2008

Sexual Selection and Alternative Reproductive Tactics in Cyprinodon Elegans

Jennifer M. Gumm
Stephen F Austin State University, Department of Biology, gummj@sfasu.edu

Follow this and additional works at: http://scholarworks.sfasu.edu/biology
Part of the Integrative Biology Commons
Tell us how this article helped you.

Recommended Citation
http://scholarworks.sfasu.edu/biology/52

This Dissertation is brought to you for free and open access by the Biology at SFA ScholarWorks. It has been accepted for inclusion in Faculty Publications by an authorized administrator of SFA ScholarWorks. For more information, please contact cdsscholarworks@sfasu.edu.
SEXUAL SELECTION AND ALTERNATIVE REPRODUCTIVE TACTICS

IN CYPRINODON ELEGANS

by

Jennifer M. Gumm

A Dissertation
Presented to the Graduate and Research Committee
of Lehigh University
in Candidacy of the Degree of
Doctor of Philosophy

in
Integrative Biology

Department of Biological Sciences
Lehigh University
11/21/08
INFORMATION TO USERS

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleed-through, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

UMI Microform 3358104
Copyright 2009 by ProQuest LLC.
All rights reserved. This microform edition is protected against unauthorized copying under Title 17, United States Code.

ProQuest LLC
789 E. Eisenhower Parkway
PO Box 1346
Ann Arbor, MI 48106-1346
Approved and recommended for acceptance as a dissertation in partial fulfillment of
the requirements for the degree of Doctor of Philosophy

November 21st, 2008
Defense Date

Dr. Murray Itzkowitz

12-03-2008
Accepted Date

Committee Members:

Dr. Anthony Echelle

Dr. Tamra Mendelson

Dr. John Nyby

Dr. Jennifer Swann
I extend endless gratitude and appreciation to my graduate advisor Murray Itzkowitz for his guidance and encouragement throughout my time at Lehigh. In addition to challenging me and fostering my scientific abilities, his mentorship has prepared me to continue on in academia. My committee members Tony Echelle, Tami Mendelson, John Nyby, and Jennifer Swann also provided support and helpful comments throughout the course of this research. This research was funded by a Texas Academy of Sciences Student Research Grant, a Howard McCarley Student Research Award from the Southwestern Association of Naturalists, a Summer Research fellowship from the Lehigh University Graduate College, and a Sigma Xi Grant-in-Aid-of-Research. Additional funding was provided by a the Allied Pilots Association, a Section 6 grant from the Texas Parks and Wildlife Department to M. Itzkowitz and the Dexter National Fish Hatchery and Technology Center. I thank the Dexter National Fish Hatchery and Technology Center for providing me with training and technical assistance. Connie Keeler-Foster, Manuel Ulibarri and the staff of the DNFHTC played an integral role in the completion of this research. I also would like to thank the staff at Balmorhea State Park for their cooperation during field experiments and my field assistants: Raelynn Deaton, Chad Hargrave, Vance Imhoff, Joe Leese, and the students from Sam Houston State University. I thank the Lehigh biology department for support and all members of the Itzkowitz lab for years of friendship, camaraderie, collaborations and sanity. Finally, I am forever indebted to Becky Gumm and Vance Imhoff for their unending love and support.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Tables</td>
<td>vi</td>
</tr>
<tr>
<td>List of Figures</td>
<td>vii</td>
</tr>
<tr>
<td>Abstract</td>
<td>1</td>
</tr>
<tr>
<td>Chapter 1: <strong>GENERAL INTRODUCTION</strong></td>
<td>3</td>
</tr>
<tr>
<td>Chapter 2: <strong>HOW DO MALES EXPRESSING ALTERNATIVE REPRODUCTIVE TACTICS FIND MATES? DIFFERENCES IN FEMALE MIMIC AND SATELLITE MALE BEHAVIOR IN <em>CYPRINODON ELEGANS</em></strong></td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>9</td>
</tr>
<tr>
<td>Methods</td>
<td>12</td>
</tr>
<tr>
<td>Results</td>
<td>17</td>
</tr>
<tr>
<td>Discussion</td>
<td>18</td>
</tr>
<tr>
<td>Chapter 3: <strong>MULTIPLE RECOGNITION PROCESSES BY TERRITORIAL MALES IN A SPECIES WITH ALTERNATIVE REPRODUCTIVE TACTICS, <em>CYPRINODON ELEGANS</em></strong></td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>27</td>
</tr>
<tr>
<td>Methods</td>
<td>31</td>
</tr>
<tr>
<td>Results</td>
<td>35</td>
</tr>
<tr>
<td>Discussion</td>
<td>36</td>
</tr>
</tbody>
</table>
Chapter 4: **EFFECTS OF DENSITY AND SEX RATIO ON THE BEHAVIOR AND REPRODUCTIVE SUCCESS OF MALE *CYPRINODON ELEGANS* EXPRESSING ALTERNATIVE REPRODUCTIVE TACTICS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>46</td>
</tr>
<tr>
<td>Methods</td>
<td>51</td>
</tr>
<tr>
<td>Results</td>
<td>57</td>
</tr>
<tr>
<td>Discussion</td>
<td>62</td>
</tr>
</tbody>
</table>

Chapter 5: **CONCLUSIONS AND SUMMARY OF FINDINGS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>84</td>
</tr>
<tr>
<td>Appendix A</td>
<td>98</td>
</tr>
<tr>
<td>Appendix B</td>
<td>99</td>
</tr>
<tr>
<td>Appendix C</td>
<td>100</td>
</tr>
<tr>
<td>Vita</td>
<td>102</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 4.1 70
Microsatellite markers used to assess paternity in *C. elegans*. Annealing temperatures (TA) are shown for each locus.

Table 4.2 71
Treatment, sample size per treatment, number of embryos and mean reproductive success (measured as percentage of offspring sired). Means and range are shown.

Table 4.3 72
Correlations between number of offspring sired and male behaviors.
LIST OF FIGURES

Figure 2.1 25
Scatterplots and regression lines depicting relationships between spawning and a) female approaches, b) chases to female mimics, c) chases to satellite males, and d) chases to neighboring territorial males.

Figure 2.2 26
Mean ± s.e.m. number of females (light grey bars), female mimics (striped bars) and satellite/territorial males (dark grey bars) that approach each stimulus treatment per 5 minute observation period. There is a significant interaction between individual identity type and stimulus type.

Figure 3.1 44
Discrimination by territorial male C. elegans. (a) Time spent near the stimuli (b) Bites performed toward the stimuli. (c) Lateral displays performed toward the stimuli.

Figure 3.2 45
Strength of preference for stimuli across all treatments. Positive values represent increasing preference for female mimics (diagonal stripes) or large female (light grey). Negative values represent increasing preferences for large males (dark grey) or small females.
Figure 4.1
The natural log of the mean number of chases performed by small (white bars),
intermediate (light grey bars) and large (dark grey bars) in high and low density.
Error bars represent standard error of the mean.

Figure 4.2
The natural log of mean number of bites performed by small (white bars),
intermediate (light grey bars) and large (dark grey bars) in high and low density.
Error bars represent standard error of the mean.

Figure 4.3
The natural log of mean number of times small (white bars), intermediate (light grey bars) and large (dark grey bars) approached spawning mops in high and low density.
Error bars represent standard error of the mean.

Figure 4.4
The natural log of mean number of chases performed by small (white bars),
intermediate (light grey bars) and large (dark grey bars) in female and male biased
OSR treatments. Error bars represent standard error of the mean.

Figure 4.5
The natural log of mean number of bites by small (white bars), intermediate (light grey bars) and large (dark grey bars) in female and male biased OSR treatments. Error bars represent standard error of the mean.

Figure 4.6

The natural log of mean number of approaches to spawning mops by small (white bars), intermediate (light grey bars) and large (dark grey bars) in female and male biased OSR treatments. Error bars represent standard error of the mean.

Figure 4.7

Relationship between number of chases performed by males and number of offspring sired in (a) male biased OSR treatment, (b) female biased OSR treatment, (c) low density treatment and (d) high density treatment.
ABSTRACT

In systems with alternative reproductive tactics (ARTs), males have discrete behaviors and morphologies that allow them to optimize their reproductive success in relation to others in the population. Males with different phenotypes directly compete with one another for mates, providing unique opportunities for studying social interactions within a species. Using the Comanche Springs pupfish, *Cyprinodon elegans*, a species with three alternative reproductive tactics, I examine how male behaviors and social interactions influence the reproductive success of each tactic.

Previous studies suggest that large territorial males have high reproductive success, while satellite males and female mimics must intrude into territories in order to spawn. In a field study, I examined the social conditions that may facilitate satellite-male and female-mimic spawning. Males exhibiting these two tactics show different association patterns. Neither female mimics nor satellites preferentially associated with high-quality territorial males, but female mimics associated with females more than satellite males. Furthermore, unlike satellite males, female mimics rely upon deception of territorial males in order to garner matings. Territorial males express sex recognition of female mimics but this aggression towards female mimics is contextual, depending on presence of other potential threats or mates in a territory. Thus, the reproductive success of female mimics appears to depend upon the social environments.

To further examine the how alternative reproductive tactics correlate with reproductive success, I integrate behavioral studies with genetic techniques in a
laboratory based experiment to show that territorial males sire more offspring than satellite males or female mimics. Further, the relative success of satellites and female mimics was influenced by demographic parameters (density and sex ratio), although demographic variation did not alter male aggressive behavior or quantitative measures of sexual selection. This study is the first to quantify success of males expressing alternative reproductive tactics in this genus. The results suggest that the effects of dynamic social environments on male behavior and reproductive success may play a role in the maintenance of alternative reproductive tactics in this system.
I. Introduction

Alternative reproductive tactics are behaviors or morphologies expressed within a sex in order to increase an individual’s reproductive success (Gross 1996). When reproductive tactics are part of an alternative strategy, they are due to a genetic polymorphism and each tactic will have average equal fitness (isopods: *Paracerceis sculpta*, Schuster & Wade 1991a, 1991b; fish: *Xiphopohrus nigrensis*, Ryan et al. 1992; Andersson 1994; birds: *Philomachus pugnax*, Lank et al. 1995; Gross 1996; Shuster & Wade 2003). By contrast, alternative tactics that are part of a conditional strategy are far more common (Andersson 1994; Gross 1996; Avise et al. 2002; Shuster & Wade 2003). Conditional strategies are characterized by 1) genetic monomorphism for the ability to switch between tactics 2) a ‘choice’ by the individual as to what tactic to express 3) the individuals’ choice being relative to some aspect of their status 4) the chosen tactic resulting in higher fitness for the individual and 5) equal fitness between the tactics occurring only at the status value when they can switch between tactics (Repka & Gross 1995; Gross 1996; Andersson 1994).

Game theory provides a model for conditional strategies through status dependent selection (Gross 1996). Under this model, there are two tactics and the use of each by an individual is determined by fitness of each tactic and that individual’s status in the population. It is important to note that the ‘status’ of an individual is related to some phenotypic quality, such as age or size, but is determined by fitness resulting from social interactions. One tactic has higher average fitness than the other
when the individual has high status in the population (Gross & Repka 1998). At lower status, the other tactic has higher fitness. Therefore, a switch-point in tactics occurs at a certain level of individual status (Gross 1996). Despite one tactic conferring a lower average fitness, this system is evolutionary stable because individuals expressing the second tactic have higher fitness than they would if they were expressing the first tactic (Gross 1984). For example, an intermediate-sized male may have low status when interacting with a large individual but would have high status if interacting with a small individual. Given this definition of status, the switch-point between tactics is under selection and can vary between individuals, among populations or within a single population over time (Leiser & Itzkowitz 2003a; Tomkins & Brown 2004).

Examples of alternative reproductive tactics are particularly varied and widespread among fishes. A recent comparative phylogenetic analysis by Mank & Avise (2006) assessed the evolution of male alternative reproductive tactics in 296 species across 86 families of ray-finned fishes. Male alternative tactics evolved independently 26-43 times in this group and a significant correlation existed between the occurrence of male tactics and male sexually selected traits (Mank & Avise 2006). Taborsky (1994) defines two main types of males based on their reproductive tactic. 1) ‘Bourgeois’ males use the primary tactic to invest in and control the resource that is limited (e.g., spawning sites or females). 2) ‘Parasitic’ males employ a plethora of alternative tactics to steal fertilizations from a bourgeois male (Taborsky 1994). These alternative tactics include female mimics who morphologically and behaviorally resemble females, sneaker males who spawn simultaneously alongside a territorial male and female, satellite males who associate with the defended site of a
territorial male but remain outside or at the margin of the territory, and cooperative males who jointly build, defend or care for a nest (Taborsky 1994). The frequency of males performing alternative tactics can range widely with up to 85% of males in a population employing an alternative tactic (Gross 1982). Expressing alternative tactics is advantageous for many males that can not express the primary tactic because it is too costly. For example, males using the primary tactic may have exaggerated sexually selected traits, large body size, or energetically costly behaviors such as territory defense (Taborsky 1994). However, males using the primary tactic usually have the highest reproductive success as well (Kodric-Brown 1986; Taborsky 1994).

One common primary tactic in systems with alternative tactics is defense of a territory. A territory is a fixed area that individuals defend in order to control exclusive access to resources such as food, breeding sites, or mates (Kaufmann 1983; Krebs & Davies 1993; Bradbury & Vehrencamp 1998). Males defending territories use advertisement displays, threats and attacks to keep out neighbors and ‘floaters’ that intrude into their territory (Kaufmann 1983). Neighbors are other territory owners while floaters are non-owners that may try to usurp the territory. Interactions between owners and floaters are non-repeated and require immediate escalation, as these intruders may be trying to secure a territory of their own. In contrast, interactions with neighbors are repeated and become stable over time (McGregor 1993).

Female mimicry is a common alternative tactic in fishes. Males using female mimicry as an alternative tactic rely upon deception of the primary male for successful reproduction (Taborsky 1994). Female mimicry occurs in more than 30
species of fish across 10 families (Taborsky 1994). Female mimics often have morphological and/or behavioral characteristics of females in order to avoid aggression by larger territorial males (Taborsky 1994). If female mimics are common, it is advantageous for territorial males to identify these mimics. Accordingly, some evidence exists that males can distinguish females from female mimics (Taborsky 1994; Gonçalves et al. 2008). For example, in a European wrasse, *Symphodus ocellatus*, male nest owners discriminate between females and similarly sized female mimics (Taborsky et al. 1987).

Alternative reproductive tactics based on male size occur in more than 30 species of endangered pupfishes in North America. Large male Comanche Springs pupfish, *Cyprinodon elegans*, defend territories around spawning sites, intermediate-sized males act as non-territorial satellites, and small males are sneakers with female-like morphology (Leiser & Itzkowitz 2002, 2003a). Individuals expressing each tactic compete for mates, with territorial males spawning significantly more than satellite or sneaker males (Leiser & Itzkowitz 2002). Alternative male mating tactics in *Cyprinodon* are conditional, based on relative male size and social interactions. The relatively largest males in the population hold territories, but in absolute size, these males may be intermediate or small (Leiser & Itzkowitz 2004). Thus, successful alternative tactics rely on accurate assessment of one’s own status and that of other males.

When alternative reproductive tactics are part of a conditional strategy, as in *C. elegans*, social interactions play a critical role in an individual’s choice of mating tactic. These interactions are mediated by aggression and communication between the
interacting individuals. My objective is to examine social interactions and their consequences in a species with alternative reproductive tactics. A comprehensive understanding of social behavior is particularly important when social interactions between individuals determine reproductive success. Additionally, understanding how males using different reproductive tactics contribute to reproductive success informs us about the role of sexual selection in shaping the mating system. Herein, I integrate behavioral studies with genetic techniques in laboratory and field-based experiments to examine social interactions among male reproductive tactics, and the resulting reproductive consequences.

In Chapter 2, I examine the complex patterns of association expressed by Comanche Springs pupfish, *C. elegans* by assessing social interactions within and between the sexes. I first test the hypothesis that there is a direct, positive relationship between territorial male reproductive success and presence of satellite males and female mimics. Second, by manipulating who is present in a male’s territory, I test the hypothesis that the presence of additional individuals in a territory influences association patterns of females, female mimics and satellite males. Examining the direct and indirect association patterns of male and female *C. elegans* in the natural population provides information about how males expressing different alternative reproductive tactics find mates.

Chapter 3 focuses on how territorial male *C. elegans* respond to variation among conspecifics in the natural environment. I test the hypothesis that territorial males discriminate between conspecifics based on differences in sex and size. Of particular interest is whether territorial males express sex recognition of female
mimics. The degree of aggressiveness to female mimics indicates if they are successful at deceiving territorial males and suggests the level of success they may have in garnering reproductive opportunities.

Finally, Chapter 4 examines the reproductive consequences of alternative reproductive tactics among male *C. elegans* and the effects of population level demographic factors on male behavior and reproductive success. I test the hypotheses that 1) density and sex ratio will alter individual male behavior 2) differences in behavior related to the expression of alternative reproductive tactics by males cause differences in reproductive success and 3) demographic parameters (density and sex ratio) alter variation in male reproductive success, producing differences in the opportunity for sexual selection.
II. HOW DO MALES EXPRESSING ALTERNATIVE REPRODUCTIVE TACTICS FIND MATES? DIFFERENCES IN FEMALE MIMIC AND SATELLITE MALE BEHAVIOR IN CYPRINODON ELEGANS

INTRODUCTION

When males compete for mates, differences in competitive ability may result in some males expressing alternative reproductive tactics to garner matings (Andersson 1994; Shuster & Wade 2003; Oliveira et al. 2008). In these systems, there is typically one primary tactic, in which males invest in, and control, limited resources (e.g., spawning sites or females) and one or more ‘parasitic’ tactics (e.g., satellite males, sneaker males, female mimics) in which males exploit the primary male’s investment and usurp fertilizations (Taborsky 1994, 2008). Alternative tactics may be advantageous to males with lesser competitive abilities if the primary tactic is too costly for them to express, for example when the primary tactic is associated with exaggerated sexually selected traits, large body size, or energetically costly behaviors such as territory defense (Taborsky 1994, 2008).

Alternative reproductive tactics are often conditional, and males can switch between them based on environmental, physical and social factors (Repka & Gross 1995; Gross 1996; Andersson 1994; Oliveira et al. 2008). Multiple tactics are evolutionarily stable due to status-dependent selection (Gross 1984, 1996; Gross & Repka 1998), under which males choose their tactic based the outcomes of male-male competitive interactions. Therefore, not only will social interactions determine a
male’s tactic but social interactions between males expressing different tactics determine the success of each tactic. Waltz (1982) developed the ‘satellite threshold model’ to generate quantitative predictions related to the expression and behavior of males using alternative reproductive tactics. For example, males with alternative tactics should be more abundant in high-quality territories or should associate with territorial males that are the most attractive to females (Waltz 1982). Thus, satellite males have more opportunities to spawn by associating preferentially with successful males than they would if they held their own territory.

Empirical evidence has supported the predictions of the satellite threshold model in that males expressing satellite or sneaker tactics often choose to associate with males that are considered high-quality mates (in insects: *Acheta domesticus*, Kiflawi & Gray 2000; anurans: *Rana catesbiana*, Howard 1978; *Spea multiplicata*, Pfennig et al. 2000; *Rana sylvatica*, Bee 2007; *Hyla cinerea*, Humfeld 2008 and fishes: *Symphodus ocellatus*, Taborsky et al. 1987; *Cyprinodon pecosensis*, Kodric-Brown 1988). For example, in fishes with alternative reproductive tactics, satellite male Azorean rock-pool blennies (*Parablennius sanguinolentus parvicornis*) associate more often with higher quality males that have more female visitors and spawnings (Oliveira et al. 2002). In the peacock blenny (*Salaria pavo*), sneaker males associate with males preferred by females (Gonçalves et al. 2003a). In addition to preferring the same males that females prefer, satellite males also evaluate males using the same cues that females use (Pfennig et al. 2000; Humfeld 2008). Male New Mexico spadefoot toads, *Spea multiplicata*, prefer to associate with conspecific over heterospecific male calls (Pfennig et al. 2000). Further, small males unlikely to be
preferred by females spent more time near stimuli with the same call rates preferred by females (Pfennig et al. 2000).

In addition to being attracted to high-quality territorial males, intersexual dynamics can influence association patterns (Henson & Warner 1997). That is, satellite and sneaker male behavior may be influenced by females or other non-territorial males (Alonzo 2008a). Communication networks facilitate reproductive and social decisions in systems with alternative reproductive tactics (Gonçalves et al. 2008). For example, nesting male *Symphodus ocellatus* spawned less when the number of sneaker males was experimentally increased (Alonzo & Warner 1999). Additionally, in the laboratory, satellite male *Salaria pavo* exhibit intersexual copying of association patterns. Males change their association preference after observing a non-preferred male in the company of a female (Gonçalves et al. 2003b). If the presence of other individuals (females or males expressing alternative tactics) alters the quality of a territory, predictions can be made about how these individuals will influence attraction of males using alternative tactics to a territory. Specifically, if the presence of females in a territory decreases its attractiveness to other females, satellite and sneaker male frequency should also decrease (Waltz 1982). Additionally, if the presence of satellite or sneaker males increases territory quality, their frequency should also increase (Waltz 1982).

This study is one of the first to assess social interaction in the context of a communication network in the genus *Cyprinodon* and examines the complex patterns of association expressed by Comanche Springs pupfish, *Cyprinodon elegans*. I examined direct and indirect association patterns of male and female *C. elegans* in the
natural population. Males of this species exhibit three alternative reproductive tactics: territoriality, satellite behavior and female mimicry. I first tested the hypothesis of a relationship between territorial male reproductive success and the presence of satellite males and female mimics. In the closely related Pecos pupfish (C. pecosensis), there is a positive relationship between satellite males and territorial male reproductive success (Kodric-Brown 1986). Thus, the same relationship was predicted for satellite male and female mimic C. elegans. Second, I tested the hypothesis that the presence of additional individuals in a territory influences association patterns of females, female mimics and satellite males. This hypothesis was tested by manipulating who is present in a territory along with the territorial male. Based upon the satellite threshold model, I predict a relationship between females in a territory and the frequency of males expressing alternative tactics. I also predict a relationship between additional males in a territory and the frequency of males expressing alternative reproductive tactics. Whether these relationships are positive or negative can not be predicted but will reflect how individuals other than the territorial male influence the attractiveness of the territory.

METHODS

Study System

*Cyprinodon elegans* has the promiscuous mating system typical of the genus. Leiser & Itzkowitz (2002, 2003a) found that male *C. elegans* performed one of three mating tactics that correlate with their size: 1) defending a territory, 2) utilizing the
areas around territories as a satellite and 3) sneaking spawns. Territorial males are usually large (50-60 mm), show blue breeding coloration on their anterior dorsal aspect and defend small areas (median territory size = 0.225 m$^2$) by chasing non-territorial conspecifics and heterospecific intruders (Leiser & Itzkowitz 2003a). Territorial males provide no direct parental care, although indirect care (i.e. protection) may be given to eggs laid inside a male’s territory (Kodric-Brown 1986). Intermediate sized males (40-45 mm) displayed a satellite tactic in which they patrolled the water column above 2-6 territories occupied by large males. Satellite males have breeding coloration similar to that of territorial males but garner matings either by interrupting spawns of territorial males, or by spawning with a when a territorial male is occupied elsewhere in the territory (Leiser & Itzkowitz 2002, 2003a). Finally, the ‘sneaker’ tactic is displayed by small males (30-35 mm). Sneakers have large ranges and frequently enter and exit the breeding territories. They retain a cryptic female-like morphology and either spawn when the territorial male is occupied or sidles and spawns alongside a large male and female (Leiser & Itzkowitz 2002, 2003a). In natural populations, individuals expressing these tactics compete for mates, but large territorial males spawn significantly more than do satellite or sneaker males (Leiser & Itzkowitz 2002).

**Field Observations**

*Cyprinodon elegans* was listed as endangered in 1967. This species occurs in Reeves and Jeff Davis Counties, TX near the town of Balmorhea. This study was conducted in the headpool of San Solomon Spring at Balmorhea State Park. The
spring outflow of about 83-98 million l/day has been walled in, creating a 180 x 21 m² public swimming pool with a naturally occurring rocky substrate. The population of *C. elegans* in the San Solomon Spring pool numbers up to 270,000 (Garrett et al. 2002). The breeding season begins in late March and extends through October; peaking in July (Itzkowitz 1974; Kodric-Brown 1986). All trials were conducted in June 2008 between 10.00 and 18.00 h.

To assess the relationship between male reproductive success and males expressing alternative tactics, large, territorial males were identified in the San Solomon Spring pool. Focal males included in the study were large in size (> 50 mm SL) and held naturally occurring territories. All territories were 0.61 - 2.44 m deep and were located 0.61 – 3.66 m from the edge of the pool. Average territory size for large males is 0.225 m² (Leiser & Itzkowitz 2003) and all focal males observed had territories of approximately this size. All observations were conducted while snorkeling and behaviors were recorded on underwater slates. Focal males were each observed for 5 min and the following were recorded: number of chases directed towards female mimics, satellites and neighbors; number of lateral displays directed at neighbors; number of females approached by the male; and number of spawning acts (see Appendix A for further descriptions of behaviors). Territorial males are very active and interact with all conspecific intruders in their territory. Thus, quantifying territorial male behavior serves as a proxy for quantifying numbers and identities of conspecific intruders.
**Territorial Manipulations**

Male and female stimulus-fish were collected by dip net each morning at least 50 m from the focal male territories. Such allocation minimized the likelihood of recent interaction with any focal males. After collection, stimulus-fish were maintained in live-well traps until used. Stimulus-fish were not marked, but it was unlikely that the same individuals were collected on subsequent days given the population size. Additionally, in previous studies no marked individuals were ever re-collected (Gumm, unpublished data).

For each trial, a stimulus fish was selected haphazardly and placed into a 133 mm x 190 mm clear plastic bottle (Nalgene, Inc.). The bottle had holes in the lid to allow for chemical communication as pupfish use chemical cues in other forms of recognition (Loiselle 1983; Strecker & Kodric-Brown 1999). The bottle was placed in the middle of a focal male’s territory followed by a 1-min acclimation period. In this period, any debris stirred up by the bottle was allowed to settle, heterospecific intruders attracted to the bottle dispersed, stimulus-fish began swimming within the bottle, and all focal males remained on their territory. After the acclimation period, a 5-min observation was conducted in which I quantified the number and identity of individuals approaching the bottled stimulus within one bottle width (133 mm) on an underwater slate while snorkeling. Given that the bottle was in the middle of the territory, the territory owner was consistently within one bottle-width throughout the observation period and his behavior was not quantified. Individuals approaching the bottle were identified as females, female mimics or satellite/territorial males. Occasionally, bottles were visited by neighboring territorial males, who have the
same breeding coloration as satellites. These neighboring territorial and satellite males were combined into one group for analysis because their mating tactic could not reliably be identified solely on appearance or behavior near the bottle. It was logistically impossible to individually identify unique vs. repeat visitors to the bottle, therefore individuals may have approached the bottle more than once, leaving the focal male’s territory between approaches. Immediately after trials were completed, stimulus-fish were released in the same area from which they were collected. Three stimulus treatments were tested: 1) bottled-male stimulus (mean SL ± s.e.m, 38.38 ± 1.60), 2) bottled-female stimulus (39.38 ± 0.84) and 3) empty bottle.

**Statistical Analyses**

Data met the assumptions for parametric testing. Each focal male was observed by one of two observers, but there was no difference between observers in total number of behaviors quantified (Unpaired t-test: n = 30, t = -0.03, P = 0.97) or total number of chases by territorial males (Unpaired t-test: n = 30, t = -1.41, P = 0.17). Thus, data were combined for statistical analysis. Field observations were analyzed using Pearson’s product moment correlation tests to examine relationships between territorial male reproductive success and the following behaviors: chases of female mimics, chases of satellite males, chases of neighboring territorial males, and number of females approached. For manipulative experiments, a 3 x 3 factorial ANOVA tested for differences in frequency of visits by different types of conspecifics based on the type of stimulus-fish (for additional statistical analyses see Appendix B). Statistics were performed using Statview v. 5.0 and SPSS v. 12.
RESULTS

Field Observations

All focal males had conspecific males enter their territory, all of which were chased by the territorial male. Most males participated in at least one spawning, although 5 males did not spawn, two of which did not have females enter the territory. Number of male spawns was not correlated with any measured variable (Figure 2.1): number of females approaching the territory (Pearson’s correlation: R = 0.08, \( P = 0.68 \), Figure 2.1a), number of chases to either female mimics (Pearson’s correlation: R = 0.08, \( P = 0.67 \), Figure 2.1b), satellite males (Pearson’s correlation: R = 0.15, \( P = 0.43 \), Figure 2.1c), or territorial males (Pearson’s correlation: R = -0.03, \( P = 0.89 \), Figure 2.1d).

Territorial Manipulations

There was no difference in size between male and female stimuli (Unpaired t-test: t = 0.55, \( P = 0.58 \)). Females were the most frequent visitors to all bottle treatments and female mimics were the second most frequent visitor. There was a significant main effect of fish type on number of approaches (Factorial ANOVA: \( F_{2, 116} = 63.781, P < 0.001 \); Figure 2.2). There was a significant interaction between stimulus type (female, male, empty bottle) and the type of approaching conspecifics (Factorial ANOVA: \( F_{4, 116} = 10.56; P < 0.001 \)). This indicates that conspecifics of different types approached bottles differentially based on stimuli in the bottle (Figure 2.2). Of all stimulus treatments, empty bottles were the least approached by females.
(mean ± s.e.m: 3.00 ± 0.64), female mimics (0.90 ± 0.18) and satellite/neighboring males (1.25 ± 0.29). When the stimulus was a bottled female, other females were the most frequent visitor (9.95 ± 1.10). Female mimics and satellite/territorial males differed in how often they approached bottled females with female mimics approaching more than satellite/territorial males (female mimic: 5.86 ± 0.70; satellite/territorial: 2.24 ± 0.35). Finally, when bottled males were the stimulus, females approached most frequently (11.55 ± 1.45). However, females mimics (2.55 ± 0.34) and satellite/territorial males (2.60 ± 0.58) did not differ in visitations to bottled males.

**DISCUSSION**

Focal observations indicated no relationship between frequency of chases towards males expressing alternative tactics and territorial male reproductive success (Fig. 2.1b, c, d). This finding does not support predictions from the satellite threshold model that satellite males and female mimics associate more with high-quality males. Additionally these results contrast with association patterns in closely related species. In both *C. bovinus* and *C. pecosensis*, satellite males associated more often with males that had higher reproductive success larger territories (Leiser & Itzkowitz 2003b; Kodric-Brown 1986). There are a number of possible explanations for these differences. First, ecological differences between the species may influence availability of breeding areas and thus behavior of females, female mimics and
satellite males. Spawning in C. bovinus was restricted to a 1 x 3 m$^2$ shelf whereas in C. elegans, spawning occurred throughout the 180 x 21 m$^2$ pool at San Solomon Spring. Increased availability of breeding area may lead to greater dispersion of satellite males and female mimics allowing them to avoid aggression from territorial males. Alternatively, the large breeding area allows a greater diversity of males to hold territories, potentially increasing the variation in reproductive success of territorial males. This gives females more mate options as they travel widely (personal observation; Ludlow & Itzkowitz 2007) assessing potential mates. Thus, female spawning and/or male reproductive success may be unpredictable by satellite males or female mimics. Thirdly, either territorial males or females may avoid spawning in the presence of satellite males or female mimics. Territorial males do express sex recognition of female mimics (unpublished data) and may alter their investment in spawning based on the presence of female mimics or satellite males (Alonzo & Warner 1999). Successful spawning by parasitic males essentially undermines a female’s mate choice so females may also avoid spawning in the presence of males expressing alternative tactics.

Male spawning success was also not related to number of females approaching a territory (Fig. 1a). Leiser & Itzkowitz (2003b) found a positive relationship between number of approaching females and number of spawns for territorial male C. bovinus. However, the relationship was influenced by territory location. Males holding territories clustered on a breeding shelf had a more direct relationship between these two variables than did males holding territories in other areas of the pond. Thus, the lack of a relationship in the current study may again be a
result of the increased area for breeding territories. Female *C. bovinus* must approach a small area specifically for breeding and may avoid this area when not spawning. By contrast, foraging female *C. elegans* may encounter males more often or may sample males without being motivated to spawn. Additionally, although I did not quantify size of females entering territories or spawning, males do express mate choice preferences for large females (Ludlow & Itzkowitz 2007) and these preferences may have masked a relationship between female approaches and spawning.

Females approached bottled males that were artificially placed in naturally occurring territories more than males did. Females may have been attracted to aggressive interactions between the bottled male and the territorial males as territorial males are more aggressive to bottled males than to bottled females when given a choice between the two (unpublished data). Presence of female *C. bovinus* also promotes aggression between neighboring territorial male, possibly facilitating the choice of a higher quality mate (Leiser et al. 2006). Alternatively, females may be attracted to additional males in a territory if they benefit from mating with males expressing alternative reproductive tactics. Female bluegills (*Lepomis macrochirus*) release more eggs in spawnings involving satellite and sneaker males (Fu et al. 2001) and female European bitterling (*Rhodeus sericeus*) actively solicit sneaker males with elaborate spawning behaviors and gain increased fertilization success with increased sneakers present at spawnings (Smith & Reichard 2005).

In addition to females being attracted to the presence of stimulus males in a territory, females also approached bottled females. Territories in *Cyprinodon* spp. are solely for breeding, so it is unlikely that foraging behaviors influence female
association within male territories. However, females may express mate-choice copying. This non-independent mate choice in which the mating behavior of a female depends on the mating behavior of other females has been documented in other fishes with male alternative reproductive tactics (*Poecilia reticulata*, Dugatkin 1992; *P. latipinna*, Witte & Ryan 2002; *S. ocellatus*, Alonzo 2008b). In a natural setting, Alonzo (2008b) showed that female *S. ocellatus* were more likely to spawn in the presence of other females and when no other females were around, were more likely to spawn in nests with higher recent mating success. I did not test the likelihood that female visitors to the bottled female would spawn with the territorial male.

Additionally, my field observations could not resolve a relationship between females approaching a territory and spawning in that territory. However, Ludlow & Itzkowitz (2007) found that high-quality males are more aggressive towards females than lower quality males. Thus, assessing or choosing high-quality males as mates incurs a cost. Mate-choice copying in pupfish may allow females to avoid assessment costs by avoiding independent assessment of males showing high levels of intersexual aggression.

Males were not attracted to bottled males in a territory but males expressing different alternative reproductive tactics responded differently to females in bottles. Female mimics approached females more than satellite/territorial males did. The observed association pattern may be a reflection of differential movement patterns between males expressing different tactics as female mimics range more widely than satellite or territorial males (Leiser & Itzkowitz 2003a). In addition, the field observations showed no predictable pattern of female spawning (Figure 1), thus
female mimics may simply follow females in order to parasitize their spawnings. However, it is surprising that satellite males rarely approached females. Satellite males initiate more aggression than female mimics (Leiser & Itzkowitz 2003a), and can defend territories if social and environmental conditions permit (Leiser & Itzkowitz 2002), whereas female mimics have never been observed holding territories in the wild. Thus, the difference between female mimics and satellites may reflect a trade off between inter- and intrasexual selection by satellite males. That is, satellite males may forgo parasitizing females in order to secure their own territory through male-male aggression as males that hold territories are much more successful than males expressing alternative tactics (unpublished data; Leiser & Itzkowitz 2003a).

Overall, the association behavior of female mimics resembles that of both males and females. Neither female mimics nor satellite/territorial males were attracted to bottled males. However, their response to female stimuli mirrored that of females in the population. By exhibiting behaviors similar to those of females, female mimics may increase their access to females and decrease aggression from territorial males (Dominey 1980; Slagsvold & Sætre 1991). Territorial males discriminate against female mimics based on visual and chemical cues (unpublished data), but the role of behavioral cues in sex recognition of female mimics is unknown. The behavior of female mimics in this system contrasts with that of female mimics in S. pavo, which associated with high-quality nests instead of females and differed from females in nest-site visiting behaviors (Gonçalves et al. 2003a). Comparative studies of the selective pressures producing variations of a single tactic (e.g. female mimicry)
across different species may provide insight into the evolution of multiple tactics within a species and vice versa.

The results of this study suggest that association patterns of *C. elegans* are often the result of indirect social interactions. I found no evidence for direct associations between males expressing alternative reproductive tactics and territorial male reproductive success. Additionally, there was no direct relationship between female visits and spawning and results suggest that females may express non-independent mate choice in this species. Finally, this is the first study to test differences relating to female mimic and satellite male behavior in relation to females. Further research should examine the effects of varying ecological factors between populations and species on the behaviors of males expressing alternative reproductive tactics. Additionally, continued study of social interactions of *Cyprinodon* in the context of communication networks may provide further evidence for the role of communication in facilitating and maintaining alternative reproductive tactics in this system.

**Acknowledgements**

I thank Murray Itzkowitz for support throughout all stages of this research. R. Deaton, C. Hargrave, A. Heim, J. Landis, E. Lamb, S. Rosado, and J. West for providing critical assistance in the field. Helpful comments on previous versions of this manuscript were provided by A. Echelle, T. Mendelson, J. Nyby and J. Swann. All methods conformed to the laws of the USA and the state of Texas with federal (permit # TE122838) and state permits (#SPR-0506-667) and Lehigh University
IACUC approval (#A3877-01). Funding for this study was provided by a Lehigh University Summer Research Fellowship and a Sigma Xi GIAR to JMG.
Figure 2.1.

Scatterplots and regression lines depicting relationships between spawning of territorial males and a) female approaches, b) chases to female mimics, c) chases to satellite males, and d) chases to neighboring territorial males.
Figure 2.2. Means ± s.e.m. of number of females (light grey bars), female mimics (striped bars) and satellite/territorial males (dark grey bars) approaching each stimulus treatment per 5-minute observation period. There is a significant interaction between type of individual and stimulus type.
III. MULTIPLE RECOGNITION PROCESSES BY TERRITORIAL MALES IN
A SPECIES WITH ALTERNATIVE REPRODUCTIVE TACTICS,
CYPRINODON ELEGANS

INTRODUCTION

Alternative reproductive tactics are behavioral and/or morphological phenotypes expressed within a sex, which may increase an individual’s reproductive success (Taborsky 1994; Gross 1996). Species expressing alternative reproductive tactics provide unique opportunities for the study of male-male competition because tactics may be associated with different social and sexual signals (reviewed in Oliveria et al. 2008). For example, males expressing the primary tactic typically have exaggerated courtship or bright colors that females assess for mate choice whereas males utilizing satellite or sneaker tactics often have more cryptic coloration. In many systems, the primary tactic relies on territoriality (reviewed in Taborsky 1994; Oliviera et al. 2008) and while interactions between neighboring territorial individuals are well characterized (Jaeger 1981; Getty 1987; Temeles 1994; Temeles 1994), studies experimentally testing communication between males expressing different tactics remain rare (Gonçalves et al. 2008).

When males expressing satellite or sneaker male tactics garner matings by intruding into a territory, it is hypothesized that they increase their reproductive success via decreased detection while territorial males increase their reproductive success by increased detection of reproductive parasites (Gonçalves et al. 2008).
conflict is exemplified when sneaker males exhibit female mimicry. Female mimics have morphological or behavioral characteristics of females (Taborsky 1994) and may benefit by increased access to females (Machias-Garcia 1994), advantages in competitive encounters (Slagsvold & Saerte 1996), or reduced aggression from territorial males (Gonçalves et al. 2005). These advantages, however, are dependent upon successful deception of territorial males and Gonçalves et al. (2008) suggest that there is an evolutionary arms race between female mimicking signaling and detection mechanisms. In general, selection is predicted to favor territorial male ability to discriminate against female mimics as they steal fertilizations in the males’ territories (Leiser & Itzkowitz 2003a), although this discrimination ability varies between species (Taborsky et al. 1987; Marco et al. 1998; Hanlon et al. 2005; Husak et al. 2004). Few studies have quantified either the morphological features of the female mimic signaling system (but see Okuda et al. 2003) or explicitly tested territorial male discrimination abilities (reviewed in Gonçalves 2008). Both aspects of female mimicry have been explored in the peacock blenny, Salaria pavo. Small sneakers lack the secondary sexual characteristics associated with large, nest holding males and mimic courtship behaviors performed by females (Gonçalves et al. 1996). Gonçalves et al. (2005) presented female mimics and size-matched females to territorial males and found that males did not respond differentially to the two stimuli and attacked and courted both equally. This shows that in some systems, female mimics successfully deceive territorial males.

In addition to recognizing and discriminating against males expressing alternative reproductive tactics, territorial males may express recognition in other
conspecific interactions as well. For example, territorial males may express mate quality recognition via mate choice preferences when they invest in courtship or parental care (Andersson 1994). Additionally, it may be advantageous for males to assess the threat posed by intruding conspecific competitors. When defended resources are limited, territorial males may be challenged by others trying to take over the territory site. Larger males may take over nests either temporarily or permanently (‘piracy’, Taborsky 1994) and threat assessment underlies one of the hypotheses explaining differential treatment of territorial neighbors vs. strange intruders (‘dear enemy’ recognition, Getty 1987). Finally, males may be faced with both potential mates and competitors. This might result in males abandoning either courtship or aggression or could result in a compromise in behavior directed at both types of stimuli (Morrell 2004). These processes are not mutually exclusive and males may be required to perform one or many of these recognition tasks at any time.

One system in which multiple recognition processes occur is pupfishes of the genus *Cyprinodon* in which males exhibit three alternative reproductive tactics; territoriality, satellite behavior, and female mimicry (Itzkowitz 1969; Kodric-Brown 1986; Leiser & Itzkowitz 2002, 2003a). Recognition involved in interactions between territorial neighbors has been studied in both laboratory and field based studies and neighboring territorial males exhibit ‘dear enemy recognition’ in which they are less aggressive to territorial neighbors than intruders (*C. variegatus*, Leiser 2003; *C. bovinus*, Leiser et al. 2006, *C. elegans*, Gumm unpub. data). Communication mediating other social interactions in this genus has typically been studied from the female perspective and focused on mate-quality and species recognition. Females
prefer larger males (*C. variegatus*, Draud 1996; *C. bifasciatus*, Ludlow et al. 2001), more colorful males (*C. pecosensis*, Kodric-Brown 1977, 1983), and males defending territories with rocky substrate over those containing only sand or silt (*C. bifasciatus*, Ludlow et al. 2001). Females from a species flock of pupfishes found in Lake Chichancanab MX prefer conspecifics males (*C. maya, C. labiosus*, Strecker & Kodric-Brown 1999; Kodric-Brown & Strecker 2001), whereas lack of female preferences for conspecifics males has promoted hybridization in other species pairs (*C. pecosensis-C. variegatus*, Rosenfield & Kodric-Brown 2003; *C. elegans-C. variegatus*, Tech & Kodric-Brown in press). These studies address how females respond to differences among males, but it is also important to consider how the territorial male respond to conspecifics in multiple contexts.

My objective was to examine multiple recognition processes by territorial male *C. elegans* by testing if males discriminate between conspecifics based on size and sex in four different treatments. (1) I hypothesized that territorial males may exhibit a compromise between courtship and aggression when given a choice between large males and females. Females are abundant in this population so the cost of losing one potential mate may be less than the cost of losing a territory to a conspecific competitor. Thus, territorial males were predicted to engage more in aggression than courtship (Santangelo et al. 2002; Gumm et al. in prep). (2) I hypothesized that males will express mate-quality recognition when given a choice between a large and small female present near his territory. In fishes, larger females are typically more fecund (Leiser 2003) and Ludlow & Itzkowitz (2007) found that male *C. bifasciatus* defending high-quality territories rejected more potential mates than did males
defending low-quality territories. Thus, I predicted that males would direct more behaviors to and spend more time near the larger female. (3) I hypothesized that males would assess the threat of conspecific competitors when presented with a large and small male near his territory. While small males may steal fertilizations, larger males pose more of a threat as they may oust a male from his territory (Getty 1987). Thus, territorial males were predicted to spend more time near and perform more aggressive behaviors to the larger male. (4) Finally, I hypothesized that males will express sex recognition of female-mimicking reproductive parasites. If territorial males discriminate against female mimics, they will direct more aggressive behaviors towards them than size-matched females. If males do not express sex recognition of female mimics, there will be no difference between their response to female mimics and size-matched females.

METHODS

Study Animal and Site

*Cyprinodon elegans*, the Comanche Springs pupfish is found in the San Solomon Spring system consisting historically of a pool at Phantom Cave and refugia at Balmorhea State Park, Toyahvale, TX, USA. The area around the headwaters of the San Solomon Spring has been excavated into a $1.3 \times 10^7$ L pool while retaining the natural substrate. The spring has an outflow of $4.12 \times 10^6$ L/hr at a constant temperature of $24.5^\circ$ C and there is no thermal stratification in the pool (Stevenson & Buchanan 1973). *Cyprinodon elegans* was listed as federally endangered in 1967 but
the population in the San Solomon refugia is healthy, numbering up to 270,000 at the height of breeding (Garrett et al. 2002).

*Cyprinodon elegans* has the promiscuous mating system typical of the genus. Leiser & Itzkowitz (2002, 2003a) found that male *C. elegans* performed one of three mating tactics that correlate with size: 1) defending a territory, 2) utilizing the areas around territories as a satellite and 3) sneaking spawnings. Territorial males are usually large (50-60 mm), show blue breeding coloration on their anterior dorsal aspect and defend small areas (median territory size = 0.225 m²) by chasing non-territorial conspecifics and heterospecific intruders (Leiser & Itzkowitz 2003a). Territorial males provide no direct parental care, although indirect care may be given to eggs laid inside a male’s territory (Kodric-Brown 1986). Intermediate sized males (40-45 mm) displayed a satellite tactic in which they patrolled the water column above 2-6 territories occupied by large males. Satellite males have breeding coloration similar to that of territorial males but garner matings by interrupting spawnings by territorial males, or by spawning with a female in a males’ territory when he is occupied elsewhere in the territory (Leiser & Itzkowitz 2002, 2003a). Finally, the ‘sneaker’ tactics is displayed by small males (30-35 mm). Sneakers have large ranges and frequently enter and exit the breeding grounds. They retain a cryptic female-like morphology and either spawn when the territorial male is occupied or sidles and spawns alongside a large male and female (Leiser & Itzkowitz 2002, 2003a). In natural populations, individuals expressing these tactics compete for mates. Males utilizing each tactic have similar levels of aggression (either initiated or
but large territorial males spawn significantly more than do satellite or sneaker males (Leiser & Itzkowitz 2002).

**Experimental Trials**

Males (n = 19 per treatment) defending natural substrate territories were identified in the pool at Balmorhea State Park near Balmorhea, TX. Each focal male was used only once. All males were over 50mm, held similar sized territories that were between 0.61 - 2.44 m deep. Stimulus fish were collected by dip net in a different area of the pool and sexed. Female mimics are identifiable as male due to the presence of a faint black bar at the edge of the caudal fin. The reliability of sex identification by this method was verified in long-term behavioral observations conducted at the US Fish and Wildlife Dexter National Fish Hatchery and Technology Ceneter, Dexter, NM, USA (Gumm, pers. obs.). Most stimulus fish were used in only one trial, however some were used in two trials. In these cases the additional trial was never the same treatment and stimulus fish were never paired together more than once. Fish were maintained in live-well traps in the pool at Balmorhea State Park until testing. Each fish was measured prior to testing and released at the collection site immediately after testing.

**Experimental Design**

Bottle presentations were conducted in the following treatments: 1) small female (mean mm ± s.e.m. = 33.44 ± 0.60) and large female (51.77 ± 0.31), 2), small male (32.74 ± 0.44) and large male (51.14 ± 0.31) 3) large male (51.36 ± 0.31) and
large female (51.70 ± 0.31), 4) small male (32.77 ± 0.44) and small female (33.24 ± 0.61. Stimuli fish were placed in cylindrical plastic bottles (133 d x 195 h; Nalgene, Inc.). Bottles were clear had holes in the top to allow visual and chemical communication. Two bottled stimuli were presented simultaneously on opposite sides of a male’s territory, approximately 45 cm apart. Territorial males were allowed to habituate for 3 min during which time all focal males visited both bottles. After habituation, each focal male was observed for 5 min in which I recorded 1) amount of time spent within one body length of each bottle, 2) number of bites at the bottle and 3) number of aggressive displays.

**Statistical Analysis**

Analyses were conducted using Statview 5.0. All data adhered to the assumptions of normality and thus, parametric statistics were used in all analyses. Within each treatment, paired t-tests were used to determine if males bit, displayed or spent time near the bottles differentially between stimuli. A strength of preference (SOP) score was calculated as the time spent near one stimulus minus the time spent near the other stimulus (for alternate calculations and analyses of SOP see Appendix C). Considering that each stimulus type was used in two different treatments, SOP scores are calculated differently between treatments and must be interpreted across trials that use the same stimulus. For treatments containing a small male, a positive SOP indicates a strong preference for the small male stimulus while a score near 0 indicates no preference. A negative SOP score indicates either a preference for the large male or small female. Similarly for treatments containing a large female, a
positive SOP indicates a strong preference for the large female whereas a SOP near 0 can be interpreted as no preference and a negative SOP indicates a preference for either a large male or small female, depending on treatment. An ANOVA was used to compare SOP for certain stimuli across different treatment groups. A Fisher’s Protected Least Significant Difference test examined pairwise comparisons across all treatments.

**RESULTS**

When size differed between females, focal males spent more time near the large female than small female (paired $t$ test: $t_{18} = 4.51, P = 0.0003$) and performed more bites at the bottle containing the larger female (paired $t$ test: $t_{18} = 3.52, P = 0.002$). Males did not display differentially to females of different sizes (paired $t$ test: $t_{18} = 1.17, P = 0.26$). When presented with stimulus males of different sizes, focal males spent more time near the larger male (paired $t$ test: $t_{18} = 4.10, P = 0.0007$) and displayed more to the larger male (paired $t$ test: $t_{18} = 4.54, P = 0.0003$). However, focal males did not bite differentially to the two sizes of stimulus males (paired $t$ test: $t_{18} = 1.09, P = 0.29$).

Territorial males also responded differentially based on sex within a size class. When stimulus individuals were large, focal males spent more time near (paired $t$ test: $t_{18} = 4.11, P = 0.0007$), performed more bites (paired $t$ test: $t_{18} = 2.21, P = 0.04$) and performed more displays to the male than to the female (paired $t$ test: $t_{18} = 3.92, P = 0.001$). In response to female mimics and size-matched females, territorial males
spent more time with the female mimics (paired $t$ test: $t_{18} = 3.52, P = 0.003$) and performed more bites (paired $t$ test: $t_{18} = 4.12, P = 0.0006$) and aggressive displays towards female mimics than size-matched females (paired $t$ test: $t_{18} = 2.28, P = 0.04$; Fig 1a).

The strength of preference (SOP), which represents the relative amount of time spent near a stimulus, was significantly different based on the treatment (ANOVA: df 3,72, $F = 20.52, P < 0.0001$; Fig. 2). Territorial males had a stronger preference for female mimics when they were presented with size-matched small females than when they were presented with large males (Fisher’s PLSD: $P = 0.0001$). There was no difference in SOP for large males that were presented with either a small male or large female (Fisher’s PLSD: $P = 0.06$). Territorial males preferred large females more when they were presented with a small female than a large male (Fisher’s PLSD: $P < 0.0001$). Finally, there was no difference in SOP for small females presented with either a large female or a size-matched female mimics (Fisher’s PLSD: $P = 0.52$).

**DISCUSSION**

Territorial male *C. elegans* discriminated between different classes of conspecifics in all treatments tested. Territorial males expressed sex recognition of female mimics, raising the question of how female mimics are maintained in the population. A possibility is that frequency dependence may influence the response of territorial males to female mimics (Gross 1991, 1996). Female mimics may attract
relatively little aggression, and potentially gain opportunities to spawn when territorial intrusions by larger males are more common. Alternatively, female mimicry may persist if the mimics are beneficial to the territorial male. A territorial male may increase his individual reproductive success if females are attracted to territories that contain males expressing alternative tactics (*Philomachus pugnax*, Hughie & Lank 1997).

By contrast, female mimics may persist if they pose little or no cost to the territorial males' reproductive success. Territorial males are preferred by females and have the highest reproductive success (Leiser & Itzkowitz 2002). Thus, if female choice plays a role in spawning behavior, females may avoid spawning with satellite or female mimicking males despite their presence on a territory (Waltz 1982). Finally, aggression towards sneakers may be too costly for territorial males if aggression results in lost mating opportunities. These hypotheses are not mutually exclusive. For example, in the European bitterling (*Rhodeus sericeus*), Candolin & Reynolds (2002) show that females spawn more quickly in the presence of multiple sneaker males. Additionally, territorial males showing increased aggression towards sneakers also have more interrupted courtships and increased latency to spawning (Candolin & Reynolds 2002).

In the second treatment, territorial male *C. elegans* discriminated between females based on size. By spending more time near and biting large females more than small females, territorial males may be expressing mate choice preferences. Female *C. elegans* are known to prefer larger males, who typically express the territorial tactic (Tech & Kodric-Brown in press). However, studies of male mate
preferences are not as common. Ludlow & Itzkowitz (2007) showed that male *C. bifasciatus* may exhibit mate choice. Large males that held high quality territories are more likely than smaller territorial males to reject females as mates, and they are more likely to reject low-quality (smaller) females, who then are accepted as mates by lower quality males. Leiser (2003) found that larger female *C. variegatus* spawned more eggs, providing a direct benefit for males preferring larger females. Thus, there is a potential benefit of increased reproductive success for males expressing preference for larger females.

Territorial males also discriminated between conspecific male competitors based on size. They spent more time near and performed more lateral displays to larger males but did not bite differentially at males of different sizes. Increased aggression towards larger intruding males may indicate that they are more of a threat to the territorial male than smaller intruding males. Previous studies show that male pupfishes differ in aggressive behaviors based on the type of competitor they face. Conspecifcics are chased more often than heterospecifics by territorial male *C. variegatus* (Itzkowitz 1974) and territorial males typically express decreased aggression to neighboring males as opposed to strange intruders (‘dear enemy recognition’, *C. variegatus*, Leiser 2003; *C. bovinus*, Leiser et al. 2006; *C. elegans*, Gumm unpublished data). It is hypothesized that ‘dear enemy’ recognition is based upon the differential threat posed by neighbors and strange intruders (Getty 1987, 1989). My results further support that threat assessment of intruders is important to territorial males.
Dear enemy recognition can be disrupted by female presence (Leiser 2003; Leiser et al. 2006) and female influence on male-male competition may also partially explain the results of the final treatment which examined territorial males behavior when faced with a potential mate and a competing male. Males spent more time near, performed more lateral displays towards and bit more toward large males than toward large females. Males appear to forgo courtship for aggression, which was predicted because losing a territory incurs a higher cost than losing a potential mate. The amount of potential reproduction lost by losing one mate is much less than the amount lost by losing control of a territory. Alternatively, male-male competition is provoked by females in *C. variegatus* and *C. bovinus* (Itzkowitz 1974; Leiser et al. 2006). Thus, the presence of a female on the territory may have led to increased territorial male aggression towards the large male in *C. elegans* as well. These possibilities can not be distinguished from this experiment. Future research should examine the effects of females on male-male aggression in this system.

Some male behaviors were only performed towards particular stimuli. Specifically, males displayed at very low levels to females across treatments but displayed differentially in all treatments that included males as stimuli. This indicates that lateral displays are not used in courting females, but are solely an aggressive behavior. Correspondingly, males directed more lateral displays towards female mimics than towards size-matched females, indicating that female mimics were identified as male competitors. Territorial males also used bites differently across treatments. Males bit more at stimulus males in treatments where both sexes were presented, however they also bit more towards large over small females. This result
suggests that bites may be involved in courtship, with a greater number of bites indicating a preference for larger females. Male harassment of females has not been explored in this species, but males are aggressive toward rejected mates (Ludlow & Itzkowitz 2007) and male harassment of females to increase the likelihood of mating is common in other fish species (Andersson 1994). Finally, territorial males did not bite differentially towards males of different sizes. If bites and displays are both expressed in aggressive interactions, males may forgo biting in order to display to males. Alternatively, bites are a more escalated aggressive behavior so males may display instead of biting to reduce the risk of injury.

There were also differences in strength of preference for various stimuli when compared across treatment groups. Specifically, territorial males had strong association preferences for female mimics when presented with size-matched females, but did not associate with female mimics when presented with larger males. Thus, sex recognition of and discrimination against certain stimuli is context dependent. That is, the treatment of a stimulus is dependent on other stimuli in the territory. Additionally, males had a stronger preference for large females when presented with small females than when presented with large males. Hence, mate-quality recognition is also context dependent. Males may only express mate preferences when they are not at risk from conspecific competitors, or mate-quality recognition may depend on having multiple females to compare. Context dependent treatment of female mimics suggests that frequency or density dependence may play a role in maintenance of alternative reproductive tactics in this system (Kvarnemo & Ahnesjö 1996). Frequency dependent selection occurs when the relative success of a
tactic depends on its frequency in the population and can facilitate male alternative reproductive tactics as an evolutionary stable strategy (Gross 1991). Density can also lead to stable alternative tactics and has been shown to mediate the success of sneaker males (Rhodeus sericeus, Reichard et al. 2004).

There are a number of caveats to this study. First, despite conducting the study in the natural environment, the bottle design creates an artificial situation that can not account for the behavior of the bottled individuals. While territorial males can discriminate in all treatments, they may not when fish are freely allowed to enter and exit the territory. For example, female mimics may have behavioral adaptations that decrease the recognition ability of territorial males and increase the success of female mimics. In this species, the behavior of female mimics does resemble that of females in certain regards. Both females and female mimics approached females placed in a territory, whereas territorial and satellite males do not (Gumm, unpublished data). Additionally, both females and female mimics frequently enter and exit territories (Leiser & Itzkowitz 2003a), which may decrease a male’s ability to assess reproductive parasites or mate quality. Finally, by conducting this study in an uncontrolled natural habitat, influences of other free swimming fishes were not excluded. Heterospecifics and other conspecifics were present in male territories (personal observation) and recent work shows that female mimics and females are attracted to non-territorial conspecifics present in the territory (Gumm unpublished data).

This study provides insight into the interactions between various social stimuli and offers a comprehensive view of the multiple selective pressures acting upon
recognition systems of territorial males. Future work should examine the proximate neural mechanisms underlying different types of recognition as different neural mechanisms may underlie responses to different social and reproductive stimuli (Cummings et al. 2008). Additionally, the role of demographic parameters should be explored as density and frequency of alternative reproductive tactics may play a role in their success and maintenance in this system.

Acknowledgements

I thank Murray Itzkowitz for support throughout all stages of this research. I thank V. Imhoff and J. Leese for providing assistance in the field. Helpful comments on previous versions of this manuscript were provided by A. Echelle, T. Mendelson, J. Nyby and J. Swann. All methods conformed to the laws of the USA and the state of Texas with federal (permit # TE122838) and state permits (#SPR-0506-667) and Lehigh University IACUC approval (#A3877-01). Funding for this study was provided by a McCarley Student Research Grant from the Southwestern Association of Naturalists, a Texas Academy of Science Student Research Grant and a Sigma Xi GIAR to JMG.
Figure 3.1. Discrimination territorial male *C. elegans*. (a) Time spent near the stimuli (b) Bites performed to the stimuli. (c) Lateral displays performed to the stimuli.

Figure 3.2. Strength of preference for stimuli across all treatments. Positive values represent increasing preference for female mimics (diagonal stripes) or large female (light grey). Negative values represent increasing preferences for large males (dark grey) or small females.
Figure 3.1
Figure 3.2
IV. EFFECTS OF DENSITY AND SEX RATIO ON THE BEHAVIOR AND
REPRODUCTIVE SUCCESS OF MALE CYPRINODON ELEGANS
EXPRESSING ALTERNATIVE REPRODUCTIVE TACTICS

INTRODUCTION

Darwin proposed sexual selection to explain phenotypic variation between the sexes (1871), however intrasexual selection among males can also result in phenotypic variation within a sex (Andersson 1994; Shuster & Wade 2003; Oliveira et al. 2008). Male alternative reproductive tactics (ARTs) are an example of such phenotypic variation and occur when males use different morphological or behavioral tactics to increase their individual reproductive success (Taborsky 1994; Gross 1996; reviewed in Oliveira et al. 2008). Reproductive tactics may be conditional throughout an individual’s lifetime and correlate directly to some phenotypic quality, such as size or aggressiveness (Gross 1996). Theoretical models demonstrating the evolutionary stability of conditional ARTs predict that different tactics will also have differences in reproductive success (Gross 1984; Repka & Gross 1995; Gross 1996; Gross & Repka 1998). For example, highly aggressive, territorial males are predicted to contribute disproportionately to the next generation. However, males using satellite or sneaker tactics almost certainly make important contributions to the population in terms of genetic diversity and effective population size. Reproductive success of males utilizing ARTs varies across species (reviewed in Avise et al. 2002). For example, in the redbreast sunfish (*Lepomis auritus*), satellite and sneaker males have low levels of
success with the majority (95%) of offspring in a nest being fathered by the nest-
attendant male (DeWoody et al. 1998). However, in a closely related centrarchid, the
bluegill (*Lepomis macrochirus*) satellite and sneaker males sire, on average, 20% of
offspring (Neff 2001).

Species specific variation in expression and success of ARTs may be linked to
ecological and demographic parameters. First, these parameters may alter how
behaviors associated with ARTs are performed. Additionally, they may alter the
evolutionary consequences of ARTs. Specifically, density dependence can alter how
selection occurs on different phenotypes, which commonly shapes life history traits
(Sutherland 1996; Moorcroft et al. 1996; Kokko & Rankin 2006; Jirotkul 1999;
Reichard et al. 2004a). Additionally, sex ratios are intrinsically tied to intrasexual
competition (Fisher 1930) and therefore predicted to mediate changes in fitness via
changes in aggressive or territorial behavior (Grant et al. 1995; Jirotkul 2000; Le
Galliard et al. 2005). There also is a direct relationship between the amount of
variance in male reproductive success and the intensity and direction of sexual
selection in a system (Bateman 1948; Shuster & Wade 2003). The ‘opportunity for
sexual selection’ is based upon variance in reproductive success and is a quantitative
measure that estimates the upper limits to change in a trait under selection (Wade
1979). When the reproductive success of most males is approximately equal, the
potential (and opportunity) for sexual selection decreases whereas when there is high
variance among males in reproductive success, there is also high opportunity for
sexual selection.
Theoretical models suggest that at low population densities, decreased competition for mates allows more males to have access to females (Eshel 1979; Shuster & Wade 2003; Kokko & Rankin 2006). Thus, aggressive behaviors decrease and, as a result, variation in male reproductive success and opportunity for sexual selection also decrease. By contrast, high densities result in increased competition and increased variation in male reproductive success as more males are excluded from breeding and resort to the less successful alternative tactics (Kokko & Rankin 2006). This relationship between density and behavior has been found in many organisms (McLain 1982; Cade & Cade 1992; McLain 1992; Jirotkul 1999; but see Head et al. 2008). However, interpreting the results of other examples is less clear. Contrary to predictions of the models, territorial male *Rhodeus sericeus* at high densities have decreased aggression and decreased increased variation in male reproductive success (Reichard et al. 2004a, 2004b). At high densities males expressing different ARTs have equal reproductive success (Reichard et al. 2004b).

The intensity and direction of sexual selection is also affected by the ratio of sexually receptive males to sexually receptive females in a population at any time (Operational sex ratio, OSR; Emlen & Oring 1977; Kvarnemo & Ahnesjo 1996). The predictions for effects of OSR on behavior are similar to those for density. A male-biased OSR should cause an increase in male competition for mates and result in high variance in male reproductive success (Emlem & Oring 1977; Kvarnemo & Ahnesjö 1996). By contrast, a female-biased OSR should result in decreased male competition and low variance in male reproductive success (Emlen & Oring 1977; Kvarnemo & Ahnesjö 1996). Thus, a female biased OSR may allow more males
opportunities to mate. Empirical data supports these predictions with high levels of aggression and also high variance in reproductive success in male biased populations (*Oryzias latipes*, Grant et al. 1995; *Clethrionomys glareolus*, Klemme et al. 2007; Mills & Reynolds 2003; Jirotkul 1999) and lower aggression and lower variance in male reproductive success in female biased populations (*Aidabennius sphinx*, Neat & Locatello 2002).

Density and OSR are dynamic parameters that can differ between populations and change temporally within a single population. If OSR and density are important factors underlying sexual selection, then ecological parameters may directly influence selection for secondary sexual characteristics and behaviors, such as those associated with the expression of ARTs (Kokko & Rankin 2006; Kasumovic et al. 2008; Reichard et al. 2008; but see Head et al. 2008).

Pupfishes of the genus *Cyprinodon* provide an ideal experimental system for testing how demographic parameters may influence the evolutionary consequences of ARTs. The breeding system of *Cyprinodon* spp. is characterized by three male reproductive tactics: territoriality, satellite behavior and female mimicry (Itzkowitz 1969; Kodric-Brown 1986; Leiser & Itzkowitz 2002, 2003a). In the natural population of the endangered pupfish, *C. elegans*, OSR varies from about equal to male biased across years (Leiser & Itzkowitz 2002). Additionally, there is variation in the effects of OSR on pupfish breeding systems. Biased OSRs have no effect on territorial aggression in *C. variegatus* (Leiser & Itzkowitz 2004) whereas a male-biased OSR results in increased aggressive interactions between territorial males in *C. pecosensis* (Kodric-Brown 1988). Additionally, a male-biased OSR results in higher
frequencies of territoriality in *C. variegatus* (Leiser & Itzkowitz 2004). A contrasting pattern is found in *C. pecosensis* where a greater proportion of males defend territories under a female-biased OSR (Kodric-Brown 1988).

The foundation of the *Cyprinodon* breeding system, territoriality, is also density dependent. High densities favor the development of territoriality over dominance (*C. pecosensis*, Kodric-Brown 1988b; *C. variegatus*, Leiser & Itzkowitz 2004). Increasing density leads to increased aggression by dominant males (*C. variegatus*, Leiser & Itzkowitz 2004), however, density does not influence the aggressive behaviors by territorial males (*C. pecosensis*, Kodric-Brown 1988b). Finally, density did not influence territorial male spawning behavior in either *C. variegatus* (Leiser & Itzkowitz 2004) or *C. pecosensis* (Kodric-Brown 1988). While these studies examined relationships between demographics and behavior associated with ARTs, behavioral observations of spawnings may not accurately indicate male reproductive success (see Leiser & Itzkowitz 2004). To date, no study has identified the fertilization success of males expressing ARTs or quantified the roles of demographics on the success of males expressing ARTs in this genus.

The objective of this research is to examine the reproductive consequences of alternative reproductive tactics among males and to examine the effects of population demographics on male behavior and reproductive success. Based upon theoretical models, I hypothesize that density and sex ratio will alter individual male behavior. However, specific predictions of how density and OSR influence behavior are not possible due to conflicting evidence for closely related species. Additionally, I hypothesize that behavioral differences related to the expression of alternative
reproductive tactics result in differences in reproductive success. Specifically, I predict that males that control resources through aggression and territoriality are will sire more offspring than males relying upon less aggressive alternative reproductive tactics such as satellite behavior and female mimicry. Finally, based upon theoretical models, I also predict that a male-biased OSR and low population densities will be associated with higher variance in reproductive success and also a greater opportunity for sexual selection than a female-biased OSR or high population densities.

METHODS

Study system

The endangered Comanche Springs pupfish, *Cyprinodon elegans*, is native Reeves and Jeff Davis counties, TX. Typical for the genus, male *C. elegans* exhibit three conditional alternative reproductive tactics that correlate with size: 1) defending a territory, 2) utilizing the areas around territories as a satellite and 3) sneaking spawns (Leiser & Itzkowitz 2002, 2003a). Territorial males are usually large (50-60 mm), show blue/green breeding coloration on their anterior dorsal side and defend small areas containing algal mats or rocks (median territory size = 0.225 m²) by chasing non-territorial conspecifics and heterospecific intruders (Itzkowitz 1969; Leiser & Itzkowitz 2003a). Territorial males provide no direct parental care, although indirect care may be given to eggs laid inside a male’s territory (Kodric-Brown 1986). Intermediate sized males (40-45 mm) display a satellite tactic in which they patrol the water column above between 2 to 6 large males’ territories. Satellite males have
breeding coloration similar to that of territorial males but garner matings by interrupting spawns between territorial males and females, or by spawning with a female in a male's territory when he is occupied elsewhere in the territory (Leiser & Itzkowitz 2002, 2003a). Finally, the 'sneaker' tactic is displayed by small males (30-35 mm). Sneakers range widely and frequently enter and exit the breeding grounds. They exhibit a cryptic female-like morphology and either spawn when the territorial male is occupied or they sidle and spawn alongside a large male and female (Leiser & Itzkowitz 2002, 2003a).

Alternative reproductive tactics in pupfish are conditional depending on male size and social interactions. Male *C. variegatus* of all sizes can be territorial under laboratory conditions (Leiser & Itzkowitz 2004), but small males do not hold territories in the field (Leiser & Itzkowitz 2004). In *C. elegans*, large males are typically territorial, although if large males are not present in the natural population, males of intermediate size defend territories while small males remain sneakers (Leiser & Itzkowitz 2003a). Thus, only the relatively largest males in the population are territorial. One assumption of ARTs as a conditional strategy is that males expressing different tactics have equal fitness only at the switch-point between the tactics (Gross 1996). Behavioral observations show that territorial males spawn more than satellite or sneaker males (Kodric-Brown 1983, 1986; Leiser & Itzkowitz 2003a).
**Maintenance of fish**

All experiments were conducted at the Dexter National Fish Hatchery and Technology Center (Dexter, NM U.S.A.). The breeding stock of *C. elegans* maintained at this facility was founded in the 1970’s. There is no evidence that this population has genetically diverged from either a second hatchery stock, the natural refugia population at Balmorhea State Park (Toyahvale, TX), or the last remaining natural population of this species (Gumm, unpublished data).

Fish were separated by sex and maintained in 435-l fiberglass cattle tanks at high densities that minimized territoriality and aggression between fish. Stock tanks had a flow-through of 15 l/min and were part of an 8000-l re-circulating system that was held at a constant temperature (24.4° C). Fishes were exposed to a 14 L:10 D cycle with fluorescent lighting to simulate daylight (CoralLife). They were fed Catfish Crumble #2 and Spirulina flake food (Aquatic Ecosystems) thrice daily and supplemented with frozen blood worms.

**Experimental design**

Trials were conducted in 151-l aquaria (92 x 46 x 43 cm) with no substrate. The test aquaria were connected to a flow-through system, which maintained constant temperature and clean water while minimizing disturbance of the fish. Spawning mops (n = 6) were placed throughout the tank, equidistant from each other. The number of spawning mops was held constant across treatments to avoid the confounding effects of resource availability on behavior (Gumm & Itzkowitz 2007). Aquaria were covered on three sides with black plastic to prevent distraction from the
surrounding environment. All trials were conducted from May 7, 2007 to August 7, 2007 with up to 4 trials being conducted simultaneously.

At the beginning of each trial, all adults were measured for standard length (SL: snout to base of caudal peduncle) and placed in one of four treatment groups: 1) Low density (1 large male, 1 intermediate male, 1 small male, 3 intermediate females), 2) Female biased (1 large male, 1 intermediate male, 1 small male, 6 intermediate females), 3) Male biased (2 large males, 2 intermediate males, 2 small males, 3 intermediate females), and 4) High density (2 large males, 2 intermediate males, 2 small males, 6 intermediate females). The densities were chosen to avoid male injury due to intense male-male aggression associated with extremely high densities. The degree of sex-ratio bias examined in this experiment is within that seen in natural habitats (Leiser & Itzkowitz 2002). All females were of intermediate size (40-50 mm) and there were no female size differences between treatment groups.

Before the trial began, all males were implanted with visible elastomer tags for individual identification (Northwest Technologies Inc.). These tags do not influence male-male interactions or female preferences for males (personal observation). Within each trial, all males received the same number and color of tags, although their placement varied for identification purposes. In female-biased and low density treatments, all males received the same color tag placed either pre-dorsally, post-dorsally or at the base of the dorsal fin. In male-biased and high density treatments, each male received one color tag pre-dorsal fin and a different colored post-dorsal fin tag. The order of the colors was randomized between the two males within a size class.
Videos (30 min.) were taken 4, 24 and 48 h after trial initiation. Videos from the 48 h observation were analyzed using JWatcher software (available: http://www.jwatcher.ucla.edu/). Each male was identified and observed individually for the last 20 min of the video. I recorded the number of chases, bites and lateral displays performed by each male as well as how often a male came within one mop-length of a spawning mop and the identity of that mop.

At the termination of each replicate, small (1 x 2mm²) fin clips were taken from the lower caudal fin of all adult individuals and fixed in 100% ethanol for later genetic analysis. All spawning mops were collected and fertilized eggs removed and placed in Petri dishes with water from their tank. Resulting embryos were allowed to develop and monitored at least every 24 h for non-developing embryos. Upon hatching, all offspring were fixed in 100% ethanol for DNA extraction at a later date.

**Paternity analysis**

DNA was isolated from parental individuals and offspring using the Quigen DNAeasy kit according to the manufacturer’s instructions. All individuals were genotyped using six tetranucleotide loci developed for the genus *Cyprinodon* (Table 2; Burg et al 2002). Loci had a mean of 12 alleles (range 8 – 20) and observed heterozygosities ranged from 0.51 to 0.91. Each 10 UL PCR reaction contained 0.875u AmpliTaq Gold® DNA polymerase (Applied Biosystems); 1X GeneAmp® 10X PCR buffer; 2.5 mM MgCl₂; 1.5 mM dNTPs; 0.5 µl each, forward and reverse primers and reagent grade sterile water. Forward primers were labeled with one of four fluorescent dyes (6-FAM, PET, NED, VIC). PCR conditions were the same for
all loci and thermal cycling (ABI 9700 Genescan thermal cycler) consisted of a touchdown protocol beginning with a denaturing step of a 95° for 9 min, followed by 33 cycles of 94° for 45s, an initial annealing temperature of 56° for 45s, and an extension at 72° for 60s. The annealing temperature decreased by 0.2°C for every cycle, and ramp time was 0.05s. The final extension cycle was 7 min at 70°C. PCR fragments were multiplexed and were resolved on an ABI 3100xl Automated Sequencer and scored using Genemapper 4.0 software (Applied Biosystems).

Parents were assigned using CERVUS 3.0 software (Marshall et al. 1998; Kalinowski et al 2007). CERVUS assigns parents using LOD scores, the log-likelihood that the putative parent is a true parent relative to other candidates (Marshall et al. 1998, Kalinowski et al. 2007). Parent pairs were assigned with 95% confidence for 432 of 461 offspring (94%) and relaxing confidence to 80% did not result in any additional parent pair assignments. Four offspring were not tested for parentage because they were genotyped at fewer than 3 loci. In 10 cases, multiple parents obtained the same LOD score and in these cases, parentage was assigned to the parent that was also assigned the majority of offspring that were collected at the same time from the same spawning mop. The natural history of the pupfish supports this spatial and temporal assignment because females typically lay 1-5 eggs sequentially with the same male (Leiser & Itzkowitz 2003a; personal observation).

**Estimating opportunity for sexual selection**

The opportunity for sexual selection is defined as $I = \frac{V_W}{W^2}$ where $V_W$ is the variance in reproductive success and $W$ is the mean reproductive success (Wade
1979; Shuster & Wade 2003). This measure represents the upper limits on the rate of change and degree of change for a trait under selection (Shuster & Wade 2003).

**Statistical analyses**

Data was assessed for normality with Kolmogorov-Smirnov tests and assessed for equal variances with F-tests and natural Log transformed when necessary. Data on percent offspring sired was arcsin square-root transformed. Analysis of variance (ANOVA) was used to test for differences in all male behaviors and percentage of offspring sired between density treatments across male size classes and between OSR treatments across size classes. Density and OSR treatments were analyzed separately to identify the independent effects of each variable. Fisher’s PLSD post hoc tests were conducted on all ANOVAs to identify differences between groups. Number of eggs spawned, number of eggs hatched, percent mortality of eggs, numbers and percentages of females spawning and opportunity for sexual selection (I) were compared between density treatments and OSR treatments with unpaired t-tests. Finally, Pearson’s product moment correlation was used to test for relationships between male behaviors and number of offspring sired.

**RESULTS**

**Effects of density on reproductive success**

High and low densities did not affect the total number of eggs spawned (Unpaired t-test: $t_{16} = -0.15$, $P = 0.89$) or percentage of egg mortality that occurred
(t_{16} = -0.75, P = 0.46). Despite higher numbers of females in the high density treatment, there was no difference in absolute numbers of females that spawned (Unpaired t-test: t_{16} = 1.85, P = 0.08) or in the percentage of females spawning (t_{16} = -1.60, P = 0.13).

Density did not influence the number of males siring offspring (Unpaired t-test: t_{16} = 1.68, P = 0.12). However, in the low density treatment, a greater percentage of males sired offspring than in the high density treatment (Unpaired t-test: t_{16} = -2.60, P = 0.02). Additionally, males in the low density treatment sired a greater percentage of total offspring/trial than did males in the high density treatment (Two-way ANOVA: F_{1,72} = 11.66, P = 0.001; Table 2). Large males sired a higher percentage of offspring than intermediate or small males (F_{2,72} = 70.03, P < 0.0001). There was a significant interaction between density and male size class (F_{2,72} = 6.94, P = 0.002). The difference in percent of offspring sired between large males and intermediate/small males was greater in the low-density treatment than it was in the high-density treatment.

**Effects of OSR on reproductive success**

Biased OSR treatments did not differ in total number of eggs spawned (Unpaired t-test: t_{16} = -1.45, P = 0.17), number of females that spawned (Unpaired t-test: t_{16} = 1.48, P = 0.16) or in the percentage of females that spawned (t_{16} = -1.93, P = 0.07). However, OSR treatments did affect egg mortality. A higher percentage of egg did not develop to late stage embryos in the male-biased OSR treatment than female-biased OSR treatment (t_{15} = -2.31, P = 0.04).
The absolute number of males or percentage of males siring offspring did not differ between male- and female-biased OSR treatments (Unpaired t-tests: Number of sires: $t_{16} = -1.51, P = 0.15$; Percent of males siring: $t_{16} = 1.11, P = 0.28$). Males in the male-biased OSR treatment, on average, had higher percent reproductive success than males in the female-biased OSR (Two-way ANOVA: $F_{1,75} = 6.79, P = 0.01$, Table 2). Additionally, large males sired a higher percentage of offspring than intermediate or small males ($F_{2,75} = 36.90, P < 0.0001$). There was a significant interaction between OSR and male size class ($F_{2,75} = 4.15, P = 0.02$). The difference in percent offspring sired by large males compared to percent sired by intermediate/small males was lower in the male-biased OSR treatment than it was in the female biased OSR treatment. That is, with more male competitors, large males were less successful compared to those competitors.

**Opportunity for sexual selection**

Density did not influence the opportunity for sexual selection ($J$) (Unpaired t-test: $t_{16} = 1.73, P = 0.10$). There was a trend for an increased opportunity for sexual selection in male- vs. female-biased OSR treatments, however, this was not significant ($t_{16} = -1.91, P = 0.07$).

**Effects of density on male behavior**

Population density had no effect upon number of chases or bites by males (Two-way ANOVA: chases: $F_{1,51} = 0.28, P = 0.60$, Figure 1; bites: $F_{1,51} = 0.28, P = 0.72$, Figure 2). Large males in high and low density treatments performed more
chases and bites than intermediate or small males (chases: $F_{2,59} = 19.58, P < 0.0001$, Figure 1; bites: $F_{2,59} = 11.17, P < 0.0001$, Figure 2). Additionally, intermediate males in both density treatments performed more chases than small males (Figure 2). There was no significant interaction for chases or bites between density and male size class (chases: $F_{2,59} = 0.11, P = 0.90$, Figure 1; bites: $F_{2,59} = 0.90, P = 0.41$, Figure 2). Lateral displays were performed mainly by large males and occurred more frequently in the high-density treatment, but were too infrequent in the low-density treatment for statistical analyses. Finally, although males in the low density treatment approached spawning mops more often than males in high density treatment, this effect was not statistically significant (Two-way ANOVA: $F_{1,59} = 3.74, P = 0.06$, Figure 3). Large males approached spawning mops more than intermediate and small males and intermediate males approached spawning mops more than small males ($F_{2,59} = 51.58, P < 0.0001$, Figure 3). There was no interaction between density and male size class in spawning mops approaches ($F_{2,59} = 1.89, P = 0.17$, Figure 3).

**Effects of sex ratio on male behavior**

The number of chases or bites performed by males did not vary as a function of OSR (Two-way ANOVA: chases: $F_{1,51} = 0.09, P = 0.77$, Figure 4; bites: $F_{1,51} = 1.05, P = 0.31$, Figure 5). Large males chased and bit more than intermediate or small in both male- and female- biased sex ratios (chases: $F_{2,51} = 13.72, P < 0.0001$, Figure 4; bites: $F_{2,51} = 7.49, P = 0.002$, Figure 5). There was no interaction between OSR and male size for bites (bites: $F_{2,51} = 1.18, P = 0.32$), however, there was a significant interaction between male size and OSR for chases (chases: $F_{2,51} = 3.82, P = 0.03$).
The difference in number of chases between large males and intermediate/small males was greater in female-biased compared to male-biased OSRs. In a female-biased OSR treatment, large males performed many more chases than intermediate or small males, whereas in a male-biased OSR treatment, the number of chases by large and small males was much more similar and were both greater than chases by intermediate males (Figure 4). Lateral displays were performed mainly by large males and occurred more frequently in the male-biased OSR treatment. Lateral displays were too infrequent in the female-biased OSR treatment for statistical analyses.

Finally, while OSR did not affect the number of times males approached spawning mops (Two-way ANOVA: F1, 51 = 1.83, P = 0.18, Figure 6), but there was a significant effect of male size class with large males approaching spawning mops more than intermediate or small males (F2, 51 = 6.18, P < 0.004, Figure 6). There was no significant interaction effect between OSR and male size class on approaches to spawning mops (F2, 51 = 2.75, P = 0.07, Figure 6).

**Relationship between aggression and reproductive success**

There were positive relationships between most male behaviors and reproductive success across density treatments. In both the low and high density treatments, the number of offspring sired by a male was significantly correlated to number of chases (Figure 7), bites and approaches to a spawning mop (Table 3). However, the relationship between lateral displays and reproductive success differed across densities. In the low density treatment, the number of offspring sired was
significantly correlated to number of lateral displays performed; this relationship was not significant in the high density treatment (Table 3).

OSR treatment did not influence the relationships between male aggressive behaviors and reproductive success. Number of chases, bites, and approaches to spawning mops were all significantly correlated to number of offspring sired by a male (Table 3). There was no relationship between lateral displays and number of offspring in either male- or female-biased OSR treatments (Table 3).

DISCUSSION

Reproductive success of male ARTs

In these experiments, aggressive males had the highest number of offspring. This result supports previous observations in *Cyprinodon* using spawning behavior to estimate male reproductive success (Kodric-Brown 1988a, 1988b, Leiser & Itzkowitz 2002, 2003). In *C. pecosensis*, males spawn more often when they are also engaged in fights (Kodric-Brown 1988b). Additionally, the presence of females increases aggression between males (*C. variegatus*, Leiser 2003) and females may incite male-male aggression in order to assess mate quality (*C. bovinus*, Leiser et al. 2006).

Among male mating tactics, territorial males are the most aggressive and they have the highest reproductive success. The unequal reproductive success across male tactics supports predictions of the status dependent model for stability of conditional male ARTs (Gross 1984; Repka & Gross 1995; Gross 1996; Gross & Repka 1998).
Males benefit from increased reproductive success as they grow and are able to maintain territories via high aggression.

Although density treatments did not result in differences in absolute number of offspring, density did influence the proportion successful males and the percentage of offspring sired by different size classes of males. In the low density treatment, more of the males participated in spawnings. Additionally, while intermediate and small males sired the same proportion of offspring in high and low density treatments, the difference between their fitness and the fitness of large males was much greater in low density treatments. Large males were about half as successful in the high density treatment as they were in the low density treatment; indicating that males exhibiting alternative tactics had different advantages in various environments. From a large-male perspective, low densities are advantageous and will result in siring most of the offspring. However, from a satellite or female mimic perspective there is a tradeoff in the benefits of different densities. These males will be more likely to spawn in low density, but have higher fitness, relative to large males, in high densities.

There are subtle differences in the effects of density and OSR treatments on male reproductive success. Across OSR treatments, there were similar numbers of offspring and proportion of males involved in spawning. Males sired higher percentages of offspring in the female-biased treatment and large males sired more offspring than intermediate or small males in both OSR treatments. However, males expressing different tactics will benefit from different OSRs. Large males have greater advantages in the female-biased OSR while intermediate and small males benefit from the male-biased OSR.
Large males sired fewer offspring in high density and male-biased OSR treatments than they did in low density and female-biased OSR treatments. These differences are likely due to the number and types of competitors present. Specifically, when only one large male was present, he was the only territorial male, whereas treatments with two large males allowed for both males to express territoriality. Previous studies have considered males lacking territorial neighbors to be exhibiting dominance instead of territoriality (Itzkowitz 1977; Kodric-Brown 1988; Leiser & Itzkowitz 2004). In C. variegatus, dominant males were involved in more spawns than territorial males (Leiser & Itzkowitz 2004). Itzkowitz (1977) suggests that it might be too costly to exert dominance at high densities and thus, males defend territories. My results suggest that the costs, in terms of aggressive behaviors, are the same at low and high densities. Thus, if males in low density and female-biased OSR treatments are considered dominant, there is no difference in aggressive behaviors between dominant and territorial males in this species. Another confounding issue is that single large males defend larger areas than multiple large males, who split the testing aquaria into two territories. Patrolling larger areas may incur a higher energetic cost but may be beneficial via increased spawning area for females. There is a positive relationship between area defended and number of male spawning events (Itzkowitz 1978; Kodric-Brown 1988). Therefore, large males in low density and female-biased OSR treatments are expected to have higher reproductive success due to defending the entire tank as a single territorial male.

Differential male success across demographic treatments could also have been influenced by female spawning behavior, however there was no difference in absolute
number of eggs spawned or hatched between treatments. Additionally, similar
numbers and percentages of available females spawned across treatments. The lack of
differences in female spawning behavior is surprising because the female-biased OSR
and high density treatments had twice as many females present than did male-biased
OSR and low density treatments. There are a number of non-mutually exclusive
explanations for this result. First, males may have aggressively rejected some females
as mates (Ludlow & Itzkowitz 2007). Females in this study were matched for size to
control for female quality, as larger females are more fecund (Leiser 2003), however,
males may have rejected some females by evaluating a different indicator of quality.
Alternatively, female-female competition may have suppressed spawning by some
females. Female aggressive behaviors have never been examined in *Cyprinodon*
although I observed some females directing aggressive behaviors towards small males
(pers obs.). Finally, there may be a limiting effect of space or spawning substrate on
female spawning. Females typically range widely and express preferences for
particular spawning substrates (Itzkowitz 1969; Kodric-Brown 1977, 1983; Ludlow et
al. 2001). The spawning mops used in these trials are artificial and while females did
lay eggs on them, they may be considered low quality and rejected by some females
as suitable spawning substrate.

**Effects of demographic parameters on male behavior**

In *C. elegans*, population density had little effect upon territorial male
aggressive behaviors. This is consistent with density effects in other species of
*Cyprinodon* (Kodric-Brown 1988, Leiser & Itzkowitz 2004). While absolute
aggression performed by males does not change, each non-territorial male garners less aggression in the high density treatment. The only aggressive behavior that did increase with density is lateral displays. This is likely due to having multiple territorial males in high density treatments. Lateral displays are common between size matched males (Leiser 2003) and in *C. elegans*, occur most often between territorial neighbors (Gumm, unpublished data).

Male size is closely associated with male reproductive tactics (territorial, satellite or female mimicry) and aggression in density treatments. Large, territorial males were the most aggressive and approached spawning mops most frequently. Intermediate males (who are typically satellites in the natural population) differed from small males (who typically exhibit female mimicry) in chasing behavior and mop approaches. Leiser & Itzkowitz (2002) found that in the natural population of *C. elegans*, the number of attacks by males differed among the three male tactics. The pattern of aggression was the same as I found, with large males most aggressive, followed by intermediate and then small males. Taken together, these results suggest that the size classes did reflect different tactics expressed by males within this study. The hierarchy of aggressive behaviors and space use (as indicated by mop approaches) was consistent across density treatments.

The effects of OSR on male behavior are more complex. There was no effect of OSR bias on chasing, biting or mop approaching behavior and large males performed all three behaviors more than intermediate/small males across OSR treatments. However, there was an interaction between OSR treatment and male size class for chasing behavior. In the female-biased OSR treatment, large males chased
about twice as much as intermediate or small males, whereas in the male biased OSR treatment, small males chased almost as much as large males. Small males typically engage in aggression with other small males while large males exhibit aggression primarily to territorial neighbors (Leiser & Itzkowitz 2002). Additionally, territorial male aggression is context dependent (Gumm, unpublished data). Thus, in the male-biased OSR treatment, large males were likely involved with neighbors, and intermediate males who pose a threat to the territory. If small males do not attract much aggression from large males, they might be free to direct aggression towards other small males. Finally, intermediate males chased less than small males, perhaps because large males direct more aggression toward them, creating an interaction between initiating and receiving aggression. That is, receiving aggression from larger males may inhibit intermediate males from expressing aggression. The effects of previous experience on male behavior is not known in this species, however becoming subordinate or losing a fight can inhibit subsequent aggression (Forkman & Haskell 2004).

**Opportunity for sexual selection**

The opportunity for sexual selection did not vary as a function of density or OSR treatments. However, there was a trend for higher opportunity for sexual selection when OSR is male-biased. This trend towards more intense sexual selection in a male-biased OSR matches predictions of theoretical models and some empirical studies (Jones et al. 2004). Given that there was no difference in male aggressive behavior based with a male-biased OSR, the increase in opportunity for sexual
selection may be due to effects of demographic parameters on female choice. Female *Cyprinodon* prefer larger males (*C. variegatus*, Draud 1996; *C. bifasciatus*, Ludlow et al. 2001), more colorful males (*C. pecosensis*; Kodric-Brown 1977, 1983), and males defending territories containing rocky substrates (*C. bifasciatus*, Ludlow et al. 2001). In some systems, increased benefits of mate choice or decreased costs of mate searching may lead to increased choosiness in male biased OSR or high density treatments (Shelly & Bailey 1992; Berglund 1994; Jirotkul 1999). However, opposite patterns are seen in systems where females encounter increased male harassment with increased male encounters (Lauer et al. 1996; Mills & Reynolds 2003). Finally, my results match those of another recent study conducted by Head et al. (2008) which found no effects of density or OSR on male-male competition behavior or opportunity for sexual selection in *Poecilia reticulata*. Further studies of *Cyprinodon* examining wider ranges of density and OSR treatments may be more conclusive.

**Conclusions**

Large males were more aggressive and sired more offspring in all experimental treatments. Additionally, there were significant correlations between common aggressive behaviors and number of offspring sired by males. Correlations between behavioral differences and differences in reproductive success among males indicate that selection is occurring, although there were no differences in opportunity for sexual selection based on variation in demographic parameters.
ACKNOWLEDGEMENTS

I thank Murray Itzkowitz for support and guidance throughout all stages of this research. The Dexter National Fish Hatchery and Technology Center provided laboratory space, equipment, training and financial support for this project. I thank S. Baker, K. Heideman, C. Keeler-Foster, R. Martin, C. Sykes and G. Zegers for their exceptional technical assistance and thoughtful insight. Helpful comments on previous versions of this manuscript were provided by A. Echelle, T. Mendelson, J. Nyby and J. Swann. All methods conformed to the laws of the USA, the state of New Mexico and are approved by Lehigh University IACUC (#A3877-01). Funding for this study was provided by a Texas Academy of Science Student Research Grant, a Lehigh University Summer Research Fellowship and a Sigma Xi GIAR to JMG.
Table 4.1. Microsatellite markers used to assess paternity in *C. elegans*. Annealing temperatures ($T_A$) are shown for each locus.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer 5' -&gt; 3'</th>
<th>GenBank</th>
<th>$T_A$</th>
<th>Repeat motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>GATA2</td>
<td>A: TCGGATGCTCAGTCAGTACG</td>
<td>AF398010</td>
<td>45/48</td>
<td>(GATA)$_{30}$</td>
</tr>
<tr>
<td></td>
<td>B: ATGAACAACGAGTCACACGC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GATA9</td>
<td>A: TCTTGGTGAAAAAGGAAGCTATACG</td>
<td>AF398012</td>
<td>50/53</td>
<td>(GATA)$_{29}$</td>
</tr>
<tr>
<td></td>
<td>B: GCGTTCTCGAGCTTGTATG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GATA26</td>
<td>A: ACCTCTCAAGGCAAAAACAACG</td>
<td>AF398018</td>
<td>50/53</td>
<td>(GATA)$_{39}$</td>
</tr>
<tr>
<td></td>
<td>B: TCCCACGATAGCTCAGACG</td>
<td></td>
<td></td>
<td>(GACA)$_{3}$</td>
</tr>
<tr>
<td>GATA39</td>
<td>A: CCTTAGGGTGCTGTGTGAGC</td>
<td>AF398019</td>
<td>50/53</td>
<td>(GATA)$_{28}$</td>
</tr>
<tr>
<td></td>
<td>B: TGGGAGGTGAACTAAGATGCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GATA104</td>
<td>A: CCATATTGCTCCCATAGCTG</td>
<td>AF398022</td>
<td>50/53</td>
<td>(GATA)$_{41}$</td>
</tr>
<tr>
<td></td>
<td>B: TTCGTCATACCTGATGCTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CmD16</td>
<td>A: CGGAAATGATATGAGCAGACC</td>
<td>AF398025</td>
<td>58</td>
<td>(GATA)$_{27}$</td>
</tr>
<tr>
<td></td>
<td>B: GGTCCTGATTTTACCCTC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2. Treatment, sample size per treatment, number of embryos and mean reproductive success (measured as percentage of offspring sired). Means and range are shown.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Small Mean (range)</th>
<th>Intermediate Mean (range)</th>
<th>Large males Mean (range)</th>
<th>Offspring Mean (range)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Density</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 male: 3 female</td>
<td>11.3 (0-19)</td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>High Density</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 male: 6 female</td>
<td>8.9 (0-40)</td>
<td>2.1 (0-33.3)</td>
<td>3.8 (0-42.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female biased OSR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 male: 6 female</td>
<td>8.9 (1-23)</td>
<td>4.28 (0-100)</td>
<td>43.4 (0-100)</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Male biased OSR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 male: 3 female</td>
<td>13.7 (1-25)</td>
<td>84.4 (0-100)</td>
<td>43.4 (0-100)</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>13.9 (1-35)</td>
<td>13.9 (1-35)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.3. Correlations between number of offspring sired and male behaviors.

<table>
<thead>
<tr>
<th>Density</th>
<th>OSR</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Density</td>
<td></td>
<td>0.690</td>
<td>0.0423</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.632</td>
<td>0.0466</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.170</td>
<td>0.0264</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.505</td>
<td>0.0153</td>
</tr>
<tr>
<td>High Density</td>
<td></td>
<td>0.698</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.748</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.722</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male biased</td>
<td>OSR</td>
<td>0.578</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.462</td>
<td>0.0071</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.327</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.998</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female biased</td>
<td>OSR</td>
<td>0.690</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.632</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.170</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.492</td>
<td>0.0037</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.505</td>
<td>0.0090</td>
</tr>
<tr>
<td>Offspring-chases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offspring-bites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offspring-displays</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offspring-Approaches</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

72
Figure 4.1. The natural log of the mean number of chases that are performed by small (white bars), intermediate (light grey bars) and large (dark grey bars) in high and low density. Error bars represent standard error of the mean.
Figure 4.2. The natural log of the mean number of bites that are performed by small (white bars), intermediate (light grey bars) and large (dark grey bars) in high and low density. Error bars represent standard error of the mean.
Figure 4.3. The natural log of the mean number of times small (white bars), intermediate (light grey bars) and large (dark grey bars) approached spawning mops in high and low density. Error bars represent standard error of the mean.
Figure 4.4. The natural log of the mean number of chases that are performed by small (white bars), intermediate (light grey bars) and large (dark grey bars) in female and male biased OSR treatments. Error bars represent standard error of the mean.
Figure 4.5. The natural log of the mean number of bites by small (white bars), intermediate (light grey bars) and large (dark grey bars) in female and male biased OSR treatments. Error bars represent standard error of the mean.
Figure 4.6. The natural log of the mean number of approaches to spawning mops by small (white bars), intermediate (light grey bars) and large (dark grey bars) in female and male biased OSR treatments. Error bars represent standard error of the mean.
Figure 4.7. Relationship between number of chases performed by males and number of offspring sired in (a) male biased OSR treatment, (b) female biased OSR treatment, (c) low density treatment and (d) high density treatment.
V. CONCLUDING REMARKS

In species with alternative reproductive tactics, social interactions determine the choice of tactic expressed by each male, and also the success of each tactic. I examined patterns of social behavior between males expressing alternative reproductive tactics and the reproductive consequences of these tactics in the Comanche Springs pupfish, *Cyprinodon elegans*.

From Chapter 2, I suggest that association patterns of *C. elegans* are often the result of indirect social interactions. I found no evidence for direct associations between males expressing alternative reproductive tactics and territorial male reproductive success. Additionally, there was no direct relationship between female visits to a territory and spawning. When stimuli were artificially placed in naturally occurring territories, females approached bottled males and also approached bottled females, suggesting that females may express non-independent mate choice in this species.

Female mimics and satellite/territorial males were not attracted to bottled males in a territory but responded to females in bottles differently. Specifically, female mimics approached females more than satellite/territorial males. By exhibiting behaviors similar to those of females, female mimics may increase their access to females and decrease aggression from territorial males. While territorial males discriminate against female mimics (unpublished data), the female mimics' behavioral adaptations may still contribute to the success of female mimicry. This is the first study to test differences relating to female mimic and satellite males behavior.
in relation to females and suggests that males using these different tactics use
different behaviors to obtain matings.

In Chapter 3, I examined how territorial males respond to various intruders in
their territory. Territorial males discriminated between conspecifics based both size
and sex. This is the first study using a dichotomous choice test design to examine
male pupfish behavior and the results suggest that territorial males express mate
choice preferences, discriminate between conspecific male competitors based on
threat and forgo courtship for aggression. Each of these results presents opportunities
for future study of territorial male behavior. Additionally, I found that territorial male
C. elegans expressed sex recognition of female mimics. However, aggression towards
female mimics was context dependent. Specifically, territorial males had strong
preferences for female mimics when presented with size-matched females, but not
when female mimics when presented with larger males. Context dependent treatment
of female mimics suggests that frequency or density dependence may play a role in
the maintenance of alternative reproductive tactics in this system. Frequency
dependent selection can facilitate male alternative reproductive tactics as an
evolutionary stable strategy (Gross 1991). Thus, female mimics may attract relatively
little aggression, and potentially gain opportunities to spawn, if they are relatively
rare compared to larger male intruders.

Finally, in Chapter 4, I tested relationships between behaviors associated with
alternative reproductive tactics and reproductive success. Additionally, given the
potential for frequency and density effects, I also examined the role of demographic
parameters on behavior and reproductive success. Male aggression in all density
treatments was based on male size, which in turn was closely associated with male breeding tactic (territorial, satellite or female mimic). However, there was no effect of density on territorial male aggressive behaviors. These results are consistent with density effects in other species of *Cyprinodon* (Kodric-Brown 1988, Leiser & Itzkowitz 2004). Despite the lack of density effects, operational sex ratio did influence male aggressive behavior. In the female-biased OSR treatment, large males chased about twice as much as intermediate or small males. However, in the male-biased OSR treatment, small males chased almost as much as large males.

In addition to being the most aggressive, territorial males have the highest reproductive success. There is positive relationship between aggression and number of fertilized offspring, supporting previous findings in *Cyprinodon* that relied on observations of spawning to estimate male reproductive success. Demographic parameters also influenced the relative success of males expressing alternative reproductive tactics. In low density, a higher proportion males participated in spawnings. Additionally, while intermediate and small males sired the same proportion of offspring in high and low densities, the difference between their fitness and the fitness of large males was much greater in low density. Large males were about half as successful in the high density treatment than they were in the low density treatment. This indicates that males exhibiting alternative tactics will have different advantages in various environments. From a large male perspective, low densities are advantageous and result in siring most of the offspring. However, from a satellite or female mimic perspective there is a tradeoff in the benefits of different densities. These males will be more likely to spawn in low density, but have higher
fitness relative to large males in high densities. A similar pattern was found due to biased sex ratios. Males sired higher percentages of offspring in the female-biased OSR and large males sired more offspring than intermediate or small males in male- and female-biased OSRs. However, males expressing different tactics will benefit from different OSRs. Large males have greater advantages in a female-biased OSR while intermediate and small males benefit from a male-biased OSR. Overall, the unequal reproductive success across male types supports predictions of the status dependent model for stability of conditional male ARTs. Additionally, the influences of demographic factors may have played a role in the evolution of or continued maintenance of alternative reproductive tactics in this system.
REFERENCES


Taborsky, M., Hudde, B. & Wirtz, P. 1987. Reproductive behavior and ecology of 
*Symphodus (Crenilabrus) ocellatus*, a European wrasse with four types of 
male behaviour. *Behaviour*, 102, 82-118.

Temeles, E. J. 1990. Northern harriers on feeding territories respond more 
aggressively to neighbors than to floaters. *Behavioral Ecology and 
Sociobiology*, 26, 57-63.

Temeles, E. J. 1994. The role of neighbors in territorial systems: when are they 'dear 

Tomkins, J. L. & Brown, G. S. 2004. Population density drives the local evolution of 

Naturalist*, 114, 742-747.

Waltz, E. C. 1982. Alternative mating tactics and the law of diminishing returns: the 

## APPENDIX A

### ETHOGRAM

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chase</td>
<td>A rapid swimming movement oriented directly toward another individual.</td>
</tr>
<tr>
<td>Bite</td>
<td>Nipping at the body or fins of another fish. This behavior can be distinguished from a chase by movement of the jaws and physical contact between the two fish.</td>
</tr>
<tr>
<td>Lateral display</td>
<td>Performed at varying distances, a male will orient himself parallel to a competitor and raise the dorsal, pelvic and anal fins. This display may also involve a C-shape position by bending of the flank and curving the tail towards the opponent while extending the dorsal and ventral fins.</td>
</tr>
<tr>
<td>Spawn</td>
<td>After a female enters a territory, she is approached by a male. The female will bite at the substrate while the male orients himself directly alongside the female with his snout near the females operculum. The pair form an S-shape and complete spawning with a jerking motion when a single demersal egg is laid (Barlow 1961; Itzkowitz 1969).</td>
</tr>
</tbody>
</table>
APPENDIX B

In Chapter 2, a 3 x 3 Factorial ANOVA was presented to test differences between the type of stimulus (male, female, empty bottle) and the type of approaching individual (satellite male, female mimic or female). One limitation of this test is the lack of pair-wise comparisons across and between all groups. This test shows that fish did not approach empty bottles as much as bottles with male and female stimuli in them. By comparing conspecifics approaches between bottles that only had male or female stimuli in them, a multivariate ANOVA will provide pair-wise comparisons between stimulus type (male and female) and identity of approaching individual (male, female or female mimic). Similar numbers of females males approached bottles containing male and female stimuli ($F_{1,39} = 0.78$, $P = 0.38$). Satellite/territorial males also approached the two stimuli at a similar frequency ($F_{1,39} = 0.29$, $P = 0.59$). Female mimics approached bottles containing females more than bottles containing male stimuli ($F_{1,39} = 17.51$, $P < 0.001$). Therefore, the interaction presented in Chapter 2 is due to the difference in female mimic behavior in response to different types of stimuli.
APPENDIX C

In Chapter 3, strength of preference (SOP) is calculated as the amount of time spent with one stimulus – the amount of time spent with the second stimulus. Standardizing SOP to account for variation in male response did not result in any differences in results of interpretation of the data. If SOP is calculated as (the time spent near one stimulus - time near the other stimulus) / (total time spent with stimuli), there are significant differences in SOP based on treatment (ANOVA: F_{3,72} = 24.74, P < 0.0001). Territorial males had a stronger preference for female mimics when they were presented with size-matched small females than when they were presented with large males (Fisher’s PLSD: P < 0.0001). There was no difference in SOP for large males that were presented with either a small male or large female (Fisher’s PLSD: P = 0.50). Territorial males preferred large females more when they were presented with a small female than a large male (Fisher’s PLSD: P < 0.0001). Finally, there was no difference in SOP for small females presented with either a large female or a size-matched female mimics (Fisher’s PLSD: P = 0.28).

Additional statistics were performed to ensure that the observed focal male association patterns were different from those expected by random chance. For each treatment, a one sample t-test assessed whether the mean SOP (calculated as a percentage of time spent with either stimulus; see previous paragraph) differed from 0. An SOP of 0 indicates no preference for either stimulus. SOP significant differed from 0 in all treatments (large male/small male: t_{18} = 6.38, P < 0.0001; large male/large female: t_{18} = 5.58, P < 0.0001; large male/small male: t_{18} = 3.27, P =
0.005; small male/small female: $t_{18} = 3.88, P = 0.001$). These results indicate that observed patterns of association by focal males were not based on random chance.
VITA

JENNIFER M. GUMM

Date of birth: October 27th, 1980
Parents: Rebecca (Power) Gumm and Lawrence Gumm (deceased)

EDUCATION

Lehigh University
Ph.D. 2009
Integrative Biology
Dissertation title: Sexual selection and alternative reproductive tactics in Cyprinodon elegans
Advisor: Dr. Murray Itzkowitz

Texas State University at San Marcos
M.S. 2004
Biology
Thesis: Species and Mate Quality Recognition in Poecilia latipinna (Poeciliidae)
Advisor: Dr. Caitlin R. Gabor

Millikin University
B.S. 2002
Biology

University of Durham, U.K.
Undergraduate work Fall 2000

PUBLICATIONS


GRANTS AND AWARDS

2008 Thorne Fellowship, Lehigh Dept. of Biological Sciences
2008 Summer Research Fellowship, Lehigh College of Arts & Sciences
2008 Wilks Award, Southwestern Association of Naturalists
2007 Sigma Xi Grant-in-Aid-of-Research
2007 Southwestern Association of Naturalists
2007 Howard McCarley Student Research Grant
2007 Texas Academy of Science Student Research Award
1998-Present Allied Pilots Association Scholarship
2003-2004 Texas State University Deans’ List
2002 Judith & G. Richard Locke Award, Millikin University
2002 Biology Honors Award, Millikin University
1998-2002 John and Ula Leighty Science Scholarship
1998-2002 Millikin University Merit Award
1999-2001 Millikin University Deans’ List,
1998-1999 National Deans’ List
1998-1999 Millikin University Freshman Honors Scholar

RESEARCH EXPERIENCE

2006 **RESEARCH ASSISTANT**, mating behavior and conservation of Southwestern pupfishes, Dr. Murray Itzkowitz (Lehigh University) Summer 2006.

2005 **RESEARCH ASSISTANT**, behavioral decisions of Beaugregory damselfish, Dr. Murray Itzkowitz (Lehigh University)

2002-2004 **RESEARCH ASSISTANT**, Texas Higher Education Coordinating Board Advanced Research Projects to Dr. Caitlin Gabor (Texas State University)

2001 **UNDERGRADUATE RESEARCH**, mating behavior of *Drosophila melanogaster*, Dr. Marianne Robertson (Millikin University)

---

**TEACHING EXPERIENCE**

2007 **LECTURER**, Department of Biological Sciences, Lehigh University
   *EVOLUTION*

2004-2008 **TEACHING ASSISTANT**, Department of Biological Sciences, Lehigh University
   *ANIMAL BEHAVIOR LABORATORY*, Spring 2008
   *GENETICS LABORATORY*, Fall 2004
   *EVOLUTION LECTURE*, Fall 2005
   *GENETICS LECTURE*, Fall 2006

2001-2002 **TEACHING ASSISTANT**, Department of Biology, Millikin University
   *ECOLOGY, EVOLUTION AND BEHAVIOR*, Fall 2001
   *ATTRIBUTES OF LIFE*, Spring 2002

2001 **MARINE BIOLOGY EDUCATION INTERNSHIP**, Mote Marine Laboratory, Sarasota, FL

2000 **MARINE ECOLOGY EDUCATION INTERNSHIP**, Marine Resources Development Foundation, Key Largo, FL


---

**INVITED PRESENTATIONS**

2007  UNIVERSITY OF TEXAS, Department of Integrative Biology. Dear enemies and female mimics: Recognition in a west Texas pupfish.

2007  SIDNEY GUTIERREZ MIDDLE SCHOOL, 7th and 8th grade science classes. Conservation and behavior of endangered pupfishes.


2006  LONG ISLAND UNIVERSITY- C. W. POST CAMPUS, Department of Biology. Species and mate-quality recognition in a unisexual/bisexual species complex.

2003  LEHIGH UNIVERSITY, Department of Biology. Species and mate-quality recognition in Poecilia latipinna.

CONTRIBUTED PRESENTATIONS


2008  Gumm, J. M. Sex recognition by pupfish: Who are female mimics fooling? SOUTHWESTERN ASSOCIATION OF NATURALISTS, Memphis, TN. Wilks Award for Best Student Paper

2008  Gumm, J. M. Sex recognition by pupfish: Who are female mimics fooling? TEXAS ACADEMY OF SCIENCES, Corpus Christi, TX. Best Graduate Student Presentation Competition


2004  **Gumm, J. M.** Asexuals looking for sex: Conflict in species and mate-quality recognition in *Poecilia latipinna* (Poeciliidae). **TEXAS STATE UNIVERSITY BIOLOGY COLLOQUIUM.** Best Graduate Presentation Competition- **HONORABLE MENTION**


2002  **Gumm, J. M. & E. Ellinger.** The effects of multiple males during mating in *Drosophila melanogaster.* Poster, **MILLIKIN UNDERGRADUATE RESEARCH SYMPOSIUM- 3rd place.**

2002  **Gumm, J. M., & E. Ellinger.** The effects of multiple males during mating in *Drosophila melanogaster.* Poster, **ILLINOIS STATE ACADEMY OF SCIENCE,** Edwardsville, IL.


---

**PROFESSIONAL AFFILIATIONS**

Animal Behavior Society, 2002-Present
American Society of Ichthyologists and Herpetologists, 2002-Present
AAAS, 2005-2007
Southwestern Association of Naturalists, 2006-Present
Texas Academy of Science, 2007-Present
Sigma Xi, 2006-Present
Sigma Zeta Natural Sciences Honor Society, 2001-Present
Tri Beta Biology Honor Society, 2005-Present

---

**PROFESSIONAL SERVICE**

Biological Organization of Graduate Students, Secretary, May 2006-January 2007
Lehigh University Graduate Student Senate Unit Representative, 2004-2005
Reviewer for Southwestern Naturalist