm production (e.g. Bateman 1948). Females increase their reproductive success through choice based on the discrimination of a combination of the genetic quality of males, and also the quality of resources they hold (Trivers 1972, 1985), i.e. their breeding-situation quality (Wittenberger 1981a).

Female-female competition, however, may determine the reproductive success of many females. For example, in polygynous mating systems females may be expected to compete for mating opportunities (Lutnesky Chapter IV). When several females share a male, they may have to compete for a share of his sperm production, the food, shelter, or breeding site he may defend, his parental care, opportunities to breed at reproductively advantageous times, or

Loss of fitness due to competition and division of the male's resources can be considered the costs of polygyny. The benefits of polygyny are found in the relative choices a female must make between males, the resources males hold, and the number and quality of other females that share the male (see Searcy and Yasukawa [1989] for a recent review).

The premise of the temporal-threshold model of polygynous mating (TMPM) (Lutnesky Chapter IV) is that female-female competition, a lesser studied component of sexual selection, plays a large role in structuring temporal patterns of mating in some polygynous animals. Male-male competition and female choice may determine who mates with whom, but the TMPM asserts that the results of female-female competition may determine when matings occur and thus potentially also their relative success. Female-female competition may therefore be more important to sexual-selection processes than previously thought.

Stated simply, the TMPM assumes that continuous mating through time would yield a fitness function that varies due to cyclical environmental factors, e.g. diel, lunar, or seasonal conditions, depending on the scale examined, that make some intervals of the cycle more advantageous for breeding (Lutnesky Chapter IV). If all the females in a polygynous mating system choose the same time interval to breed, e.g. the optimal time due to environmental conditions alone, their average fitness could decrease due to accrued polygyny costs (Lutnesky Chapter IV). The TMPM asserts that females can offset these polygyny costs by choosing to mate at times for which fewer females compete.
The purpose of this chapter is to test the specific prediction of the TMPM that polygynous females will compete for breeding order, and that synchrony in mating will decline with female group size as a result of this competition. The basic idea is that females disperse their mating to avoid the polygyny costs associated with female-female competition (Lutnesky Chapter IV).

METHODS

System

The pomacanthid angelfish *Centropyge potteri* was used to test the TMPM. *C. potteri* is endemic to the Hawaiian islands and Johnston Atoll (Randall et al. 1985). It is territorial (Lutnesky 1989, Chapter III), and lives in harems that consist of one male and from one to eight females (Lobel 1978a, Lutnesky 1989, Chapter III).

Lobel (1978a) made the original description of spawning, and its temporal pattern. At dusk, a male spawns with only one female at a time, with the females of his harem. Pelagic eggs are broadcast; no parental care is given. Spawning mostly occurs between December and June (Lobel 1978a), but can also occur in other months (Lobel 1989, M. Lutnesky personal observation). Each month, spawning may occur daily starting 8 days prior to full moon, and continue until full moon (Lobel 1978a, but see Lutnesky Chapter IV, and below). Females only spawn once per night (Lobel 1978a, M. Lutnesky personal observation).

During courting and spawning, female *Centropyge* may exhibit agonistic behavior toward one another (Bauer and Bauer 1981, Moyer 1981, Lutnesky Chapter II). Preliminary observation indicated that *C. potteri* females "interfere" with the courting and spawning of other females in their harem.
Direct interference consists mostly of a female placing herself, often in the tail-up spawning posture (Lutnesky Chapter II), between the male and the female he is courting, but it can also include driving other females away from the male, and biting and chasing them (Lutnesky Chapter II). Indirect interference involves a female "soliciting" the male while he courts another female. She adopts the tail-up spawning posture in midwater near the place the male is courting another female. The male often leaves the female he is courting and spawns with the posturing female (Lutnesky Chapter II).

Given that C. potteri was described as a periodic spawner for a daily temporal pattern of mating, the TMPM could be tested in this time scale over a reasonably short time period.

Simultaneous observations of females living in harems of different sizes, but in the same habitat, are needed to properly test the model (Lutnesky Chapter IV). Preliminary data (Lutnesky Chapter IV) indicated that females spawned in enclosures set in a tidal pond (Lutnesky Chapter III). Enclosures in the tidal pond provided a semi-natural environment; the fish were exposed to natural physical stimuli (e.g. light, tide, current, weather, etc...), some of which may be cues for spawning.

Seven floating enclosures were constructed. The enclosures were made of wire mesh (12.7 mm²), and were suspended at the surface with styrofoam floats. Six were 1.8 m long x 0.7 m wide x 1.5 m deep, and the seventh was 4.0 m x 1.1 m x 1.5 m. Each of the smaller enclosures housed one male and one female, and the larger enclosure housed one male and six females. All enclosures provided the same 0.9 m³ of water per fish.

The six smaller enclosures were tied together by their long sides, with an opaque plastic sheet attached between each enclosure. The plastic sheet served
as a visual barrier between enclosures. This set of six enclosures was placed on one side of a deck (5.6 m long x 3.5 m wide) that sits on concrete pillars about 1.3 m above the water given average tidal fluctuation. The large enclosure was placed on the side of the deck opposite from the set of six enclosures. A visual barrier between the set of six enclosures and the other enclosure was formed by an oyster-encrusted screen that serves as a fish barrier in the tidal pond.

From the vantage point of the deck, one has an unobscured view into either the entire set of six enclosures, or the large enclosure. The enclosures were allowed to accumulate algae for at least a month before fish were added to the system. C. potteri feed on the algae that naturally grows on these enclosures (Lutnesky Chapter III).

Using two observers, the design was to make observations of the time of spawning, or the lack of spawning, that occurred for each of the paired and harem females each day over an entire lunar month. In this way the TMPM could be tested in a daily time scale. Although a single lunar cycle does not allow a formal test of the model in a monthly time scale, the data give insight into the problem, and allow for some discussion concerning this time scale. Formal analyses, however, are limited to the first 21 days of the sample because on the twenty-second day the largest female in the harem was found dead with a traumatic head injury of unknown origin. The largest female in a harem likely plays an important role in female-female competition.

To avoid interobserver bias, we collected data only on unambiguous behavior patterns, we made preliminary observations together to "calibrate" our definitions, and had periodic reviews to avoid drift in our assessments. The
haremic females could be distinguished by size and fin clips; paired females were also fin clipped to avoid any associated bias.

Fish were captured and sexed as described in Chapter II. The six females that went into each treatment were roughly matched for size, i.e. two small (\( \leq 60 \text{ mm} \)), two medium (\( 60 \text{ mm} < x < 66 \text{ mm} \)), and two large (\( > 66 \text{ mm} \)) females for each treatment. Males were always larger than the females with which they were haphazardly placed. The fish were given about two weeks to adjust before data collection began. Data were collected from 7 April to 2 May, 1990.

We could not simultaneously observe more than 12 individually-known females because of limitations in equipment and trained personnel. Although we had six paired females and six haremic females for comparison, all haremic females were in the same harem. For this reason we make no claims beyond this single harem for this experiment.

The a priori design in testing the model was to construct an artificial system in which the predictions of the TMPM ought to hold, if the animals are capable of behaving by such "rules." The data are useful to determine if \textit{C. potteri} in our experimental harem behaved by these rules, and also to determine if the TMPM is potentially useful in the study of natural systems. To this end, we have also collected some corroborative evidence from the field (see below).

Significant results are indicated by \( P < 0.05 \), and all statistical analyses follow the procedures given in Zar (1984).

Enclosures

Each afternoon observations began about 90 minutes before sunset. Time of spawn (minute) for all females, and time (minute) and frequency of
interference behavior for each haremic female were collected. To simplify data collection, all behavior described above as interference behavior was lumped.

As one measure of synchrony, the TMPM predicts that the variance in time of mating will be greater for larger groups of females (Lutnesky Chapter IV). To make comparisons valid, however, the calculation should first be adjusted for handling time, i.e. the time required for a male to mate with a female, and then be ready to mate with another female (Lutnesky Chapter IV). This ensures that the dispersion of matings through time examined is due to the choice by females of "available" time, not time waiting because the male is occupied with another female. An adjusted time of mating for the $i^{th}$ female is given by $g_i$, $i = 1, 2, ..., n$ (Lutnesky Chapter IV), where

$$g_i = A_i - \sum_{j=1}^{i} H_j.$$ 

$A_i$ is the actual time of mating for the $i^{th}$ female, and $H_j$ is the handling time for the $i^{th}$ female. The handling time of the $i^{th}$ female is also removed from her actual time of mating when calculating the adjusted time of mating if actual time of mating is measured at the end of mating, otherwise handling time would be summed from $j = 1$ to $j = i - 1$ instead of from $j = 1$ to $j = i$.

All C. potteri matings were assigned a handling time of one minute. This estimate is conservative because mating actually only requires a few seconds (Lutnesky Chapter II), and preliminary observations showed that males can mate with two females within the same minute.

The variance in adjusted time of mating was examined by calculating the mean adjusted time of mating over the 21 day sample for each female. The
variance in this mean adjusted time of mating between paired and haremic females was compared using a variance ratio test.

A second way to analyze the data was to simply use the variance in adjusted time of mating for each female as a variable in a Mann-Whitney test that compared paired and haremic females. That is, to see if not only the group of haremic females had a larger variance in adjusted time of mating, but also to see if a larger variance occurred as an individual response as well. The individual response is expected over several cycles because temporal thresholds occur in pairs, i.e. there are positions in time that yield the same average fitness on either side of the optimal time to mate due to cyclical environmental conditions (Lutnesky Chapter IV). When the best position is taken (first female during the optimal time interval), a haremic female could achieve the same fitness by choosing several positions of equal average fitness (Lutnesky Chapter IV).

A Wilcoxon paired-sample test was used to examine if the mean rate of interference per day was significantly different for the haremic females before versus after each interfering female spawned. Additionally, Kruskal-Wallis and nonparametric multiple comparison tests were used to examine if mean interference per day was significantly different for the individual haremic females.

Two types of mean spawning order among the haremic females were each examined by Kruskal-Wallis and nonparametric multiple comparison tests. The first type was sequential spawning order, and was based on who spawned first during the day. The second type was spawning order relative to optimal environmental conditions. Paired females were assumed to track only environmental cues for spawning due to their social isolation. Their average
time of spawning was assumed to reflect an optimal environmental time. Haremic females were thus examined to see if there was a nonrandom order of spawning relative to this "optimal" time.

Field

Because the behavior of largest female in the harem appeared to be so important in structuring the daily pattern of spawning in the experimental harem, I designed an *a posteriori* test to see if the behavior was an artifact of the system. Again, two observers were used.

During May - July, 1990, we made eight dives on five separate days (Lutnesky Chapter II) at a depth of about 16 m offshore of Kaneohe Bay, Oahu, Hawaii. We were at the study site at least 45 minutes before sunset, and usually stayed until about 20 minutes after sunset. We observed a total of seven harems, each containing one male and on the average 4.0 ± 0.2 (SE) females.

Females with which the male interacted were designated as his harem because females rarely interact with males outside their harem (Lutnesky, unpublished data). The largest female could be identified based on her size and markings; all other females in the harem were lumped together as "other" for these short observations.

Using a Wilcoxon paired-sample test, a comparison of mean interference per day between the largest female and "other" females was made. This data would help us determine if largest females exhibit significantly more interference behavior than the other females combined, and are thus integral to the formation of temporal spawning patterns.
RESULTS AND DISCUSSION

As predicted by the TMPM, the variance in mean adjusted time of spawning was greater for haremic females than for paired females (Fig. 5.1, \( P < 0.05 \), variance ratio test).

The largest female in the harem (hereafter referred to as the alpha female) appeared to track the same environmental cues as the paired females (Fig. 5.2). The spawning pattern of the paired females is suggestive of an asymmetrical fitness function (Lutnesky Chapter IV). Peak spawning occurs about 0.5 h before sunset, but falls off rapidly (Fig. 5.2).

Subordinate females in the harem spawned in time periods long before the alpha female spawned, and after the alpha female spawned (Fig 5.2). This caused the individual variance in adjusted time of mating to be greater in subordinate haremic females than in paired females (Fig. 5.1, \( P < 0.05 \), Mann-Whitney test). Although the sample sizes generating the variances (see below) are not equal for all females, sample variance is a measure of dispersion unbiased by sample size (Sokal and Rohlf 1981).

Interference behavior appears to provide a secondary polygyny cost that subordinate females may be avoiding by dispersing the temporal pattern of mating. It is secondary in that the females appear to be fighting over a primary polygyny cost(s) that has not been identified. All haremic females exhibited interference behavior, and it occurred almost exclusively before the interfering female spawned (Fig. 5.3A, \( P = 0.025 \), Wilcoxon paired-sample test).

Interference behavior was performed predominantly by the alpha female (Fig. 5.3B). She interfered significantly more often than any of the subordinates, none of whom were significantly different from each other in this
Figure 5.1. Mean adjusted time of mating for *Centropyge potteri* in a daily time scale. Closed circles indicate individual means; open circles indicate grand mean of individual means. Harem females use more of the environmental period for mating than do paired females. Error bars show standard deviation. Females in each treatment are arranged by size rank, the largest female at the left. Thickened ordinate shows range of sunset during observation. $A = \alpha$ female.
Figure 5.2. Frequency of spawning in ten-minute time intervals. The paired females and the alpha haremic female show similar patterns of spawning.
behavior (Fig. 5.3B, $P < 0.001$, Kruskal-Wallis test, and nonparametric multiple comparison tests).

The frequency of the alpha female's interference behavior significantly increased until she spawned (Fig. 5.3C, $P < 0.0025$, $r_s = 0.89$, Spearman rank correlation). As indicated by the general pattern, after spawning her interference behavior was negligible (Fig. 5.3C).

Interference behavior often disrupted the courting and spawning of females. The alpha female would even occasionally break-up nuzzling behavior (Moyer and Nakazono 1978, Lutnesky Chapter II) moments before a spawn. Smaller females usually moved off readily, but occasionally they were chased or bitten.

Polygyny costs could occur because of interference behavior. A female could be injured if it chose to directly contest a larger female for a mating opportunity. Additionally, a polygyny cost may occur if the highly coordinated spawning behavior and gamete release is disturbed by interference such that a low fertilization rate occurs. A simple way for the subordinate females to avoid these potential costs is to spawn at a less-contested time. This is what the TMPM predicts, and it also appears to be what the females in this experiment did.

The field data also indicate that the alpha female is the predominant user of interference behavior. Alpha females interfered more than all other females in their harems combined (Fig. 5.4, $P < 0.01$, Wilcoxon paired-sample test).

Given the experimental results, and the field data, alpha females may be important in the determination of temporal patterns of mating in C. potteri. Although there are likely several other polygyny costs, it appears that interference behavior is an important source of polygyny cost in this system.
Figure 5.3. Haremic females interfere when other females are courted by the male. A) Interference occurs before the interfering female has spawned. B) All females interfere, but the largest female (alpha female) interferes significantly more than the other females. The other females show no significant differences in interference behavior. C) The alpha female's interference rate significantly increases until she spawns, and then becomes negligible (open circle). All error bars indicate standard error of the mean.
Figure 5.4. Field data (n = 7 harems) shows that alpha females interfere significantly more than all their other harem members combined. Error bars indicate standard error of the mean.
OFFSHORE INTERFERENCE DATA

Mean Interference / Day

0 1 2 3 4

Alpha female Other females
The TMPM indicates that if females are equally competitive they will form an ideal-free distribution (Fretwell 1969, 1972) as they disperse their mating to avoid polygyny costs. If dominant females can exclude subordinate females, however, an ideal-despotic distribution (Fretwell 1972) is more likely (Lutnesky Chapter IV). Dominants should choose the optimal environmental time interval while subordinates should choose less advantageous time intervals.

The haremic females showed no significant differences in sequential spawning order (Fig. 5.5A, Kruskal-Wallis test). There was, however, a significant difference in spawning order relative to the optimal time (Fig. 5.5B, P < 0.025, Kruskal-Wallis test). The alpha female is closest to the optimal time, but nonparametric multiple comparison testing showed that only she and the second largest female were significantly different on this index.

Subordinate females sometimes spawned within a minute or two after the alpha female and thus sometimes spawned near the presumed optimal time. Additionally, smaller females would sometimes "intercept" the male through indirect interference just before the alpha female spawned.

These tactics sometimes allowed subordinates to successfully compete with the alpha female. Spawning before the alpha female carries a high risk of interference, but whether or not spawning directly after the alpha female incurs other costs such as sperm depletion is unknown.

One might expect a correlation between size rank and spawning order (either index) if larger females were capable of excluding smaller females from advantageous time intervals. No correlation exists (Fig. 5.5). Additionally, both the experimental harem and field data indicate that only alpha females have a high-interference tactic. Perhaps this is because direct interference is the most
Figure 5.5. Spawning order as related to size rank. A) No significant differences occurred for females in sequential spawning order. B) There was a significant difference in spawning order relative to optimal time. The largest (alpha) female was the closest to the optimal time, and significantly different from the second to the largest female. The alpha female was largely unsuccessful in excluding other females from the optimal time. Error bars indicate standard error of the mean.
common tactic, and most often directed toward a smaller female. Whatever interference behavior subordinates may employ among themselves, they must still contend with the alpha female during the optimal time interval. Perhaps this makes a high-interference tactic unprofitable for all but the alpha female.

So what does an alpha female gain from a high-interference tactic? Alpha females may not gain exclusive access to the optimal time interval, but it appears that the shift of subordinates away from the optimal time interval may result in fewer competitors at that time.

The cost of interference behavior for an alpha female is probably very small. Both the experimental harem and field data indicate that alpha females are rarely challenged when they interfere. It is likely that subordinates simply move off because in the most extreme cases there may be close to an order of magnitude difference in wet weight between the smallest female and the alpha female. The rare "challenges" observed were passive in that the subordinate moved away so slowly that the alpha female chased or bit her. It thus appears that the interference behavior carries little risk for the alpha female; most of it consists of simply displacing smaller females that readily move away.

Spawning occurred almost continuously throughout the lunar month in both the paired and haremic female treatments (Fig. 5.6). However, both treatments did appear to follow some environmental cue. A lull in spawning appeared to occur independently in both treatments just after full and new moon (Fig. 5.6).

Although the sample covered only one lunar cycle, the paired females used almost the entire environmental period. Thus it appears that the haremic females could not achieve less synchrony by dispersion of temporal mating patterns in a monthly time scale.
Figure 5.6. Spawning was nearly continuous in both paired and haremic treatments over a lunar month, but was less frequent after full (open circle) and new (closed circle) moon.
April 1990
Harem females were, however, less synchronous than paired females (Fig. 5.6). The harem females appeared to solve the synchrony problem in a different way: subordinate females spawned significantly less often than the paired females (Fig. 5.7, $P < 0.005$, Mann-Whitney test). Although the alpha female spawned all 21 days, the subordinates ranged from only 3 - 9 days while the paired females ranged from 9 - 15 days (Fig 5.7).

It is a common phenomenon among mammals that aggressive interactions between females reduces the number of offspring produced by subordinate individuals (Dunbar 1980, Dublin 1983, Wasser 1983, Smuts 1987). Whether or not fewer eggs were produced by the subordinate females in this study, or simply the same number of eggs produced with fewer spawning acts, is unknown. In releasing the same number of eggs, there is a negative correlation between number of spawning acts and number of females present in a polygynous freshwater fish, *Hyphessobrycon pulchripinnis* (Burt et al. 1988).

Perhaps numerous uncontested spawning acts, with fewer eggs per spawn, increases the probability of fertilization. This would make a continuous spawning tactic (Bauer and Bauer 1981) advantageous if it outweighed the potential change in fitness due to changes in environmental conditions over the lunar month at this study site. Only the harem alpha female, however, employed a continuous spawning tactic. All other females had short or long breaks in spawning activity.

A potentially fruitful area for future study may be whether the interference behavior and spawning tactics of alpha females constitutes mate guarding. The problem is confounded by protogynous sex change, however, and how this may also influence a female’s interactions with the male and her frequency of spawning (Moyer and Zaiser 1984; Lutnesky Chapter III).
Figure 5.7. Subordinate haremic females spawned significantly less often than paired females. Error bars indicate standard error of the mean. Boxes indicate range. Number of spawns for the alpha female in the harem is shown for comparison.
Alpha female — •

Number of Spawns

Pairs

Harem (Subordinate females)
CONCLUSIONS

The predictions of the TMPM hold up well for Centropyge potteri in a daily time scale. Females compete for mating order as indicated by their interference behavior. Competition results in subordinate females often competing for less contested time intervals, and thus haremic females spawned less synchronously than paired females. The interference behavior, predominantly by the alpha female, appears to be an important polygyny cost and may structure temporal patterns of mating in the daily time scale.

Over a single lunar month, haremic females spawned less synchronously than paired females, but not through dispersion of the temporal pattern of mating. Instead, subordinate females spawned less often than paired females. Tests of the TMPM are needed in this time scale, but it is unlikely that the complexity of temporal patterns of mating in this time scale are fully accounted for by the TMPM.
CHAPTER VI
CONCLUDING REMARKS AND FUTURE RESEARCH

Overall, the results presented in this dissertation indicate that the pomacanthid angelfish, *Centropyge potteri*, is an excellent study animal to explore problems in behavioral ecology. Its polygynous mating system, nonpermeable social groups (Ross 1990), territories that are often compact, and readily identifiable sexes make it an ideal candidate for observation in both the field and under the more controlled conditions of an enclosure study. This study demonstrates the importance of using a complimentary approach, utilizing both field and laboratory techniques, in the study of problems in behavioral ecology.

The only major problem in using *C. potteri* is that scuba divers must often work in fairly deep water, and in locations that may have strong currents. Studies in diverse habitats are important to fully understand reproductive strategies in *C. potteri* and other coral-reef fishes, but also may be hazardous to the researcher. Studies are often biased because samples are only taken in the range of habitats in which it is safe to work. It is important to be aware of this bias. For example, the findings of this study would be much different had the sampling been limited to habitats inside of Kaneohe Bay where *C. potteri* lives under low-density conditions in shallow and calm water. This is one reason I am so grateful to my dive buddies (see Acknowledgements) for helping me explore the sometimes rough water offshore of Kaneohe Bay.

The descriptions of sexual dimorphism, protogynous hermaphroditism, and social behavior of *C. potteri* provided in Chapter II set a foundation for the
dissertation, and future studies of morphology and behavior that utilize *C. potteri*.

The results of Chapter II open an interesting avenue for studies of morphology and color pattern. Analyses using the color-pattern index indicate that males and females significantly differ in color pattern; males have proportionally more dark blue pigment on their sides. Casual observation suggests that this is true within any population, but there may be differences between populations. For instance, females inside of Kaneohe Bay appear more "masculine" in color pattern than do females either offshore of Kaneohe Bay, or at Portlock. This would be simple to investigate quantitatively.

The most interesting point concerning future research on color pattern, however, is that populations that appear to differ in color pattern also appear to differ in social organization. Offshore Kaneohe Bay and Portlock are higher-density populations characterized by larger harems and smaller territories, while inside of Kaneohe Bay are lower-density populations characterized by smaller harems and larger territories. Could it be that color pattern is related to social organization and encounter rates? Might the relative differences in stimulatory and inhibitory stimuli (Lutnesky Chapter III) influence how "male" or "female" individuals appear? The problem is complex, however, as differences in habitat or the condition of the fish may also influence color pattern. This problem could be investigated using controlled enclosure studies.

The data and arguments presented in Chapter III show that fish density is important to the proximate cues that trigger sex change in *C. potteri*, and that fish density is also potentially important to the sex-change processes of other territorial-harem fishes. Social-group composition is known to be important in the induction of sex change (Shapiro and Lubbock 1981, Ross et al. 1983),
but the results in Chapter III show that for *C. potteri* the density of a social group may be as integral to the sex-change process as social-group composition. Together, social-group composition and density may influence proximate stimuli in a systematic way that allows one to predict the potential for sex change within a social group.

Of several hypotheses tested, the absolute encounter-rate threshold model was the only hypothesis to survive the experimental paradigm in Chapter III. This study is a good start, but similar studies are needed on other territorial-harem fishes. I do not expect that all of these sex-changing fishes will behave as did *C. potteri* because they come from such diverse phylogenies (e.g. Warner 1984).

Concerning further tests with *C. potteri*, the next step in testing the absolute encounter-rate threshold model is a field test. Addition of females to harems in low-density social groups should stimulate sex change in the alpha female by increasing the frequency of encounters that stimulate sex change, assuming the frequency of encounters that inhibit sex change is below threshold (Lutnesky Chapter III). The addition of females to high-density groups should not stimulate sex change because presumably stimulation through smaller-female encounter is already above threshold, but inhibitory stimuli through encountering the male is preventing sex change (Lutnesky Chapter III). One should carefully monitor territory borders when conducting such manipulations. Changes in territory size would alter encounter rates (Lutnesky Chapter III), and the manipulations may not have their intended effect.

Aldenhoven (1984) induced sex change by adding females to natural harems of *Centropyge bicolor*. She concluded that social-group composition
(harem size > 4) was implicated in the induction of sex change, but acknowledged "availability of space" might also have been important. In each case (n = 3 manipulations), the sex-changing fish started to use previously unused terrain in the formation of his harem. With an encounter-rate interpretation, one might say that it was not the addition of females alone that caused sex change, but the combination of added females (increased stimulation), and larger territory for the original male to cover (decreased inhibition) that allowed sex change to occur. Field manipulations in which encounter rates were monitored could resolve how sex-change stimuli are actually altered in a natural setting and further test the encounter-rate threshold (ERT) models.

So, if the absolute ERT is the operating mechanism and a female is in a high-density population where the level of inhibitory stimuli is above threshold, what does she do? She either does not change sex or she leaves if she is able. The latter point will probably make field manipulations of the sort discussed here difficult. Moyer and Zaiser (1984) discuss the difficulty of field manipulations with Centropyge.

Casual observation suggests that social-group organization is fairly homogeneous within patches of habitat occupied by C. potteri territories. One does not find a male with a harem of two females next to a male with a harem of eight females. Perhaps one could add "potential for sex change" to the components of breeding-situation quality (Wittenberger 1981a, Lutnesky Chapter IV) when discussing why females choose certain males/territories for territorial-haremic fishes that change sex. Although rare in adults, movement of fish between territories could lead to this homogeneity. I have already mentioned juveniles moving between territories (Lutnesky Chapter II), but I
have also seen adults (3 occasions) swim across a reef only to be successively chased by the residents they encounter.

The most exciting aspect of future research indicated by Chapter III is the potential use of encounter rates in the study of general sexual plasticity in teleosts. It appears that sexual plasticity in juvenile teleosts may be the rule rather than the exception (Francis 1992). If sex determination and protogynous sex change are the same process occurring during different times in the ontogeny of a fish (Francis 1992), much of what has been learned about sex change can be applied to the more fundamental problem of sex determination.

Encounter rates could be used to assess the relative levels of stimulation (encounter smaller) and inhibition (encounter larger) experienced by undifferentiated individuals. If protogynous sex change and sex determination are similar processes, encounter rates and sex determination are probably correlated. In species or situations where it might be advantageous to be a certain sex, encounter rate data can be analyzed to see if individuals take encounters randomly, or actively seek or avoid certain encounters. There are many variations on this theme, and the ERT models form a starting point.

The temporal-threshold model of polygynous mating (TMPM) in Chapter IV is a general model that may be useful in explaining why females in polygynous mating systems choose to mate at certain times. It is exciting because of its potential for broad applicability. Additionally, female-female competition has been studied much less intensively than male-male competition, but under the conditions of the TMPM female-female competition may be an important component of sexual selection (Lutnesky Chapters IV and V). The specific
predictions of the TMPM should allow testing of the model in diverse mating systems (Lutnesky Chapter IV).

One test of the TMPM (Lutnesky Chapter V) shows that haremic female \textit{C. potteri} do compete for reproductive access to their male each day. Subordinate females move away from presumed optimal environmental conditions near dusk, and mate at times that would not be expected in the absence of female-female competition. Subordinate females appear to avoid agonistic encounters with the largest female of the harem by mating at times she defends less vigorously. The resource over which the females fight was not identified in this study. The fighting itself must be considered a secondary cost to the loss of this resource. It could be the fertility of the male, a higher probability of being eaten by predators if not spawning near dusk (the presumed optimal time), or other factors. Although the behavior of \textit{C. potteri} females in this study is consistent with the predictions of the TMPM, a study which identified the primary resource would help clarify female-female competition in this species.

It is my hope that someone working with birds or mammals will be interested in testing the TMPM. The model can also be used as a template for similar situations where fitness costs due to physical factors are balanced against fitness costs due to social factors. There are probably many kinds of temporal thresholds. I have already alluded to how young male African elephants, \textit{Loxodonta africana}, may avoid the cost of male-male competition by mating at less contested, and presumably less advantageous, times (Lutnesky Chapter IV). In addition to this, the model may be useful in explaining temporal patterns of mating in polyandrous spotted sandpipers, \textit{Actitis macularia} (L. Oring personal communication). Here, some males could offset polyandry costs by mating at
less advantageous times. It should be apparent that the model has the potential for several variations on its original theme.
LITERATURE CITED


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