THE EFFECT OF ENVIRONMENTAL CONTAMINANTS ON MATING DYNAMICS AND POPULATION VIABILITY IN A SEX-ROLE REVERSED PIPEFISH

A Dissertation

by

CHARLYN GWEN PARTRIDGE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2009

Major Subject: Zoology

THE EFFECT OF ENVIRONMENTAL CONTAMINANTS ON MATING DYNAMICS AND POPULATION VIABILITY IN A SEX-ROLE REVERSED PIPEFISH

A Dissertation

by

CHARLYN GWEN PARTRIDGE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	Adam G. Jones
Committee Members,	Duncan MacKenzie
	Gil Rosenthal
	Thomas J. DeWitt
Head of Department,	U. J. McMahan

December 2009

Major Subject: Zoology

ABSTRACT

The Effect of Environmental Contaminants on Mating Dynamics and Population
Viability in a Sex-Role Reversed Pipefish. (December 2009)
Charlyn Gwen Partridge, B.S., Nicholls State University;
M.S., University of South Alabama
Chair of Advisory Committee: Dr. Adam G. Jones

Understanding how anthropogenic activity impacts the health and viability of wildlife populations is one of the most important tasks of environmental biology. A key concern related to bi-products of human activity is the accumulation of environmental pollutants within aquatic environments. Pollutants such as endocrine disruptors and heavy metals have the potential to impact both human and wildlife populations in contaminated areas. While much research has focused on how these compounds impact natural selection processes, such as viability and reproduction, their effect on sexual selection processes is not as clear. The goal of this dissertation was to address how environmental contaminants impact sexual selection processes in a sex-role reversed pipefish and evaluate how these effects may impact long-term population viability. Here we show that short periods of exposure to environmentally relevant concentrations of a synthetic estrogen result in male pipefish with female-like secondary sexual traits. While these males are capable of reproduction, exposed males are discriminated against by females in mate choice tests. In natural populations, this type of discrimination could reduce male mating opportunities, potentially reducing their reproductive success. In an additional component of this dissertation, it was discovered that pipefish populations around Mobile Bay, specifically Weeks Bay, are currently being exposed to significantly elevated levels of mercury. These populations are genetically distinct from coastal populations but moderate levels of gene flow occur among sites, and gene flow between contaminated and non-contaminated population may be influencing how environmental contaminants are impacting genetic diversity and population viability. In the case of endocrine disruptors, migration between contaminated and non-contaminated sites may negatively impact population viability. Morphological traits induced with exposure to contaminants may be maintained for extended periods of time, therefore, the effect the exposed phenotype has on mating dynamics and sexual selection could be carried to non-contaminated sites if exposed individuals move to new populations. On the other hand, immigration of individuals from non-contaminated sites into contaminated areas may help maintain genetic diversity within exposed populations. In conclusion, the work presented in this dissertation shows that the presence of environmental toxins can significantly impact sexual selection processes, which in turn can have profound effects on the viability and future evolutionary trajectory of populations. Future work in this area should not only address how these toxins impact individual fitness, but should also address how population structure may be influencing the severity of these compounds on natural populations.

DEDICATION

To my son, Brayden

Proof that anything is possible

ACKNOWLEDGEMENTS

I sincerely thank my advisor, Adam Jones, for all of his guidance and giving me the opportunity to pursue this field of study. Thank you for believing in my abilities even when I didn’t. I could not have asked for a better advisor. Also, a special thanks to my committee: Gil Rosenthal, Duncan McKenzie, and Thomas DeWitt for your help and guidance.

Thanks to all of the past and present members of the Jones lab: Kenyon Mobley, Kim Paczolt, Clay Small, Nick Ratterman and Sunny Scobell. Thank you for the wonderful conversations, your critical and valuable scientific input, the laughs, the coffee, the beer and most importantly your friendship. You have make these years most enjoyable. But better yet, you all have made me a better scientist.

Thank you to everyone in the Department of Biology at the University of South Alabama, especially Anne Boettcher. Thank you for putting up with my needs and demands. Thank you for always finding space for my fish even though there was never space available. And finally, thank you for your valuable scientific input and time. This project would not have been possible without your help. Thank you to the Department of Marine Sciences at the University of South Alabama, especially Nancy Merrill, for allowing me to take over your conference room everyday for the past year.

Thank you to all of my pipefish collectors, feeders and fellow collaborators over the years: Emily Boone, Dan Martin, Molly Mintz, Brenna Elmn, Melaine Rodriguez, Zach Cress, Delayne Ferguson, Tammy Wells, Melissa Kominek Smith, Nadia Joiner and

Elizabeth May. You have made working in Alabama in the middle of the summer while fighting alligators most enjoyable.

Thank you to the guys at Weeks Bay National Estuarine Research Reserve and Grand Bay National Estuarine Research Reserve, especially Scott Phipps and Chris May, for providing boats and being patient while I tried to collect water samples without falling face first in the mud. Also, thank you to David Evans of NOAA for agreeing to run all of our fish tissue samples.

Tack to everyone at Kristineberg Marine Research Station in Fiskebacskil, Sweden. Ingrid Ahnesjö, Anders Burglund, Charlotta Kvarnemo, Sarah Robinson-Wolrath, Krammagnus Robinson-Wolrath, Maria Lissåker, Melanie Raimondo, and Simo Rintakoski. Thank you for all of your help and providing great insight for this project. A special thanks to Gunilla Rosenqvist and Krammagnus Robinson-Wolrath for taking the time to help me when I needed it the most.

Thank you to my parents, Charles and Lynda Partridge, for always believing in me and supporting me in every way possible. It has been through your strength and support that I have been able to make it this far.

Thank you to my son, Brayden Partridge, for making me laugh and reminding me that there are more important things in life than work.

Lastly, thank you to my fiancé, Doug Haywick. Words cannot even begin to describe how much your love and support mean to me. I love you.

This research was approved by the Animal Care and Use Committee at both the University of South Alabama (AUP#: 06035 and 09021) and Texas A&M University (AUP#: 2004-253).

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

TABLE Page

CHAPTER I

INTRODUCTION

Natural and sexual selection are the dominant forces driving evolution in natural populations. Natural selection mechanisms consist of factors associated with an individual's ability to compete for limited resources, thus impacting individual survivorship and reproductive ability (Darwin 1859). Sexual selection mechanisms involve processes that influence an individual's ability to compete for access to mates, thus impacting their reproductive fitness only (Darwin 1859, 1871). Both natural and sexual selection mechanisms are influenced by various behaviors and traits that contribute to variance in fitness, the currency on which these selective forces act. For example, naturally selected traits may consist of the ability to effectively forage, the ability to escape predation or disease resistance, while sexually selected traits may consist of individual attractiveness and preference or the ability to physically gain access to mates. Understanding how and to what degree these traits contribute to the overall variance in relative fitness is vital to understanding how they contribute to species evolution. In addition, when environmental disturbances, such as global warming or water pollution, interfere with an individual's competitive ability by altering these selected traits, their relative fitness can be significantly affected. The effects of these disturbances can also be observed on a population level if the impact is broad enough to affect a significant proportion of the individuals within the population.

This dissertation follows the style of *Behavioral Ecology and Sociobiology*.

One of the greatest concerns facing the environment is the impact of anthropogenic activity on the health of natural populations (Schwarzenbach et al. 2006). One bi-product of continued human activity is the accumulation of environmental contaminants, such as pesticides, heavy metals and endocrine disrupting compounds, which are released into the ecosystem. These contaminants are typically synthetic exogenous compounds and can interfere with normal physiological or genetic mechanisms in wildlife and human populations (Jorgenson 2001; Neri et al. 2006; Hotchkiss et al. 2008; Crump and Trudeau 2009). Many of these compounds are known to have deleterious effects on wildlife populations. Understanding how they affect reproductive fitness and population viability is essential to determining their impact on the ecosystem. These contaminants can impact population viability on multiple levels by affecting both natural selection processes, through decreased survivorship and reproduction (Hayes et al. 2002, 2003; Hill and Janz 2003; reviewed in Reish et al. 2006; Menza et al. 2007; Kidd et al. 2007; Maunder et al. 2007; Peters et al. 2007; Schäfers et al. 2007; Cevasco et al. 2008; Haeba et al. 2009; Haxton and Findlay 2008; Rippey et al. 2008; Brunelli et al. 2009; Guimaraes et al. 2009; Xu et al. 2009), and sexual selection processes, by impacting mating dynamics (Crews et al. 2007; Arellano-Aguilar and Garcia 2008; Coe et al. 2008; Saarisota et al. 2009). While much effort is currently being placed on assessing the impact of environmental contaminants on aspects of natural selection processes, such as development and reproductive ability, the impact of these compounds on sexual selection processes are not as well documented. However, because sexual selection is a major selective force driving evolution, factors that disrupt this mechanism can also have long-term effects on evolutionary processes and population viability. Thus, only by determining the extent which particular environmental contaminants affect both natural and sexual selection processes can we begin to fully comprehend their impact on natural ecosystems.

One class of environmental contaminants that has received a large amount of attention due to their persistence in the environment and because of the negative impact on aquatic populations is endocrine disruptors. Endocrine disruptors are typically synthetic compounds that interfere with normal hormone function. Many pharmaceuticals, such as 17α-ethinylestradiol (EE2), and industrial compounds, such as bisphenol A, have been shown to have adverse effects through disruption of the endocrine system in many organisms (reviewed in Lintelmann et al. 2003; reviewed in Hotchkiss et al. 2008; Cevasco et al. 2008; Schoenfuss et al. 2008; Vandenberg et al. 2009). Xenoestrogens, synthetic estrogen mimics, affect animals by binding to estrogen receptors and eliciting inappropriate estrogenic responses (Lutz and Kloas 1999). Males inhabiting contaminated areas have been shown to have decreased sperm production, increased liver weights, and increased vitellogenin levels (Munkittrick et al 1994; Sumpter 1995; Sumpter and Jobling 1995; Folmar et al. 1996; Harries et al. 1997; Allen et al. 1999; Rangel 2000). Also, female and male lab reared zebrafish exposed to xenoestrogens as larvae had delayed gonadal and behavioral development compared to unexposed larvae (Hill and Janz 2003). In addition to regulating gonadal development, sex hormones also regulate traits that influence mate selection (reviewed in Folstad and

Karter 1992; Andersson 1994; Parker et al. 2002; McGraw et al. 2006) and thus exposure to xenoestrogens may have an effect on sexual selection processes.

Sexual selection mechanisms often lead to the development of elaborate ornaments that are used by individuals to assess mate quality and expression of these traits is often correlated with mating success (reviewed in Andersson 1994). Many of these secondary sexual traits are under the control of sex steroids, such as estrogen and testosterone, and expression is dependent on hormone concentration (reviewed in Folstad and Karter 1992; Andersson 1994; Parker et al. 2002; McGraw et al. 2006). Because secondary sexual trait expression varies with hormone levels more than that of primary sex traits (i.e., gonads), these secondary sex traits may be more sensitive to small levels of environmental pollution, particularly when these pollutants are endocrine disrupting compounds. Recent studies have shown that exposure of individuals to environmental contaminants, such as pesticides and synthetic hormones, can disrupt expression of these traits by either suppressing expression (Arellano-Aguilar and Garcia 2008) or inducing expression of the trait in the opposite sex (Ueda et al. 2005). While current research has focused on the effects of endocrine disruptors on primary sexual organs (Hill and Janz 2003; Moncaut et al. 2003; Toft and Baatrup 2003; Vandenbergh et al. 2003; Park and Kidd 2005; Xu et al. 2008), fewer studies have examined the impact of these compounds in regard to sexual signaling and selection (Bell 2001; Bjerselius et al. 2001; Oshima et al. 2003; Coe et al. 2008; Saarisota et al. 2009). In this study, the impacts of exposure to a synthetic estrogen on multiple aspects of reproductive success in a sex-role reversed pipefish were evaluated. This is currently the most comprehensive study to evaluate the impact of an endocrine disruptor on factors that significantly affect reproductive success and the first study to evaluate how exposure affects reproduction in a sex-role reversed organism.

In addition to understanding how environmental contaminants affect an individuals' relative fitness, it is also essential to understand how these effects translate into population viability. The genetic structure of populations within and around contaminated areas may influence how strong of an effect environmental pollutants may have on genetic diversity and population health. For example, environmental contaminants may have a significantly larger effect on smaller populations with little migration, because the negative effects of environmental contaminants on genetic diversity will be compounded by the populations' susceptibility to inbreeding and genetic drift. Euryhaline organisms are a particularly interesting group in which to address these types of questions. Euryhaline fish can readily move between estuarine and coastal environments, with some species even inhabiting freshwater systems. Studies have shown that some sub-populations of euryhaline fish have become genetically distinct from their coastal counterparts even when a high potential for high gene flow exists (Beheregaray and Sunnucks 2001). This "divergence-with-gene-flow" model suggests that local selection pressure between these distinct ecosystems allows for significant divergence between these populations. Since environmental contaminants tend to be more concentrated in freshwater and estuarine environments because they are normally closer to the source of the contamination and they experience less dilution by non-contaminated water sources, isolated populations in these areas may be more impacted by the presence of pollutants than if these populations maintained significant gene flow with their coastal counterparts. This is because continued gene flow between the estuarine and coastal populations may help to offset the loss of genetic variation normally experienced in populations with high levels of environmental contamination. In this dissertation, the genetic structure of coastal, estuarine and freshwater populations of a euryhaline pipefish were addressed. This information was then used to infer how environmental toxins may be impacting population viability in contaminated areas.

Study Species

For this dissertation, I examine how exposure to a synthetic estrogen, 17α-ethinylestradiol (EE2), impacts sexual selection processes in a sex-role reversed pipefish and I suggest how population structure may be influencing the affect of these environmental contaminants on long-term population viability. Some of the sampling areas where these pipefish were collected are known to contain historically high levels of mercury, thus, I also assessed the total mercury concentration of multiple pipefish populations in these areas.

In order to determine how EE2 may be affecting sexual selection processes, the components of sexual selection that contribute most to the variance in relative fitness had to be established. For this study, the broad-nosed pipefish, *Syngnathus typhle*, was used as the model system. *S. typhle* has historically been one of the dominant model systems for understanding questions concerning sexual selection theory in sex-role reversed organisms. Because of the amount of data that are currently available for *S.*

typhle in terms of mate choice mechanisms, this species was the most appropriate of the pipefish species for examining the degree to which pre - and post-copulatory selection mechanisms influence the opportunity for sexual selection.

Once the major factors influencing the opportunity for sexual selection were obtained, we utilized the Gulf pipefish, *Syngnathus scovelli*, as the model system for addressing questions concerning how environmental estrogens may be impacting sexual selection processes and how population structure may influence the affect of these contaminants. *S. scovelli* was selected as the model species for these studies for multiple reasons. First, *S. scovelli*, provides an excellent model for examining how endocrine disruptors impact mating dynamics because this species experiences very strong sexual selection on females (Jones et al. 2001) and the secondary sexual traits in females appear to be regulated by estrogen pathways (Ueda et al. 2005). The mating system of this species is polyandrous and intraspecific competition occurs between females rather than males (Jones et al. 2001). In *S. scovelli*, intraspecific competition and strong sexual selection have led to extreme sexual dimorphism compared to *S. typhle*, resulting in larger and more ornamented females. Furthermore, genetic evidence has shown that mating is not random in Gulf pipefish populations and that mating females are larger and more ornamented than average females, suggesting that these characteristics are under sexual selection (Jones et al. 2001). As previously stated, ornamentation of female pipefish appears to be regulated by estrogenic hormones and exposure of pipefish to EE2 causes males to develop secondary sex traits normally only found in females (Ueda et al. 2005). This suggests that estrogen mimicking compounds may affect mating dynamics by affecting the expression of secondary sex traits in these pipefish. Second, the Gulf pipefish provides an excellent system to evaluate how population structure may influence the effect of environmental contaminants on population viability. *S. scovelli* is a euryhaline pipefish and the most common pipefish found along the Gulf of Mexico. While most populations of *S. scovelli* are located either in marine or brackish environments, freshwater populations have been found in every state along the northeastern Gulf of Mexico (McLane 1955; Whatley 1969; Targett 1984; Viola 1992). Currently there are no data evaluating population structure of this species. However, because of the presence of multiple populations across varied ecological habitats, it is possible that these groups may be genetically distinct. Also, some groups within Mobile Bay are located near areas that have been historically known to contain high levels of mercury, a heavy metal pollutant (Novoveska, 2005). Thus using this species allowed me to determine where pipefish populations are being impacted by environmental contaminants and how patterns of gene flow between pipefish populations may be influencing the effect of these contaminants.

Dissertation Justification

The ultimate goal of this study was to understand how environmental contaminants may be affecting sexual selection processes and how these effects may impact long-term population viability. The central hypothesis of this dissertation is that environmental contaminants may be affecting population viability by disrupting mating dynamics and that population structure may play a significant role in determining how severe this impact is on natural populations. In order to address this question I first determined which factors contribute most to the opportunity for sexual selection in pipefish. Second, I examined which behavioral and morphological factors contributed to male and female mate preference, since variation in mating success should strongly influence the opportunity for sexual selection in *S. scovelli*. Third, I evaluated how exposure of a synthetic estrogen, EE2, affected multiple components of reproductive fitness, including mate choice, male mating success and male reproductive success. In addition, I examined how exposure to EE2 affected the expression of secondary sexual traits in males and female courtship behavior. Fourth, since population structure may impact how severely environmental contaminants affect population health, I evaluated the population structure of *S. scovelli* throughout the Gulf of Mexico and Mobile Bay. Lastly, I examined whether wild populations of Gulf pipefish around Mobile Bay were being impacted by the presence of environmental contaminants.

Chapter Outline

In CHAPTER II, I examined the relative importance of pre-copulatory and post-copulatory sexual selection processes in the broad-nosed pipefish, *S. typhle*, to determine if post-copulatory processes, particularly male cryptic choice, significantly affected offspring survivorship. In addition, I assessed the relative importance of various pre- and post-copulatory processes on the opportunity for sexual selection.

In CHAPTER III, I explored how morphological and behavioral traits influenced mating preferences of male and female pipefish given that data from Chapter II suggested that variance in female mating success contributes significantly to the opportunity for selection in pipefish.

In CHAPTER IV, I conducted a comprehensive study to examine how short term exposure to environmentally relevant concentrations of an endocrine disruptor, 17α-ethinylestradiol (EE2), impacted multiple levels of reproductive fitness, including mate choice, male mating success and male reproductive success in *S. scovelli.* In addition, I examined how exposure to EE2 affected the development of secondary sexual traits in male pipefish. Finally, I evaluated whether exposure of female pipefish to EE2 significantly affected female behavioral traits that are associated with male preference.

In CHAPTER V, I evaluated patterns of gene flow of the Gulf pipefish throughout the Gulf of Mexico and Mobile Bay in order to determine whether estuarine and freshwater populations were genetically distinct from their coastal counterparts.

In CHAPTER VI, I surveyed populations of pipefish and other small planktivorous fish within Weeks Bay, Mobile Bay and Grand Bay, MS to determine if pipefish populations are being impacted by the presence of environmental contaminants. For this study I concentrated mainly on the presence of mercury since specific locations within Weeks Bay have historically shown high levels of mercury.

In CHAPTER VII, I summarize the results from the previous chapters and relate how these components may impact long-term population viability and selection processes in populations located in areas contaminated by environmental pollutants.

CHAPTER II

THE EFFECT OF PERCEIVED PARASITE LOAD ON POST-COPULATORY MATE CHOICE IN A SEX-ROLE REVERSED PIPEFISH*

Introduction

Post-copulatory sexual selection has become a subject of considerable discussion in animal behaviour (Eberhard 1996; Birkhead and Møller 1998; Birkhead and Pizzari 2002). One area that has been much debated over the past few years is cryptic mate choice, a mechanism by which members of one sex (usually females) bias offspring parentage during or after mating in favour of mates with a preferred phenotype. In organisms with conventional sex roles, cryptic choice can occur in a number of different ways, some of which include termination of copulation, prevention of sperm transfer, discarding sperm, sperm choice, reduction of offspring number, and differential allocation of resources (Eberhard 1996; Snook 2005). Although many important studies during recent years have started to clarify some of these processes, many of them still are not well understood.

Particular studies of cryptic choice usually focus on one or a few possible cryptic choice mechanisms, and some of these mechanisms have been better documented than others. For example, one of the least controversial mechanisms is differential allocation

---

*With kind permission from Springer Science+Business Media: *Behavioral Ecology and Sociobiology,* The effect of perceived female parasite load on post-copulatory male choice in a sex-role reversed pipefish, 63, 2009, 345-364, C Partridge, C Kvarnemo, I Ahnesjö, K Mobley, A Berglund and AG Jones, Figures 1-4.

(Cunningham and Russell 2000; Sheldon 2000; Kolm 2001), in which a female mated to a less preferred male may allocate fewer resources to those offspring in order to retain resources for future mating events, possibly with superior males. Other types of cryptic female choice that are slightly more controversial than differential allocation, but are still reasonably well documented, include female influence on the number of sperm transferred by a male (Gronlund et al. 2002; Pilastro et al. 2002; Tallamy et al. 2002) and differential storage of sperm (Otronen 1990; Pizzari and Birkhead 2000, Bussière et al. 2006). The most controversial mechanism, sperm choice, results from a bias in the use of sperm by the female. In this case, females preferentially choose sperm from more attractive males, and this choice can be independent of sperm number or quality. Sperm choice is controversial for several reasons (Telford and Jennions 1998; Birkhead 2000; Eberhard 2000; Kempenaers et al. 2000; Pitnick and Brown 2000; Birkhead and Pizzari 2002; Eberhard 2004, Bussière et al. 2006). First, it is often difficult to disentangle female effects from male effects based upon the outcome of fertilization patterns. Second, sperm are extremely difficult to observe, so it can be difficult to determine whether patterns of paternity are a product of the number of sperm transferred by rival males or a consequence of choice by the female. Third, for species in which physical sperm removal by the female does not occur, it can be difficult to envision mechanisms by which a female could choose among sperm once they are already in her reproductive tract.

Despite the opportunities that sex-role reversed taxa afford with respect to the study of sexual selection, they have been studied very little with respect to cryptic choice or gamete competition. The present study was motivated by the expectation that in sex-role reversed taxa with male brooding and strong sexual selection on females, cryptic male choice or some form of egg competition might evolve in such a way as to reinforce the operation of pre-copulatory choice. As implied above, it can be difficult to conduct an experiment that provides definitive evidence for post-copulatory sexual selection and clearly distinguishes between male and female effects (Birkhead 2000; Eberhard 2000; Birkhead and Pizzari 2002). However, Pitnick and Brown (2000) offered one solution when they suggested that manipulation of perceived mate quality could be used to delineate these effects. Thus, the approach suggested by Pitnick and Brown (2000), while not a panacea, provides one solution to the challenge of disentangling male from female effects during post-copulatory sexual selection.

We take advantage of this approach in the broad-nosed pipefish, *Syngnathus typhle*, a sex-role reversed species that has been a useful model for the study of numerous topics in sexual selection (Berglund and Rosenqvist 2003; Wilson et al. 2003). Organisms in which both males and females mate multiply have a high potential for sexual conflict and thus have the potential for cryptic choice to evolve (Gowaty 1997; Jones 2002). The broad-nosed pipefish is such a species. In addition, like all other species in the family Syngnathidae, *S. typhle* exhibits male pregnancy (Dawson 1985; Wilson et al. 2001; Jones and Avise 2003; Stölting and Wilson 2007). During copulation, females deposit eggs into the male's brood pouch, where the eggs are fertilized, and males carry the embryos until parturition. The brood pouch is a highly vascularized structure that provides aeration, protection and osmoregulation (Quast and Howe 1980; Watanabe et al. 1999; Carcupino et al. 2002), and may serve as a site for nutrient transfer (Haresign and Shumway 1981; Berglund et al. 1986a) for the developing offspring. In *S. typhle*, both males and females exhibit a high rate of multiple mating, with 90% of males obtaining multiple mates for a single brood, and sexual selection acts more strongly on females than on males (Berglund et al. 1988; Berglund et al. 1989; Vincent et al. 1992; Jones et al. 1999).

The phenomenon of male pregnancy makes the broad-nosed pipefish an exceptionally good model for studies of post-copulatory sexual selection for two important reasons. First, sexual selection acts more strongly on females, so the gametes of relevance to cryptic choice are eggs, which compared to sperm are large and easy to observe, an attribute that makes it possible to track the fate of each individual gamete throughout the male pregnancy. Second, this species exhibits a significant level of brood reduction (Ahnesjö 1992a). In other words, a substantial fraction of the eggs (approximately 30 percent on average) transferred to the male fail to result in offspring. There is some evidence that eggs from larger females are more likely to result in progeny at the end of the pregnancy than those from smaller females, suggesting that some sort of post-copulatory sexual selection may be at work in this species (Ahnesjö 1996). However, no experiment has yet addressed whether this maternity bias is based on male or female effects.

An additional key factor in the choice of *S. typhle* as the model for the present study is that the perceived quality of mates can be experimentally altered (Rosenqvist and Johansson 1995). Pipefish and other fishes are intermediate hosts of the trematode

Cryptocotyle lingua. This parasite penetrates the skin of the fish and encysts, resulting in small black dots produced by host pigment. Pre-copulatory mate choice experiments have shown that males prefer to mate with females that have a lower parasite load (Rosenqvist and Johansson 1995; Mazzi 2004). Furthermore, manipulation of perceived parasite load through the tattooing of females with small black dots produces the same preference effect (Rosenqvist and Johansson 1995). This approach allows for an experimental alteration of female attractiveness without affecting any other aspect of actual female quality or condition.

The present study was inspired by an interest in gamete choice, but given the lack of data on cryptic choice in pipefish, we used an experimental design covering multiple mechanisms of cryptic choice, including male effects on the number of eggs transferred, differential fertilization of eggs, and some forms of differential allocation of resources to offspring. Hence, this experiment had three objectives. First, by experimentally manipulating the apparent parasite load of females, we aimed to address if and to what extent a male's perception of female quality influences post-copulatory sexual selection via cryptic male choice. Second, we wished to take advantage of variation in female quality to investigate the role of female quality and egg size on embryo survivorship. Finally, we compiled data from a number of *S. typhle* studies to quantify, for the first time, the various potential sources of variance in relative fitness in females during sexual selection.

Methods and Materials

Field Sampling and Mating Experiment

Male and female *S. typhle* were collected in early May 2004, prior to their reproductive season, from the Gullmar Fjord on the Swedish west coast (58° 15' N, 11° 28' E). Pipefish were collected from shallow (1 – 6 m water depth) eelgrass (*Zostera marina*) beds using a beam trawl with a 2 mm mesh. Adult individuals were segregated by sex and maintained in 225 litre barrels connected to a flow-through natural seawater system at Kristineberg Marine Research Station.

We chose pairs of size- and colour-matched females and tattooed each female to produce one experimental and one control female per pair. The experimental female was tattooed with black dots using carbon-based tattoo ink (8-10 dots per side), and the control female was tattooed with the same number of colourless dots using deionized water, the solvent for the tattoo ink. Tattooing was performed by hand with a fine tattoo needle on fish anaesthetized in 2-phenoxyethanol (50 µl/L). A single male was allowed to mate with the two matched females (either a tattooed female followed by a control female or vice versa, Figure 2.1) one female at a time in a mating tank (40x25x70 cm). Each male thus represented a single replicate of the experiment, and we had two treatments that differed only with respect to the order of mating of tattooed versus control females (Figure 2.1).

All mating trials were observed, and the first female in each replicate was allowed to mate with the male until his brood pouch was approximately half full, at which time the female was removed from the tank. Most females required multiple copulation events to

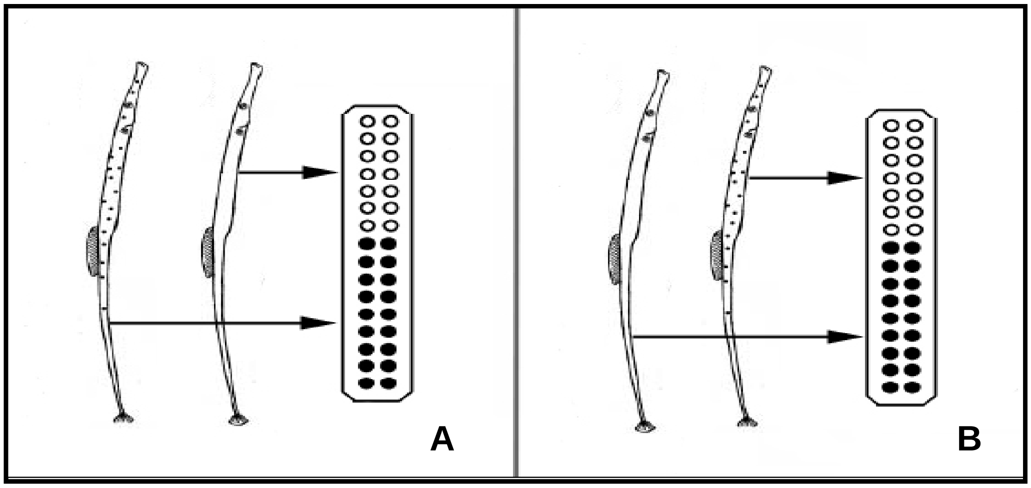


Figure 2.1: Experimental mating design. Each focal male (represented by brood pouch diagrams) mated with a pair of females. The experiment consisted of two treatments. In the first treatment (A) the black-dot tattooed female, altered to look parasitized, mated first, filling the lower part of the male's pouch with eggs, and the control female (tattooed with colorless dots) filled the remainder of the pouch. In the second treatment (B) the control female filled the lower part of the male's pouch and the black-tattooed female filled the remainder of the pouch.

fill their half of the male's brood pouch and at no time did we physically interrupt a copulation event. We recorded the number of copulations required to partially fill the male, which allowed us to calculate the number of eggs transferred per copulation, one potentially important factor in cryptic choice.

After a male's pouch was approximately half-filled by the first female, we partially anaesthetized the male by submerging his head and trunk (but not his brood pouch) in 2-phenoxyethanol (50 µl/L) and counted the number of eggs transferred by the first female by gently lifting the pouch skin folds. After the counting procedure, the male was placed in a tank with the second female and allowed to mate until the male's brood pouch was full. We again observed all copulations to determine the number of eggs transferred per copulation event and counted the total number of eggs transferred by the females into the brood pouch. At the end of each mating trial, we took caudal fin clips from females and preserved these tissue samples in 95% ethanol for genotyping. Mature eggs (approximately 10) were gently pressed out of the ovaries of each female and dried (60ºC, 24 hrs) for egg dry-weight measurements. Males were allowed to brood the embryos until well-defined eyespots were visible through the brood pouch. Finally, pregnant males and embryos were sacrificed by using a lethal dose of 2-phenoxyethanol (2 ml/L), and they were preserved in 95% ethanol.

Maternity Analysis

To determine the maternity of the offspring, we extracted DNA from all developing embryos using a 5% chelex/proteinase K digestion (Miller and Kapuschinski 1996).

Because eggs from individual females are positioned together within the brood pouch (Jones and Avise 1997a; Jones et al. 1999), it is not necessary to genotype every individual embryo to determine the position of the transition between embryos from the first and second female. Hence, we genotyped every sixth developed embryo within the brood pouch by using three to four hypervariable microsatellite loci developed by Jones et al. (1999) for *S. typhle*. Once we identified the approximate location of the spatial boundary between maternal genotypes within the brood pouch, we genotyped an additional 12 embryos around the boundary to determine the exact maternity of all embryos within the pouch. All fragment analysis was performed on an ABI Prism® 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) and peaks were called using ABI Prism® GeneMapper™ software (Applied Biosystems, Foster City, CA). The proportion of eggs that resulted in well-developed offspring was then calculated for each female by dividing the number of developed embryos that were genetically compatible with that female by the number of eggs initially transferred to the male by that female. In addition, the numbers of undeveloped and unfertilized eggs within the brood pouch were recorded, and their maternity was inferred by their spatial locations relative to genotyped embryos.

Statistical Analysis

A total of 34 males completed the study; however, data from six males were removed due to documented errors in egg counts, resulting in a total of 28 males statistically analyzed. Additionally, some analyses had sample sizes lower than 28 males due to missing data. Of the 28 males, 12 males (147.0 ± 7.2 mm, standard length ± SEM) mated with a colourless dot female first and a black dot female second; while the remaining 16 males (156.4 ± 8.1 mm) mated with black dot females first followed by colourless dot females. We performed transformations on the data set to obtain normality prior to analysis. Proportion of eggs surviving was exponentially transformed. We used natural log transformations on eggs transferred per copulation and the number of undeveloped eggs. Prior to transforming the number of undeveloped eggs, these values were increased by one to allow for transformation of zeros. The overall number of eggs accepted by the male per female was square root transformed. All statistical analyses were performed with JMP IN statistical software (SAS Institute, Carysfor, NC).

Results

Our results support the notion that brood reduction is an important phenomenon in this pipefish. On average, only about 70 percent of the eggs transferred to the brood pouch resulted in well-developed embryos (Table 2.1). This level of brood reduction is similar to what has been found in previous studies of this species (Ahnesjö 1992a).

The primary goal of the experiment was to look for cryptic male choice based on perceived female parasite load in *S. typhle*. First, we asked whether or not the male's perception of female parasite load affects the number of eggs a male receives per copulation. A female typically requires several mating attempts to partially fill the brood pouch of a male. Because we observed the mating trials and recorded all copulations, we were able to count the number of copulations required to transfer the

Table 2.1: The effects of treatment and order on the proportion of embryos that survived, the number of undeveloped eggs, the total number of eggs deposited per female, the number of eggs transferred per copulation, female length and egg dry weight. The table shows untransformed means ± standard errors of the means, but the p-values are derived from a paired t-test on data that were transformed to obtain normality (see text). α = 0.05.

	Treatment				**Order**			
Variable	**Clear tattoo**	**Black tattoo**	**No. males**	**t-test p-value**	**First female**	**Second female**	**No. males**	**t-test p-value**
Proportion of eggs surviving	0.65 ±0.07	0.75 ±0.05	28	0.31	0.76 ±0.06	0.64 ±0.07	28	0.23
Number of undeveloped eggs	4.5 ±0.9	5.8 ±1.6	26	0.83	4.8 ±1.1	5.5 ±1.5	26	0.74
Total number eggs transferred/female	39.9 ±3.8	42.9 ±4.0	28	0.55	45.7 ±4.3	37.1 ±3.2	28	0.10
Number of eggs per copulation	24.4 ±2.8	29.0 ±3.7	27	0.41	31.5 ±3.2	22.0 ±3.1	27	0.006
Female length (mm)	169.5 ±5.0	168.2 ±5.4	26	0.58	166.5 ±4.9	171.3 ±5.4	26	0.03
Egg mass (mg)	1.07 ±0.05	1.04 ±0.06	21	0.75	1.01 ±0.06	1.09 ±0.05	21	0.29

eggs and calculate the number of eggs transferred per copulation. If males were exercising cryptic choice at this stage, we would predict that they would receive more eggs per copulation from control females than from experimentally altered females, and no such pattern was evident (Table 2.1).

Second, cryptic male choice may operate through differential fertilization of eggs. Males appear to retain unfertilized eggs in their brood pouches until the end of the pregnancy, so this hypothesis predicts that we would find more undeveloped eggs from those transferred from the experimental females compared to that of the control females. Again, we saw no evidence for such a pattern (Table 2.1).

Finally, cryptic male choice could occur as a result of non-random embryo absorption by the male or by preferential allocation of resources to the embryos from control females. Under this hypothesis we would predict that eggs from the "non-parasitized" control females would experience a greater probability of producing viable offspring than would eggs from tattooed females. Once more, we found no evidence for cryptic male choice based on perceived female parasite load. Eggs from control females were not more likely to produce viable offspring compared to eggs from females tattooed to look more parasitized (Table 2.1). Thus, overall we detected no evidence for cryptic male choice based on perceived female quality.

Our study does not suggest that post-copulatory processes are completely unimportant in pipefish, however, as we do find evidence that some important post-copulatory processes may be occurring. Our data on number of eggs transferred per copulation show a first-female advantage in pipefish with respect to the number of eggs transferred per copulation. Regardless of tattoo status of the female, there was a tendency for the first female that mated with a male to transfer more eggs per copulation than the second female (Table 2.1). Note that the number of eggs per copulation is different than the total number of eggs transferred per female, because most females copulated several times during a given mating trial. Thus, the total number of eggs transferred by a female to a male was artificially equalized between first and last females by the experimental design, but the number of eggs transferred per copulation was not similarly equalized.

Our study also shows some potential for interesting post-copulatory processes related to pipefish body size. Males and females in our experiment exhibited enough variation with respect to total length that we were able to test for correlations between length and other variables of interest. We tried to avoid pairings involving extreme size differences between males and females, thus causing male and female sizes to be positively correlated across mating pairings in our experiment ($r = 0.80$, $n = 28$, $p < 0.001$). Consequently, some of the effects that appear to be a result of female size could be a consequence of male size. We pooled our data across treatments in the following analyses. We found a significant positive relationship between female length and the proportion of embryos that survived in the brood pouch ($r = 0.30$, $n = 54$, $p = 0.03$, Figure 2.2). However, it is possible that error in egg counts could be driving this significance (See Discussion). Embryo survivorship was not, however, significantly correlated with male length ($r = 0.28$, $n = 28$, $p = 0.15$). This suggests that if there was a correlation between female size and the proportion of embryos surviving holds true that

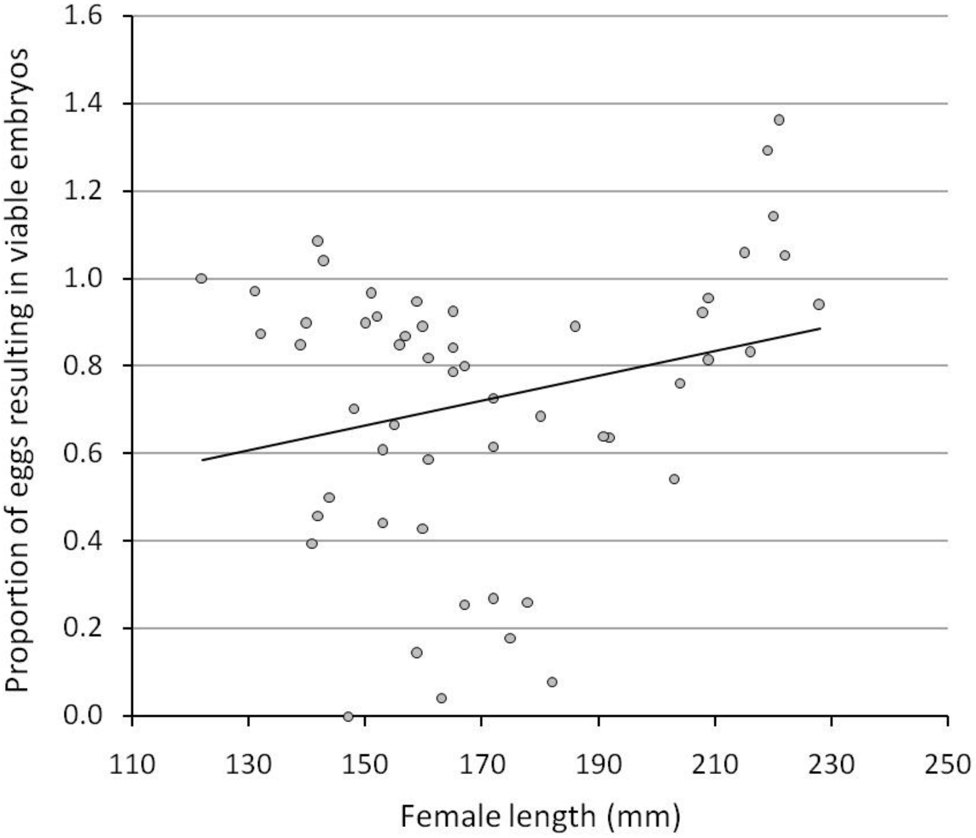


Figure 2.2: Embryo survivorship was positively correlated with female length (r = 0.30, n = 54, p = 0.03). Non-transformed data are shown. Embryo survivorship was not, however, correlated with male length (r = 0.28, n = 28, p = 0.15), despite a positive correlation between male and female length in our experimental design (see text). Points indicating survivorship greater than one are due to experimental error in egg counts. However, this error is expected to be random and should not produce a spuriously positive regression.

this effect is due to some aspect of female quality, such as the tendency for larger males to produce larger eggs, as female length was positively correlated with egg dry weight (r = 0.30, n = 47, p = 0.04).

We also found that female length was positively correlated with her number of eggs transferred per copulation (r = 0.50, n = 53, p = 0.0003, Figure 2.3), indicating another possible mechanism of sexual selection. In this case, male standard length also was positively correlated with the average number of eggs transferred per copulation (r = 0.57, n = 28, p = 0.002). Thus, this suggests that larger mating pairs of pipefish transfer more eggs per copulation, but additional research will be necessary to determine whether this effect is due to male or female length.

One important question in this analysis is how large an effect of cryptic male choice based on perceived female quality would be consistent with our data. To address this issue, we calculated the difference in survivorship between embryos originating from the control and treatment females for each male. The mean difference in the proportion of eggs surviving is 0.10 (Table 2.1), so the point estimate actually indicates an effect in opposition to what would be expected if cryptic choice were occurring, and the 95 percent confidence intervals range from -0.06 to 0.28. Hence, the maximum average decrease in survivorship due to cryptic male choice consistent with our data is six percent. We can put this figure into perspective by considering it relative to other sources of variance in fitness related to sexual selection in this pipefish. Figure 2.4 shows the variance in relative fitness that would be expected in a *S. typhle* population

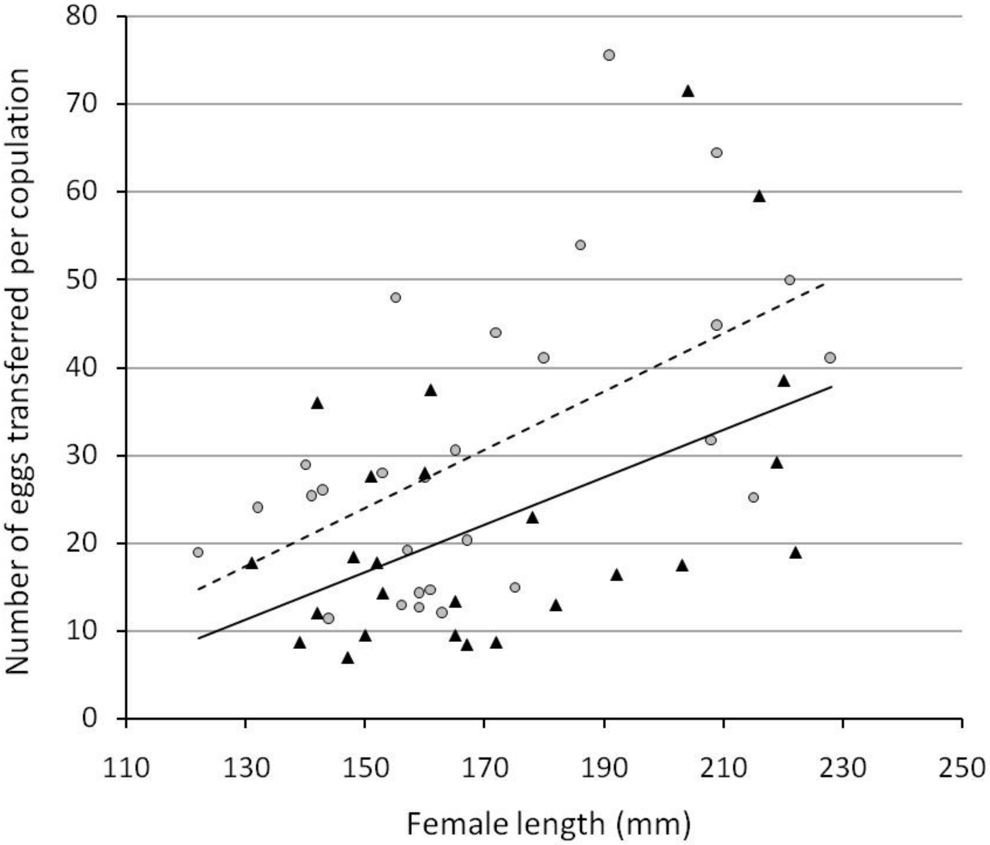


Figure 2.3: The significantly positive relationship between female length and number of eggs transferred per copulation (r = 0.50, n = 53, p = 0.0003; all females pooled). Our experimental design resulted in a positive correlation between male and female length within mating pairs (see text), so the relationship shown in this figure indicates that larger mating pairs transferred more eggs per copulation. The dashed line (corresponding to the circle) shows the least-squares regression for females mating first, and the solid line (triangles) shows the regression for females mating last. Male length also was positively correlated with number of eggs transferred per copulation (r = 0.57, n = 28, p = 0.002). Non-transformed data are shown.

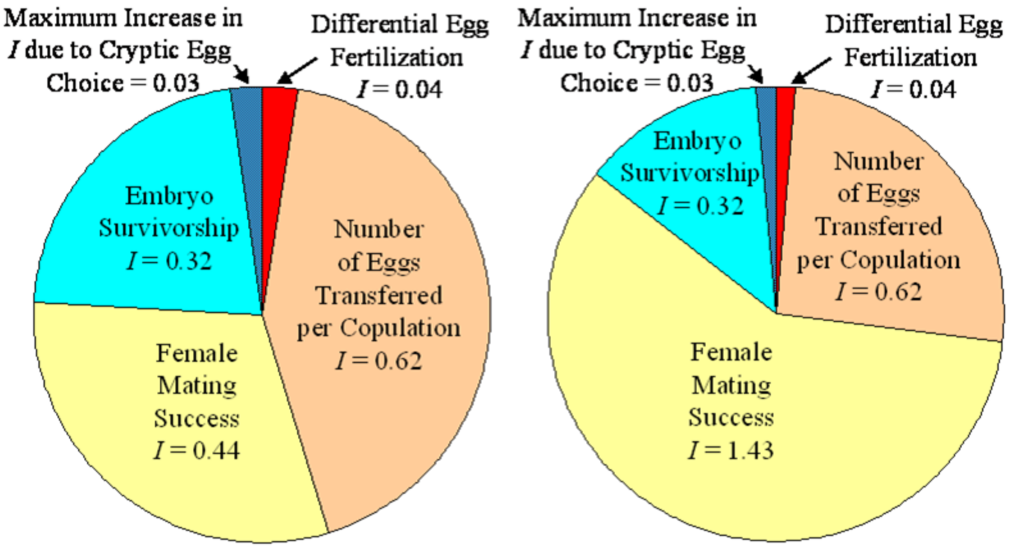


Figure 2.4: The sources of variance in relative fitness related to sexual selection in females of *S. typhle*. Sexual selection on females, the sex experiencing the most intense sexual selection, could arise from several different sources, including number of mates per female, number of eggs transferred per copulation, differential fertilization of eggs, and embryo survivorship within the brood pouch. This figure displays the amount of variance in relative fitness (i. e., the opportunity for selection, *I*), that would arise from each of these sources, holding variance from the other sources constant. The left panel shows fitness variance resulting from mating success for an equal operational sex ratio and the right panel shows results for a 3:1 (female:male) sex ratio, which probably is more relevant to natural populations (Berglund and Rosenqvist 1993; Vincent et al. 1994). Data for number of eggs transferred and female mating success are from Berglund et al. (1986b) and Jones et al. (2005), respectively. Data for differential egg fertilization and embryo survivorship are from the present study. The increase in *I* due to cryptic male choice assumes the strongest possible cryptic male choice consistent with our data, thus using a six percent decrement in embryo survivorship in parasitized females. Of course, our point estimate of the effects of cryptic choice actually indicates a result in opposition to expectations, suggesting that cryptic choice based on perceived quality of females does not occur in this system. A similar exercise based on number of eggs transferred per copulation showed a negligible (less than 0.01) increase in *I* for this component of sexual selection.

when all other sources of variance in fitness are held constant. For example, if pre-mating choice were the only source of variance in fitness, then the opportunity for selection (*I*, Wade and Arnold 1980; Arnold and Wade 1984) in pipefish would be 0.44with an operational sex ratio of unity and 1.43 with a female-biased operational sex ratio as more typically is observed in nature (Berglund and Rosenqvist 1993; Vincent et al. 1994). The operational sex ratio in the field is usually heavily female biased because most males are pregnant and unavailable to mate most of the time (Vincent et al. 1994). The variance in relative fitness in the absence of cryptic male choice (i.e., in our control females) due to embryo survivorship is 0.32. If cryptic male choice of the maximum magnitude consistent with our data is included in the calculation, then the variance in relative fitness increases to 0.35, an increase in the opportunity for selection of 0.03. Thus, our data suggest that cryptic male choice based on perceived parasite load has the potential to make at most a very small contribution to the intensity of sexual selection. In contrast, other forms of post-copulatory sexual selection could be extremely important, because the number of eggs transferred per copulation and embryo survivorship make substantial contribution to the opportunity for selection at both operational sex ratios (Figure 2.4).

Discussion

Our results show that a male's perception of female parasite load does not detectably affect post-copulatory processes in males of *S. typhle.* In principle, cryptic male choice of eggs or embryos could occur at several different phases of the reproductive cycle, possibly acting through variation in the number of eggs transferred, differential fertilization, differential survivorship of embryos, and differential allocation (Eberhard 1996; Eberhard 2000; Pilastro et al. 2004). In our experiment, perceived female parasite load had no significant effect at any of these stages. Our observations regarding the number of eggs transferred per copulation showed that the male's perception of female parasite load did not affect the number of eggs transferred per copulation. Secondly, differential fertilization did not occur since the number of unfertilized eggs within the brood pouch did not differ between females of different treatments. Similarly, there was no significant difference in the proportion of embryos that survived between those eggs given by females experimentally altered to look parasitized and control females, indicating that differential embryonic survivorship based on perceived female parasite load is not occurring at a detectable level. This latter result indicates there is no effect of perceived female parasite status on either male gamete choice or any forms of differential allocation by the male that influence offspring survivorship. Hence, the overall outcome of this study is that manipulation of the perceived parasite load of females had no measurable effect on any aspect of post-copulatory egg or embryo success.

Of course, we could not address all aspects of egg competition and cryptic male choice in a single study, so several caveats are worth mentioning. One feature of this study is that it does not cleanly distinguish between differential allocation and gamete choice. If either gamete choice or differential allocation of resources (e.g., nutrients or oxygen) to offspring based on maternity were occurring in this system, we might predict a decrease in offspring survivorship for the less preferred of the two females. Thus, if we had found a significant effect of our tattooing treatment, such an effect could be due to either of these processes. Since we did not find an effect, we can conclude that neither of these processes occurs at a sufficiently high rate to affect the probability that deposited eggs will result in viable embryos at a level that we could detect in our study. However, one mechanism of cryptic male choice that was not addressed in this paper is differential allocation at a level that changes offspring quality without affecting embryo survivorship. The resolution of this question will await studies that consider traits of offspring, such as size and growth rates, that are potentially related to fitness (Ahnesjö 1992b; Sandvik et al. 2000).

The other caveat in the present study is that perceived parasite load is not a secondary sexual trait, but rather it is a possible indicator of female quality. Rosenqvist and Johansson (1995), for example, found that heavily parasitized females had reduced fecundity relative to non-parasitized females, suggesting that males gain direct benefits by avoiding parasitized mates. The male's perception of female parasite load does affect the male's assessment of female attractiveness, in that males preferentially choose to mate with females they perceive to have lower parasite loads during pre-copulatory mate choice (Rosenqvist and Johansson 1995). However, parasite load is merely one aspect of total female attractiveness, and other aspects of the female phenotype could conceivably result in larger effects. If males use post-copulatory mechanisms only to discern between females with larger ornaments, for example, then the results of our experiment may not provide a comprehensive description of the true nature of the phenomenon. Future studies manipulating other aspects of female quality would be welcome.

A third caveat is that the present study addressed only within-brood phenomena. If males allocate resources or abort offspring at the level of whole broods based on whole-brood value in order to conserve resources for the next brooding event, then we would not have detected such an effect in the present study. For example, a male could allocate more resources to a brood mothered by all attractive females compared to one mothered (partially or wholly) by unattractive females. Such a strategy would have been undetectable in our study.

Even though we found no evidence for cryptic male choice, we did see potential for some post-copulatory processes to be important in this pipefish. Both the order in which females mated and the average size of mating pairs had a significant effect on the number of eggs transferred per copulation. Within our mating trials, female size was significantly correlated with male size, making interpretation of this latter pattern difficult, but other studies of *S. typhle* have also shown that larger females transfer more eggs per copulation, indicating that this pattern is probably a general feature of this species' reproductive ecology (Berglund et al. 1986b). The effect of order on the number of eggs transferred per copulation could be called "first-female advantage", with first females transferring more eggs per copulation than second females. This first-female advantage probably stems from the fact that the first female to mate is depositing eggs into a completely empty pouch and is therefore less constrained by the amount of space remaining in the pouch than are subsequent females. Thus, while the number of eggs transferred does not affect the proportion of embryos surviving, females that are able to mate more often with empty males could increase their total fitness by depositing more eggs within the brood pouch than subsequent females. While the potential for this type of post-copulatory choice exists, whether this first female effect is mediated by males, by allowing the first female encountered to deposit more eggs than subsequent females, or whether it is mediated by females, with females differentially investing eggs depending on a male's reproductive status, is unclear and should be evaluated further.

It is still unclear as to whether or not a significant correlation exists between female size and the proportion of embryos that survived through the brooding period. While our data did show a significant correlation between female size and the proportion of embryos that survived, it is possible that counting errors in the number of eggs transferred per female may be driving this significance. If there was a bias in counting error, where the number of eggs transferred from larger females was underestimated more often than those of smaller females, than this bias could have led to the significant relationship observed. However, if this error is random, than a significant relationship should truly exist between female size and the proportion of embryos that survived. Unfortunately, even if this significant relationship exists, it is extremely difficult to distinguish between male and female effects on survivorship as a consequence of egg size, as larger eggs could survive better as a consequence of their intrinsic quality or as a consequence of some form of cryptic male choice supporting larger eggs. Interestingly, if males are cryptically choosing to allocate more resources to larger eggs, such a process could actually reinforce their pre-copulatory preferences, since males prefer to mate with larger females and larger females produce larger eggs (Berglund et al. 1986b; Berglund and Rosenqvist 2003). However, further work is necessary to determine whether or not cryptic choice based on female size is occurring in *S. typhle*.

Consideration of the potential sources of female fitness variation during the process of reproduction sheds some light on potential factors influencing sexual selection in sex-role reversed pipefish (Figure 2.4). Clearly, a large amount of the variance in relative fitness of females is due to differences among females in mating success, so this species exhibits a large potential for pre-copulatory sexual selection, mediated by dominance hierarchies or male choice. However, broad-nosed pipefish exhibit high variance in embryo survivorship and number of eggs transferred per copulation, such that even in the absence of variation in mating success, differences among females in embryo survivorship and egg transfer ability could result in strong directional sexual selection. Thus, we would expect strong selection on traits in females that enhance egg transfer or embryo survivorship. Results from the present experiment suggest that a male's perception of female parasite load makes at most an extremely small contribution to post-copulatory fitness variation, however. Overall, the high variances in embryo survivorship and eggs transferred per copulation indicate a very high potential for post-copulatory sexual selection in pipefish, but if post-copulatory sexual selection is occurring, perceived parasite load plays a very small role.

In principle, cryptic choice has the potential to be an important mechanism of sexual selection allowing for directional selection that may profoundly influence the evolution of secondary sexual characteristics (Eberhard 1996; Birkhead and Pizzari 2002; Pilastro et al. 2004). If we interpret our results in terms of a male's overall perception of female quality, it appears unlikely that male *S. typhle* use post-copulatory mechanisms to directly select for visible aspects of the adult female's phenotype, such as perceived parasite load, ornamentation or body size. However, we only addressed perceived female parasite, so this assertion is an extrapolation that should the subject of additional work. These results lead to the conclusion that if cryptic male choice is occurring in pipefish, such choice most likely acts upon gamete characteristics rather than upon traits of the adult producing the gametes. Such a situation also seems to hold in the guppy, *Poecilia reticulata* (Evans et al. 2003), in which artificially inseminated females were shown to bias offspring survival in favor of preferred males rather than non-preferred males without previous exposure to either male. Choice in this case is due to characteristics of the gametes themselves, since it is unlikely that haploid sperm are able to express male attractiveness (Evans et al. 2003).

While many studies have suggested the presence of cryptic mate choice in a number of species (Edvardsson and Arnqvist, 2000; Ward, 2000; Pilastro et al., 2004), methodological limitations often make interpretation of patterns difficult. As a consequence, some mechanisms of cryptic choice, such as differential allocation, are reasonably well supported, but others, such as gamete choice in the absence of inbreeding, remain controversial (Pizzari and Birkhead 2000; Bussière et al. 2006). Our results contribute another example with no clear evidence of cryptic choice. However, we are still in need of additional research regarding several critical potential mechanisms of cryptic choice, and sex-role reversed taxa have the potential to contribute to this endeavour.

CHAPTER III

MALE AND FEMALE MATE PREFERENCE IN THE GULF PIPEFISH, *SYNGNATHUS SCOVELLI*

Introduction

Since Darwin first proposed the theory of sexual selection (Darwin 1859, 1871) researchers have been fascinated by the concept of mate choice. Typically choice of mates is performed by females since large investments in offspring limit their potential reproductive rates compared to males (Trivers 1972). In these cases, availability of females limits male reproductive success, resulting in males competing over access to a limited number of females, while females are selective when choosing potential mates. Researchers have long associated the evolution of many elaborate traits and behaviors in males with female choice mechanisms. Some such examples include the elongated tail of the widowbird (Andersson 1982) and the elaborate bowers constructed by bower birds (reviewed in Frith and Frith 2004). In many cases, the sole purpose of these elaborate ornaments is to increase mating success and potential reproductive fitness by attracting additional mates. This is because mating and reproductive success are highly correlated for the sex experiencing strong sexual selection (Bateman 1948) and thus, any gain in mating success by individuals may pay-off in terms of increased relative reproductive fitness.

In most cases, female choice for particular male traits has led to the development of morphological and behavior differences between the two sexes (reviewed in Andersson

1994). Of the morphological differences, body size is one of the most easily recognizable. Selection for increased male body size has been attributed to both intrasexual competition, where larger male body size is beneficial during physical contests, and through female choice mechanisms (reviewed in Andersson 1994), where females may benefit by mating with larger males if they are able to gain access to better resources than smaller males.

Behavioral traits used in courtship rituals have also been shown to be related to mating success (reviewed in Andersson 1994; Boake and Hoikkala 1995; Kotiaho et al. 1996; Hoikkala and Suvanto 1999; Wogel et al. 2005; Demary et al. 2006; Murai and Backwell 2006; Baird et al. 2007; Delaney et al. 2007). Many of these traits have been shown to be condition dependent in a number of different taxa (*fish*: Nicoletto and Kodrick-Brown 1999; *birds*: Borgia et al. 2004; *insects*: Ahtiainen et al. 2001; Kotiaho 2002; *amphibians*: reviewed in Wells 2001; *crustaceans*: Backwell et al. 1995) and thus provide an honest signal for females to assess male health and viability. However, the benefit to females in selecting males with more intense courtship behavior is still debatable. In some cases, females may receive direct benefits from these individuals if males in better condition are able to provide additional resources. Yet, in instances where males only contribute gametes with no other form of provisioning, the benefit becomes less clear. Many studies have suggested that by choosing these males, females may gain indirect benefits through an increase in the mating and reproductive success of offspring (reviewed in Andersson 1994). Some of these models include the “good genes” models (Zahavi 1975) and the “sexy sons hypothesis” (Weatherhead and

Robertson 1979). In these cases, the female's relative fitness does not change; however, by providing her offspring with genes that may be more resistant to disease or parasites (good genes model) or with genes that insure her male offspring are attractive and have higher than average mating success (sexy sons), the female can increase her indirect fitness. However, problems concerning many of the models still exist and the relative contribution of these models toward the evolution of female choice is debatable (reviewed in Jones and Ratterman 2009). In addition, it is likely that multiple mechanisms are contributing to the evolution and maintenance of female mate choice.

While much of the research concerning mate choice has centered around organisms with traditional sex roles, mate choice mechanisms also exist in organisms that are sex-role reversed. In these situations, sexual selection acts more strongly on females than on males and the morphological and behavioral traits used to assess mate quality evolve in females (Clutton-Brock and Vincent 1991). The reason for this reversal of sex roles could be due to a number of factors such as higher potential reproductive rates in females (Clutton-Brock and Vincent 1991; Clutton-Brock and Parker 1992; Ahnesjö et al. 2001), female-biased operational sex ratios (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996), higher investment in offspring care from males relative to females (Trivers 1972), or costs of breeding (Kokko and Monaghan 2001; Simmons and Kvarnemo 2006). Regardless of the reason for this reversal, availability of males limits female reproductive fitness and thus leads to an increase in competition between females over access to males.

Strong competition among females over access to males is particularly common in polyandrous bird systems. In these cases, operational sex ratios tend to be skewed toward females since males can only mate with one female, while females are able to mate with multiple males. However, intense female competition has also been observed in polygynadrous (Berglund et al. 1989; Jones et al 1999), monogamous (Kvarnemo et al. 2007; Sogabe and Yanagisawa 2007) and in some instances polygamous mating systems (Silk et al. 1981; Fedigan 1983; reviewed in Clutton-Brock 2007). In some cases, this competition has lead to the development of female weapons, ornaments and behavioral traits that allow females to gain access to or gain the attention of perspective males (reviewed in Clutton-Brock 2007). Similar to male secondary sex traits, expression of these traits in females has been shown to significantly impact female reproductive success through increased mating opportunities due to either male preference (Cumming 1994; Berglund et al. 1997; Amundsen and Forsgren 2001; LeBas and Marshall 2000; Berglund and Rosenqvist 2003; Torres and Velando 2005; Griggio et al. 2005), or increased competitive ability over rival females (Berglund and Rosenqvist, 2001). Male preference for females may directly impact male reproductive success as well, since it has been shown that mating with preferred versus non-preferred females directly impacts offspring viability in house mice (Drickamer et al 2003; Gowaty et al. 2003).

In cases where the condition of both parents directly impacts offspring fitness it is possible for both sexes to show some form of preference for mates, even when sexual selection is stronger on one sex than the other (Kokko and Monaghan 2001; Kokko and

Johnstone 2002). This type of mutual mate choice normally occurs when both sexes contribute significantly to offspring quality and can evolve in systems regardless of the mating system and/or direction of sexual selection (Kokko and Johnstone 2002). For example, in organisms with male parental care, females should choose males who are best able to provide resources for the offspring, while males should choose females with high quality eggs, since egg quality and offspring viability tend to be correlated (Kjorsvik et al. 2003; Gagliano and McCormick 2007). In these cases, since both females and males invest in offspring quality, females by producing costly eggs and males by providing care to the offspring, both sexes may show some type of mate preference. Thus, while pressure to obtain mates may be stronger on one sex, it cannot be assumed that this competition robs them of any choice.

In this study, both male and female mate preferences were assessed in a polyandrous sex-role reversed pipefish. Pipefish are one of the most studied model systems for examining sexual selection theory in sex-role reversed organisms. In addition, pipefish are one of the most interesting groups in which to examine not only mate choice mechanisms but also to evaluate how mating systems may influence choice since the mating system is extremely varied throughout this group (Wilson et al. 2003). All pipefish species exhibit male parental care and in many cases males have developed a specialized pouch where the offspring develop. During mating, female pipefish deposit eggs into the brood pouch of males where the eggs are fertilized and maintained during development. Males provide oxygen (Hout 1902), osmoregulation (Leiner 1934; Linton and Soloff 1964; Quast and Howe 1980; Watanabe et al. 1999; Partridge et al. 2007) and nutrients to the developing young (Haresign and Shumway 1981; Berglund et al. 1986a; Ripley and Foran 2009). At birth, the juveniles are released into the environment where no other paternal care takes place. The most commonly used pipefish for assessing mate choice mechanisms is the broad-nosed pipefish, *Syngnathus typhle*. *S. typhle* is a polygynandrous species, where males and females both mate multiply within a breeding bout (Jones et al. 1999). Some degree of dimorphism exists in this species in that females induce an ornament during courtship and intrasexual competition (Berglund et al. 2005). In addition, this ornament appears to be an honest indicator of female quality since it has been shown that female fecundity is related to female ornamentation (Berglund et al. 1997). Males can also temporarily display this ornament but to a lesser degree than females (Berglund et al. 2005). Studies have shown that *S. typhle* shows some degree of mutual mate choice with individual size, ornamentation and dominance all contributing to both male and female preference (Berglund et al. 1986b; Berglund 1995; Berglund and Rosenqvist 2001; Sandvik et al. 2000; Berglund et al. 2005). Choice of mates also has a direct influence on reproductive fitness in this species given that individuals who mate with chosen partners tend to produce higher quality offspring than individuals forced to mate with less preferred partners (Sandvik et al. 2000).

While *S. typhle* is the most studied of the pipefish species, the mating preferences of most of the other 200 species of pipefish are still relatively unknown. One particularly interesting species in which to examine mate choice mechanisms is the Gulf pipefish, *S. scovelli*. *S. scovelli* is strongly polyandrous and this species shows one of the largest discrepancies between the variance in male and female mating success of any polyandrous species to date (Jones and Avise 1997b; Jones et al. 2001). Because of the mating system of this species, theory would predict that female-female competition would be stronger and male choice would be more stringent in *S. scovelli* than in *S. typhle*. As expected from their mating systems, *S. scovelli* is more sexually dimorphic than *S. typhle*. Females are larger than males and have a deeply keeled abdomen with iridescent transverse stripes (Jones and Avise 1997b; Jones et al. 2001). This dimorphism becomes even more distinct during courtship because females develop a striking breeding coloration that consists of alternating black and white stripes and the dorsal fin darkens and becomes erect. In addition, females exhibit specific courtship behaviors that may be used by males to assess potential mating partners. Similar to what has been observed for *S. typhle*, females of this species may also benefit by being selective in their choice of mate. Since male pipefish are responsible for all parental care and additional nutrient provisioning (Berglund 1986a; Ripley and Foran 2009) and the number of eggs a female can deposit within the brood pouch of the male may limit her reproductive success, females may benefit by choosing larger, high quality males as mates. Thus, because of the strong investment presented by males and females of this species, mutual mate choice may also be present in this system. In this study, we focus on addressing the factors that influence male mating preference in this polyandrous sex-role reversed pipefish, since factors influencing mate choice have yet to be addressed in this species; however, the influence of male size on female preference is also assessed since females may benefit from mating with larger males in terms of increase reproductive success.

Methods and Materials

Pipefish Collection and Maintenance

Male and female S. *scovelli* pipefish were collected from a low salinity environment at Meaher State Park, Baldwin Co., AL (30°66624, 87°92731). Pipefish were collected using a hand seine from shallow *Vallisneria* beds from August – November 2004 and again in July – September 2007. Males and females were segregated upon return to the lab and maintained in separate 50 gallon barrels connected to a recirculating system. Water conditions within the laboratory were prepared and maintained so as to match the water conditions from their natural low salinity habitat (see Partridge et al. 2007). Pipefish were fed *Artemia* nauplii twice daily and diets were supplemented with copepods collected from a local pond. Pipefish were acclimated to laboratory conditions for at least 3 days before taking part in a trial.

Mate Preference Trials

All preference trials were performed in the morning 10 minutes after the beginning of the 12 hour daylight cycle. Either a Cannon Optura 500 MiniDV or a Sony HDV 1080i MiniDV video camera was used to videotape all mate choice trials for 1 hour. Behaviors were analyzed using JWatcher version 1.0 animal behavior software (2006, University of California Los Angeles, Los Angeles, CA: http://www.jwatcher.ucla.edu/).

Male Preference. In order to determine if female size influenced male mate choice, a standard mate choice design was used in which focal males were allowed to simultaneously assess both a small and large stimulus female. Prior to the trials, the standard length (cm) and maximum body depth (cm) of female pipefish were obtained. Females were then paired based on size and color. Female pairs consisted of one large female and one small female with large females being at least 5 mm larger than their small female partner. Both the focal male and the stimulus females were placed into mate-choice tanks the night before the trial began. The preference choice tests were conducted in 10 gallon tanks that were divided lengthwise by a transparent barrier and the back chamber was divided in half by an opaque divider that was orthogonal to the transparent divider. The two stimulus females were placed in the tank with the small female in one small chamber (25W x 28H x 12.5D cm) and the large female in the other small chamber (25W x 28H x 12.5D cm). Since the two smaller chambers were divided by an opaque barrier, stimulus females were not allowed to visually assess one another but they were allowed to observe the male. The focal male was placed in the front longer compartment (50W x 28H x 12.5D cm), from which he could visually observe the females in the two small compartments (Figure 3.1).

For male choice trials, behaviors of both the focal male and each stimulus female were analyzed. The proportion of time the focal male spent performing the following behaviors were examined: 1) swimming near the divider of each female (defined as when the males swims in a Z-like pattern up and down the transparent divider), and 2) dancing (defined as when a male performs an up and down bobbing motion). In addition, the number of twitches (defined as abrupt shaking of the individual lasting for less than a second) that a male performed toward each females was recorded. For a

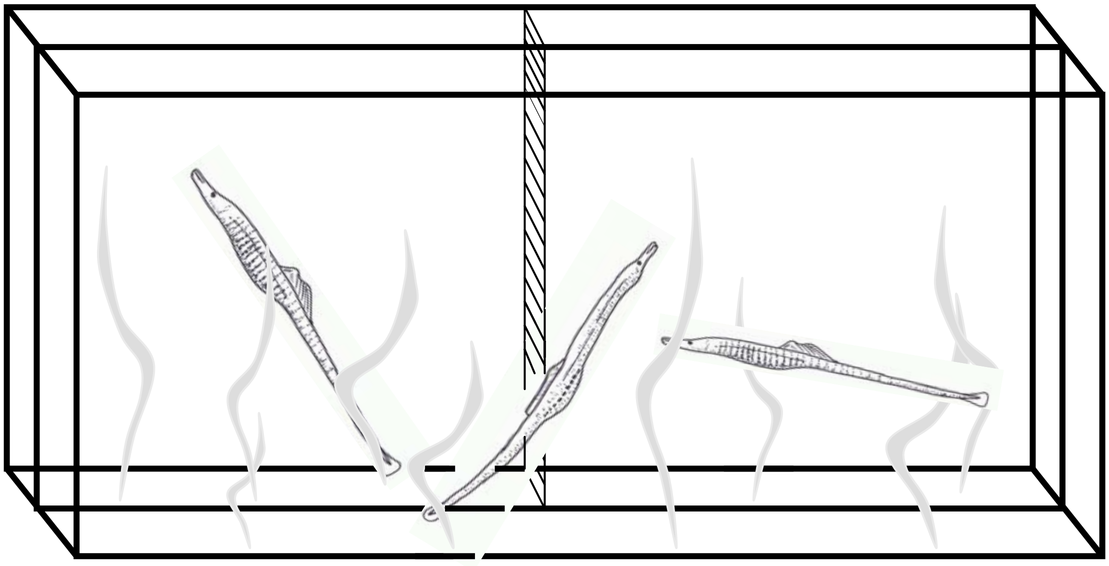


Figure 3.1: Diagram of mate choice tank. For male choice trials (pictured above), the focal male was placed in the front compartment while stimulus females were each placed within the separate back compartments. The focal male was separated from stimulus females by a clear perforated plexi-glass divider to allow for both visual and chemical cues to be detected. Stimulus females were separated by an opaque plexi-glass divider to prevent visual contact between the individuals. For female choice trials the mate choice design was similar except that the focal female was placed within the front compartment while the stimulus males were each placed in the separate back compartments.

quantitative measure of choice, we lumped the male behaviors of swimming near the divider and dancing into a more inclusive category termed “male response time”. We also recorded the proportion of time each focal male spent on the same side of the tank with each female. To define male preference, the stimulus female that the focal male spent the majority of the time responding to was designated as the preferred female, while the other female was deemed the non-preferred female. Finally, we recorded and analyzed the behavior of each female in the trial (see below) to determine if female courtship behavior had an effect on male choice.

Female Preference. Similar to that described for the male preference test, this study also examined the influence of male size on female mate choice. Prior to the trials the standard length (cm) and maximum body depth (cm) of each male was measured. Males were then paired based on size and color. The male pairs consisted of one large male and one small male, with large males being at least 5 mm larger than their small male partner. The same experimental design was used as in the male-choice trials (see above), except the focal female was place in the front longer compartment, while the stimulus males were each placed in the separated back compartments (Figure 3.1). Stimulus males were separated from one another by an opaque barrier that inhibited visual contact between them. The proportion of time the focal female spent performing the following behaviors toward each male was recorded: 1) color display (defined as the female displaying a darkened, erect dorsal fin and high contrast transverse stripes along the body), 2) posing (defined as the female moving sharply into a vertical position and assuming a distinct posture with the abdomen protruding), and 3) dancing (defined above). We also recorded the number of twitches the focal female performed toward each male. In *S. scovelli*, the female color display appears to be a static mating behavior, possibly indicating female receptivity, as this behavior often occurs in the absence of males. However, posing and dancing are behaviors that females actively perform toward males. Thus, the proportion of time females spent posing and dancing were grouped to represent "active female courtship behavior". Finally, the proportion of time focal females spent on the same side of the tank with each male was recorded.

Male and Female Interaction. In many cases, when male and female behaviors are significantly correlated it is informative to determine which sex may be driving this interaction. Because all behaviors that were recorded in Jwatcher during the mate choice trials were time stamped, we were able to examine the number of times a change in female courtship behavior elicited a change in male response behavior and the proportion of time a change in male response behavior elicited a change in female courtship behavior. For this component, we recorded the number of times a change in male response behavior (i.e., male changing from swimming to swimming near the divider) occurred within 10 seconds after a change in female courtship behavior (i.e., a female switched from posing to dancing). Similarly, the number of times a change in female courtship behavior occurred within 10 seconds after a change in male response behavior was recorded. We then took the number of times a change in male response behavior followed a change in female courtship behavior and divided that by the total number of changes in female courtship behavior to determine the proportion of time a change in female behavior elicits a change in male behavior. The proportion of time a change in male response behavior elicited a change in female courtship behavior was determined by dividing the number of times a change in female courtship behavior followed a change in male response behavior by the total number of changes in male response behavior.

Statistical Analysis

Prior to analysis, all data was appropriately transformed to achieve best approximate normality. When normality was not achievable, appropriate non-parametric tests were employed. Tests of the mean were used to determine if the proportion of total time focal males spent with each female, the relative proportion of time focal males spent responding to each female, the total proportion of time focal females spent with each male, the relative proportion of time focal females spent in color display in front of each male, and the relative proportion of time focal females actively courted each male differed from a random expectation of 0.5. Paired t-tests were used to determine if the relative proportion of time females actively courted and the number of twitches females performed to males differed significantly between preferred and non-preferred females. A Wilcoxon two-sampled signed rank test was used to determine if the number of twitches focal males performed to each female, the number of twitches focal females performed to each male and the number of twitches stimulus females performed to focal males significantly differed. In order to determine the factors that were most associated with male mating preference a stepwise multiple regression analysis examining both female size and behavioral data was employed. In these trials, since one male chose between two stimulus females, we evaluated whether the difference in the relative proportion of time males responded to the two stimulus females was correlated to the difference in size of the two stimulus females and the difference in the proportion of time stimulus females spent performing specific courtship behaviors. We also examined whether mating preference was based on size assortative mating in this species. For this we ran a linear regression analysis to determine if the size of the focal individual was correlated with size of the individual most preferred. In order to determine whether changes in female behavior were influenced by changes in male behavior or vice versa we examined the proportion of times a change in female courtship behavior induced a change in male response behavior and the number of times a change in male response behavior induced a change in female courtship behavior. A paired t-test was then used to determine if the induced changes in behavior differed between males and females.

Results

The results of this study suggest that female behavior may be the dominant factor influencing male choice; however female size may also be contributing to male preference. The proportion of time males spent by females and the relative proportion of time males responded to females did not differ between large and small females; however, males did perform significantly more twitches to larger females than to smaller females (Table 3.1). Larger stimulus females also spent significantly more time in their

Table 3.1: The effect of female size on male mate choice and female courtship behavior. Transformation on the number of twitches by males and females was unable to satisfy the assumption of normality, so a Wilcoxon two-sampled paired signed rank test was performed. All data are reported as mean ± S.E. of untransformed data.

Male Behaviors	*Small Female*	*Large Female*	*N*	*T-Test*	*P-value*
Prop. of Time w/ Female	0.50±0.06	0.50±0.06	17	$\lvert t \rvert_{1,16} = 0.06$	0.88
Rel. Prop. Time Responded to Female	0.42 ± 0.07	0.57 ± 0.067	17	$\lvert t \rvert_{1,16} = 1.08$	0.3
Number of Twitches to Female	2.1 ± 0.8	6.3 ± 1.4	17	$\lvert z \rvert_{1,16} = 2.44$	0.015
Female Behaviors	*Small Female*	*Large Female*	*N*	*Paired Test*	*P-value*
Rel. Prop. Time in Color Display	0.32 ± 0.09	0.68 ± 0.09	16	$\lvert t \rvert_{1,15} = 2.15$	0.048
Rel. Prop. Time Actively Courting	0.34±0.09	0.66± 0.09	16	$\lvert t \rvert_{1,15} = 1.87$	0.081
Number of Female Twitches	4.2 ± 1.6	8.2 ± 1.9	17	$\lvert z \rvert_{1,15} = 1.50$	0.132

color display than smaller stimulus females; yet, the relative proportion of time females spent actively courting and the number of twitches females performed did not differ between the two (Table 3.1). While large females did spend more time in their color display than small females, this did not impact male response time since there was no significant correlation between male response time and the proportion of time females spent in color display ($r^2 = 0.10$, $n = 17$, $p = 0.25$).

Data from female choice trials suggest that female preference is not significantly influenced by male size. Male size did not influence the total proportion of time focal females spent in front of each male, the relative portion of time focal females spent in color display in front of each male, the relative proportion of time focal females spent actively courting each male or the number of twitches focal females preformed toward each male (Table 3.2).

Stepwise multiple regression analysis showed that female behavior significantly influenced male response time. The factors that were significantly correlated with male response time were the relative proportion of time females actively courted and the interaction between female active courtship and the number of twitches a female performed (multiple regression: overall $F_{7,16} = 4.42$, $r^2 = 0.57$, $n = 17$, $p=0.001$; relative proportion of time females actively courted: $|t| = 3.63$, $p = 0.002$; interaction between relative proportion of time females actively courted and number of twitches performed: $|t| = 2.41$, $p = 0.03$). As would be expected, preferred females spent significantly more time actively courting focal males (Figure 3.2); however there was no significant

Table 3.2: The effect of male size on female mate choice. Transformation of the number of twitches performed by females did not satisfy the assumption of normality, so a Wilcoxon two-sampled signed rank test was performed. All data are reported as mean ± S.E. of untransformed data.

Female Behavior	*Small Male*	*Large Male*	*N*	*T-test*	*P-value*
Prop of Time w/ Male	0.51 ± 0.08	0.49 ± 0.08	8	$\lvert t \rvert_{1,7} = 0.27$	0.79
Rel Prop of Time in Color Display	0.51 ± 0.10	0.49 ± 0.10	8	$\lvert t \rvert_{1,7} = 0.09$	0.93
Rel Prop of Time Actively Court Male	0.66 ± 0.13	0.34 ± 0.13	8	$\lvert t \rvert_{1,7} = 1.44$	0.19
Number of Twitches to Male	2.4 ± 1.3	3.8 ± 1.3	8	$\lvert z \rvert_{1,7} = 0.76$	0.45

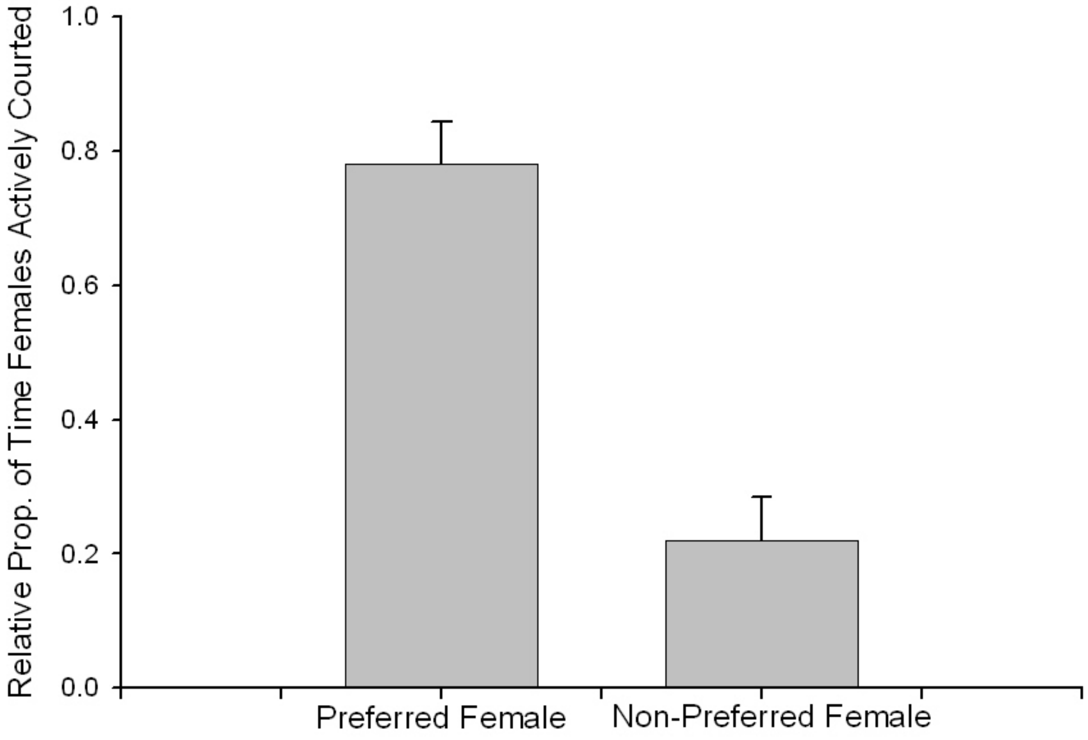


Figure 3.2: The relative proportion of time preferred and non-preferred females spent actively courting focal males were significantly different ($|t|_{1,16} = 4.39$, $n = 16$, $p = 0.0005$). The stimulus female that the focal male spent the majority of the time responding to was designated as the preferred female, while the other female was deemed the non-preferred female. Error bars represent standard error of the mean.

difference in the number of twitches preformed by preferred and non-preferred females (Figure 3.3).

In seahorses, positive size assortative mating occurs and may have played an important role in driving speciation in this group (Jones et al. 2003). However, this does not appear to be the case for *S. scovelli*. Focal male size and the size of the preferred stimulus female were not significantly correlated ($r^2 = 0.05$, $n = 17$, $p = 0.38$). Similarly, female size and the size of the stimulus male of which she most actively courted were not correlated ($r^2 = 0.01$, $n = 8$, $p = 0.77$), suggesting that assortative mating based on female preference does not occur.

Often when male and female behaviors are correlated, such as the interaction between male response time and female courtship behavior, it is important to determine which sex is driving the interaction. In other words, does female courtship behavior drive male response behavior or are females responding to changes in male behavior. Based on our data, we found that the interaction between male response time and female active courtship behavior appears to be driven most often by the female. Changes in female active female courtship behavior elicited a change in male response behavior significantly more than a change in male response behavior elicited a change in female active courtship behavior (proportion of time a change in female courtship behavior elicited a change in male response behavior: 0.48±0.05 (mean ± S.E.), proportion of time a change in male response behavior elicited a change in female courtship behavior: 0.30±0.04; $n = 24$, $|z|_{1,23} = 3.04$, $p = 0.002$). However, while female behavior may be driving male choice, changes in male behavior did elicit changes in female courtship

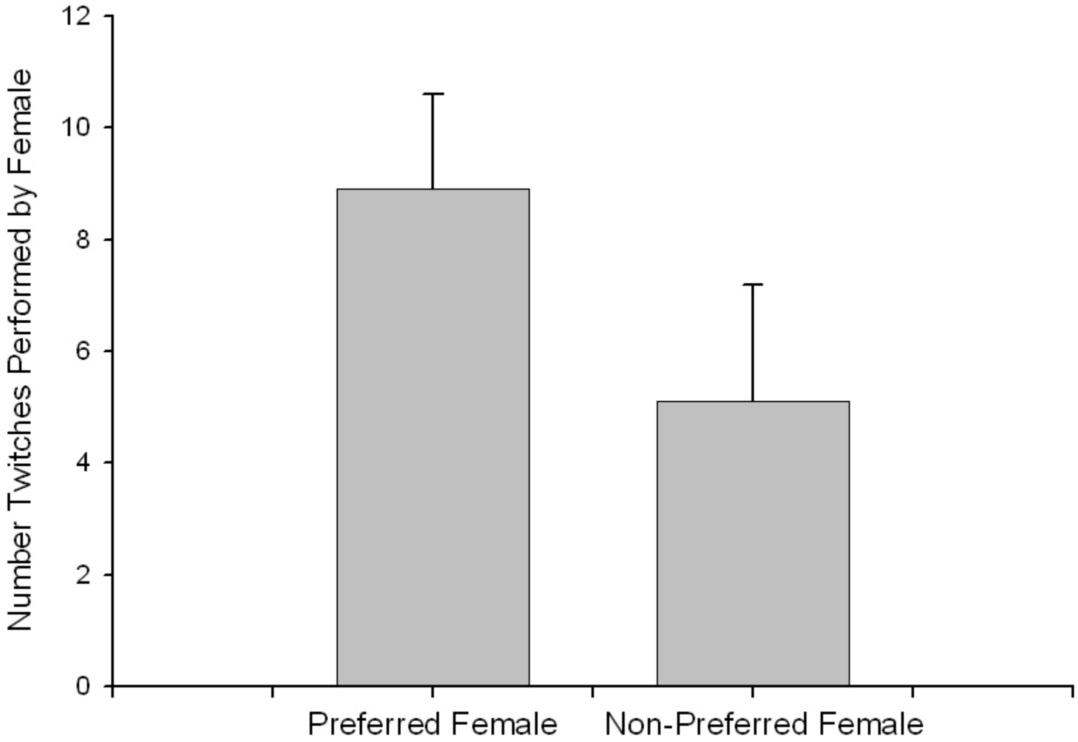


Figure 3.3: The number of twitches preferred and non-preferred females performed toward focal males. The stimulus female that the focal male spent the majority of the time responding to was designated as the preferred female, while the other female was deemed the non-preferred female. While the number the number of twitches performed by preferred and non-preferred female was not significantly different ($|z|_{1,14} = 1.85$, $n = 15$, $p = 0.06$), the interaction between the number of twitches performed and female active courtship behavior was significantly correlated to male response time ($|t| = 2.41$, $n = 17$, $p = 0.03$). Error bars represent standard error of the mean.

approximately 30% of the time, suggesting that both males and females may be helping to maintain this interaction.

Discussion

The main goal of this study was to (1) determine the morphological and behavioral factors that may influence male preference and (2) determine if male size impacts female preference in the Gulf pipefish. Based on our data, female behavior is most likely the dominant factor driving male preference. Female active courtship behavior and the interaction between female active courtship behavior and the number of twitches females performed were significantly correlated with male response time. However, female size may also contribute to male preference since males twitched significantly more toward large females than smaller females. The fact that female size did not have more of an impact on male mate choice is surprising since data from Jones et al. (2001), which evaluated mating success of *S. scovelli* in natural populations, showed that larger, more ornamented females had increased mating success and size preferences have been observed in other *Syngnathus* species (*S. typhle,* Berglund et al. (1986b); *S. abaster,* Silva et al. (2007)). One potential explanation for the discrepancy between our data and that of Jones et al. (2001) is that we did not allow for female-female competition in this study. Stimulus females in the mate choice tanks were separated by an opaque divider to prevent both visual and physical contact, thus preventing the two females from interacting. In natural populations this female-female interaction is likely to occur during matings and females have been observed to interrupt the mating attempts of other females (personal observation). In this case, female size could play an important role in determining mating success. Interestingly, while the amount of time females spent in color display was not significantly correlated with male response time, larger females were able to maintain this coloration longer than smaller females. This lack of an effect on male response may be because the color display serves as a general indicator of female receptivity, while more active behaviors, such as dancing and twitching, may provide a more honest signal of female condition if they are more energetically costly. However, the fact that larger females can display the color change longer than smaller females may allow these females to broadcast their willingness to mate longer, possibly providing them with more mating opportunities.

In many systems, traits that indicate female fecundity or condition are commonly those that influence male preference. While no one has examined the energetic cost of the female behaviors that are associated with male choice in this species, such as posing, dancing and twitching, it is likely that female display rate and condition are correlated and indeed a similar relationship has been observed in *S. typhle* (Berglund et al. 1986b). Also, in *S. scovelli* a positive linear relationship between female body depth and female gonadal somatic index (GSI) exists, suggesting that female body depth may be an indicator of female fecundity (Bolland and Boettcher 2005). However, we did not find an effect of female body:depth to length ratio on male response time, suggesting that female behavior may override most physical traits that could influence male preference. However, female size may still have some influence on male preference since males performed more twitches to larger females than to smaller females.

While female size may have some effect on male preference, female preference was not influenced by male size. This is again somewhat surprising considering that larger males may be able to accept more eggs from females, thus increasing female reproductive fitness values. The lack of female size preference in *S. scovelli* may be the result of both strong female-female competition and the fact that limited access to males may restrict a female's ability to fully assess male quality prior to mating. The operational sex ratio of the population from which we sample is heavily skewed toward females (4 receptive females per non-pregnant male) (Bolland and Boettcher 2005). Thus, females may be forced to mate with any receptive male since the benefit of being choosy may not offset the risk of losing limited mating opportunities.

One major caveat to this study is that we were not able to fully assess stimulus male behavior during these trials, and thus do not know the impact of male behavior on female mate choice. However, it does appear that females may be influenced by male behavior since changes in male behavior induced changes in female courtship behavior at least 30% of the time. This suggests that while female courtship behavior may be driving more changes in male behavior than the reverse, male behavior is likely influencing female behavior as well. Increased male response behavior may be providing positive reinforcement to courting females, thus allowing females to determine whether males are interested in mating. If courtship behavior is costly to females, this type of positive feedback loop would allow females to conserve energy by reducing the amount of time they spend courting nonresponsive males. Therefore, due to the strong interaction between female and male behavior, future studies should take male behavior into account when assessing female preference.

Traditionally, *S. typhle* has been the model organism used to assess mate selection in sex-role reversed pipefish. However, striking morphological and behavioral differences between *S. typhle* and *S. scovelli* exist, suggesting that factors contributing to mate preference may not be universal for all pipefish species. *S. scovelli* and *S. typhle* differ significantly in the degree of sexual dimorphism that exists between the two species. This difference could easily be explained by the fact that sexual selection acts more strongly on female *S. scovelli* as compared to *S. typhle*, thus leading to more distinct secondary sexual traits in this species (Jones et al. 2001). However, mate choice mechanisms may also influence the degree of variation in sexual dimorphism between species. Mutual mate choice has been suggested to lead to a reduction in the degree of sexual dimorphism, potentially leading to the two sexes becoming monomorphic (Clutton-Brock 2007). While this should continue to be tested, the difference in the presence of mutual mate choice between *S. typhle*, which shows some degree of mutual mate choice (Sandvik et al. 2000; Berglund et al. 2005), and *S. scovelli*, may also contribute to the differences in sexual dimorphism between the two species. In *S. typhle* both males and females display courtship ornaments, although the display of the male is significantly less than that of the female and both sexes show a preference for mates that display the ornament (Berglund et al. 2005). Continued selection by both sexes for the same trait would eventually lead to a decreased sexual dimorphism in the group. While size preference has been observed in both sexes of *S. typhle*, this does not appear to be the case for *S. scovelli*. In addition, while female *S. typhle* show a preference for more ornamented males, *S. scovelli* males show no sign of ornamentation during courtship and induction of female ornaments in males actually leads to female discrimination during choice trials (see Chapter IV). Males do, however, exhibit some courtship behaviors that are similar to females, such as dancing and twitching, which may be used by females to assess male condition and may influence female preference. Therefore, as stated previously, the effect these behaviors have on female mate preference should be further evaluated.

From this study we show that male mate choice is significantly influenced by female courtship behavior in a polyandrous sex-role reversed pipefish. In addition, while other studies have shown that female size significantly impacts mating success (Jones et al. 2001), we suggest that this size effect may be due to female-female competition and may only play a limited role in male mate choice. However, studies addressing the impact of female size on intra-sexual competition should be employed. We found no affect of male size on female mate choice. However, it is possible that females are using behavioral traits to assess male quality and we cannot absolutely rule out the possible of female mate choice in the species. Finally, we show that changes in female courtship behavior can drive changes in male response behavior but that male behavior may also aid in reinforcing female courtship behavior. Future work on this species should include determining the cost of female display behavior and further analysis in assessing how male behavior may be impacting female choice.

CHAPTER IV

SHORT TERM EXPOSURE TO A SYNTHETIC ESTROGEN DISRUPTS MATING DYNAMICS IN A SEX-ROLE REVERSED PIPEFISH

Introduction

Sexual selection is a major evolutionary mechanism that often results in the evolution of secondary sex traits, which play a significant role in competition for access to mates. Mate choice mechanisms often lead to the development of elaborate ornaments that are used by individuals to assess mate quality and experimental alterations of these traits can significantly impact male mating success. While expression of a secondary sexual trait is usually limited to one sex, the genes influencing their development are usually present in the genomes of both sexes (Andersson 1994). Sex-specific expression is often the result of the relevant genes being under the control of sex-steroid hormones, testosterone in males and estrogen in females (reviewed in Folstad and Karter 1992; Andersson 1994; Parker et al. 2002; McGraw et al. 2006). Because secondary sexual trait expression varies with hormone levels more than that of reproductive ability, these traits may be more sensitive to small levels of environmental pollution, particularly when the pollutants are endocrine disrupting compounds, such as bisphenol A, polychlorinated biphenyls and synthetic hormone mimics.

Endocrine disruptors are compounds that interfere with normal hormone function and have been shown to greatly impact population viability by interfering with reproduction (Kidd et al. 2007). In addition, studies have shown that exposure to environmental pollutants can affect the development of secondary sexual traits, causing them to be suppressed (Arellano-Aguilar and Garcia 2008) or expressed in the opposite sex (Ueda et al. 2005; Larsen et al. 2008). While most of the research concerning the impact of endocrine disruptors on population health has focused on their effects on primary sexual organs, particularly the gonads (Allen et al. 1999; Hill and Janz 2003; Moncaut et al. 2003; Weber et al. 2003; Vandenbergh et al. 2003; Palace et al. 2006; Pettersson et al. 2006; Brown et al. 2008) and reproductive ability (Hill and Janz 2003; Maunder et al. 2007; Peters et al. 2007; Schäfers et al. 2007), the number of studies concerning the effect of these compounds on pre-copulatory mating behavior is generally increasing (Bell 2001; Bjerselius et al. 2001; Oshima et al. 2003; Robinson et al. 2003; Crews et al. 2007; Saarisota et al. 2009). However, very few studies have assessed how exposure to endocrine disruptors affect secondary sexual trait expression (Ueda et al. 2005; Larsen et al. 2008) and how that may in turn impact mate choice mechanisms (Arellano-Aguilar and Garcia 2008). This situation is particularly surprising since the ability of individuals to maintain secondary sexual traits significantly influences mating dynamics and mating success. One potential reason for this gap in knowledge is that many of the model species used for the study of endocrine disruptors do not maintain obvious traits involved in mate choice. However, mate choice and sexual selection are key components of reproduction and in turn population viability, so there is a critical need to understand the effects of endocrine disruptors on these evolutionary processes. In this study, we examine how short-term exposure to environmentally relevant concentrations of a synthetic estrogen affects multiple aspects of reproductive fitness, including mating opportunity, mating success and reproductive success, in a sex-role reversed pipefish characterized by strong mating preferences and sexual selection. Our work provides one of the most comprehensive studies of the effects of an endocrine disrupting compound on reproductive behavior and the only investigation of the effects of such contaminants on a sex-role reversed organism.

One endocrine disruptor that has received a large amount of attention is 17α-ethinylestradiol (EE2). EE2 is a synthetic estrogen that is one of the major components of oral contraceptives. A high resistance of EE2 to degradation in the human body is one feature that makes it useful in contraceptives. However, this characteristic also allows large amounts of EE2 to pass into the environment through domestic waste water (Hill and Janz 2003; Lintelmann et al. 2003), and high concentrations of EE2 have been found in rivers within the United States and Europe (Kolpin et al. 2002; Vajda et al. 2008). Of the vast array of potential endocrine disrupting chemicals, EE2 is one of the most troubling because it binds estrogen receptors with high affinity and is relatively stable in the environment. The amount of EE2 in wastewater treatment plant effluent is variable depending upon season and effectiveness of water treatment but can range from 0.1 – 10 ng/L (Kolpin et al. 2002; Clauzot et al. 2008; Vajda et al. 2008). Surface waters around treatment plants commonly show concentrations of EE2 of 0 – 5 ng/L (Clauzot et al. 2008; Vajda et al. 2008). However, a study by Koplin et al. (2002), which evaluated EE2 concentrations in 139 U.S. contaminated rivers, found maximum concentrations of EE2 to be 820 ng/L, and concentrations approaching 35 ng/L in Europe have been reported (Pojana et al. 2007). These levels are of significant concern considering that exposure to 0.1 ng/L EE2 can induce vitellogenin production in males of some species, and a whole-lake experiment showed that 5-6 ng/L EE2 caused a population of fathead minnows to collapse after only two seasons of exposure (Kidd et al. 2007). This sort of population collapse could result from effects of EE2 on gonads or other primary sexual traits (Bell 2001; Bjerselius et al. 2001; Robinson et al. 2003; Balch et al. 2004; Larsen et al. 2008) or by disrupting mating patterns so severely that population viability is reduced. Thus far, very few studies have evaluated how EE2 impacts mechanisms of sexual selection and mate choice, so our goal was to study EE2 exposure in a system characterized by ritualistic courtship behaviors, elaborately ornamented secondary sexual characters, and strong sexual selection.

The Gulf pipefish, *Syngnathus scovelli*, is characterized by reversed sex roles (Jones and Avise 1997b; Jones et al. 2001). Males possess a specialized pouch on their ventral surface for the brooding of offspring. During mating, a female deposits unfertilized eggs into the male's brood pouch, the male fertilizes the eggs by releasing sperm into the pouch, and the male carries the developing embryos for about two weeks until they are born as independent juveniles. Male Gulf pipefish typically receive eggs from one female per "male pregnancy", but females are capable of filling multiple pouches (Jones and Avise 1997b; Jones et al. 2001). Consequently, males are a limiting resource for reproduction, and females compete for access to males. The result is strong sexual selection on females and weak sexual selection on males (Jones and Avise 1997b; Jones et al. 2001), a reversal of the usual direction of sexual selection. As might be expected from the mating patterns, Gulf pipefish are sexually dimorphic. Females are larger than males and possess a number of sex-specific characteristics, such as a deeply keeled abdomen, intense silvery-blue lateral stripes, a large dorsal fin, and striking temporary breeding coloration. Ornamentation of female pipefish appears to be regulated by estrogenic hormones, as males exposed to high concentrations of EE2 (100 ng/L) develop iridescent stripes on the abdomen, a trait normally only expressed in females (Ueda et al. 2005). The questions we address in this study are: (1) Do environmentally relevant concentrations of EE2 produce males that are feminized with respect to secondary sexual traits? And (2) are these effects of sufficient magnitude to disrupt mating patterns and sexual selection? These questions are addressed by examining the effects of EE2 in several different experiments, including a test of the effects of EE2 exposure on male morphology, mate choice trials involving males and females choosing between exposed and unexposed individuals of the opposite sex, and measurement of the effects of EE2 exposure on the ability of males to mate and to carry their pregnancy to term.

Methods and Materials

Pipefish Collection and Maintenance

We used a hand seine to collect pipefish from submerged vegetation at the northern end of Mobile Bay in Meaher State Park, Baldwin County, AL (30º66624, 87º92731). Pipefish were collected between July and November in the years 2006 through 2008. Males and females were housed separately in 30 L aquaria connected to a recirculating filtration system. Pipefish were fed a diet of *Artemia* nauplii twice daily and their diets were supplemented with live copepods biweekly.

Mate Choice of Exposed versus Non-Exposed Individuals

Exposure. Control and treatment tanks were maintained on separate recirculating systems. The control system consisted of fifteen 30L aquaria, while the two treatment systems consisted of nine 30 L aquaria each. All tanks were divided into two 15L compartments by a perforated barrier. The control system was initially spiked with 510 µL of 95% ethanol, the solvent for the EE2 solution, to account for the 450L of water within the tanks and a 60L sump for a total of 510L. Treatment systems were initially spiked with 330 µL of either 1mg/L EE2 (for 1ng/L system) or 100 mg/L EE2 (for 100 ng/L system) to account for the 270L of water within the tanks and 60L sumps for a total of 330L. Same sex individuals were size and color matched and paired. One of the individuals of the pair was placed into a 15L compartment in the control system, while the other individual was placed in a 15L compartment in either the 1 ng/L or 100 ng/L EE2 system. All individuals were exposed for a total of 10 days. To maintain consistent concentrations of EE2 in all systems, we performed 2% water changes (including appropriate amounts of either 95% ethanol or EE2 in 95% ethanol for control and exposure tanks, respectively) and cleaned tanks daily.

Body Morphology. Before exposure, we measured the standard length of each male. We also used a Nikon Coolpix 5000 digital camera to photograph each male in lateral view against a size standard. From the photographs, we used the computer program *Image J* (NIH, Bethesda, MD, www.rsbweb.nih.gov/ij/) to measure maximum body depth. We then calculated the depth:length ratio by dividing the maximum body depth by the standard length. We measured each individual a second time at the conclusion of each exposure period and mate choice trial.

In order to quantify the effects of EE2 on male coloration, the images taken of the lateral view of each male both prior to and after exposure (see above) were analyzed using *Image J*. The red color was removed from all pictures in order to obtain images that provided the best color differentiation to detect changes in the banding pattern. The maximum and mean color intensity was then obtained for an area approximately 1.3 cm long and 0.02 cm high along the lateral side of the male, where the iridescent stripes develop. The maximum color intensity, defined as the value of the brightest pixel within that area, was then divided by the mean color intensity of the area. Because the areas within the iridescent bands produce the highest color intensity, by evaluating the relationship between maximum color intensity and mean body color intensity along the area where these iridescent stripes develop, we can assess whether areas of iridescences develop in exposed males.

<u>Mate Preference Trials</u>. All preference trials were performed in the morning 10 minutes after the beginning of the 12 hour daylight cycle. We used either a Cannon Optura 500 MiniDV or a Sony HDV 1080i MiniDV video camera to videotape all mate choice trials for 1 hour. Behaviors were analyzed using JWatcher version 1.0 animal

behavior software (2006, University of California Los Angeles, Los Angeles, CA: http://www.jwatcher.ucla.edu/).

Male Preference. The effect of each treatment on male preference was determined by using a mate-choice design in which the focal male had the ability to assess control and exposed females but the females could not interact with each other. Each mate-choice tank was divided lengthwise by a transparent barrier. One of the resulting long chambers was divided in half by an opaque divider orthogonal to the transparent barrier. Two females were placed in the tank, with an exposed female in one small chamber (25W X 28H X 12.5 D cm) and a control female in the other small chamber (25W X 28H X 12.5 D cm). The male was placed into the longer compartment (50W X 28H X 12.5 D cm), from which he could see into the smaller compartments. The females, separated by the opaque divider, could not see each other but they could observe the male. The same behaviors described in CHAPTER III were used to assess male preference in this study. Briefly, the proportion of time focal males spent swimming near the divider and dancing were recorded and lumped together to provide a quantitative measure of choice that was termed “male response time”. In addition, the number of twitches focal males performed to each stimulus female and the proportion of time focal males spent on the same side of the tank with each female was recorded. Finally, we recorded and analyzed the behavior of each female (see CHAPTER III) to test for effects of exposure to EE2 on female courtship behavior.

Female Preference. The effect of each treatment on female mate choice was determined by evaluating the relative proportion of time females spent actively courting each male and the number of twitches females performed toward each male. We used the same experimental set up as in the male-choice trials (see above). Thus, each trial involved a focal female and two males, one of which had been exposed to EE2 and one of which was a control male. The female was able to visually assess both males and males were able to assess the focal female; however, males were separated from one another by an opaque divider. The female behaviors previously described in CHAPTER III were used to assess female choice. Briefly, the proportion of time the focal female spent posing and dancing to each male were grouped together to represent female active courtship behavior. In addition, the total proportion of time focal females spent on each side of the tank, the proportion of time focal females spent in color display, and the number of twitches focal female performed toward each male were also recorded.

Mating Success and Reproductive Success

Mating and reproductive success were evaluated two different ways. For years 2006 and 2007, after each mating trial, control and exposed males were placed individually in 15L compartments to which a female that had not been involved in a mating trial was added. We checked males each morning to determine whether or not they were pregnant. Once impregnation occurred, we removed the female. Pregnant males were allowed to brood their offspring, and we recorded the amount of time that elapsed from impregnation until birth.

In 2008, we exposed ten non-pregnant males each to control (95% ethanol) water, 1ng/L EE2 water, and 100 ng/L EE2 water. After ten days, males were moved to clean tanks containing females. Given that females can impregnate multiple males (Jones et al. 2001), we housed two females with five males in each tank. Males were checked twice daily for pregnancy. Pregnant males were removed and maintained individually in 15L aquarium compartments, where we monitored the progression of their pregnancies. After three days, females from each tank were rotated to ensure that any male's failure to become impregnated was not attributable to the females with which he was housed. Pregnant males nearing parturition, which occurs after 12-15 days of brooding, were moved to individual 2L birthing chambers. For each male, we counted the number of offspring born as a measure of his reproductive success.

Statistics

For our statistical analysis, we first transformed our data by using arcsine (square root) transformations on all proportion data and square-root transformations on the number of twitches females performed to each male, the number of twitches males performed to each female and the maximum/mean color intensity of males. We compared male body depth:length ratio and maximum/mean color intensity between exposed and unexposed males prior to and after exposure by using paired t-tests. T-tests of the mean were used to determine if the proportion of time focal females spent with each male, the relative proportion of time focal females actively courted males, the proportion of time focal males spent with each female and the relative proportion of time focal males responded to a female differed from a random expectation of 0.5. In the male mate choice experiments (two females with one male per trial), we used paired t-tests to compare control and experimental females with respect to: (1) number of twitches performed, (2) extent of color display, and (3) amount of active courtship. A Wilcoxon two-sample paired signed ranks test was used to determine of the difference in number of twitches control and exposed females performed in male choice tests differed from 0. A chi-square analysis of a 3 × 2 contingency table was used to examine whether or not a male's ability to become pregnant was dependent upon exposure. Finally, we used an analysis of variance to compare the mean number of offspring produced by males subjected to different treatments.

Results

With respect to morphology, we found that short-term exposure to EE2 had a significant effect on male phenotype. After only 10 days of exposure, males from both low (1 ng/L EE2) and high (100 ng/L EE2) exposure treatments showed significant changes in body morphology, which resulted in males developing secondary sexual traits that are associated with female morphology. Reproductively active female pipefish have a larger body depth than males due to the space needed for egg production and after exposure to EE2, the body depth: length ratio of exposed males was significantly greater than their control partners (Table 4.1).

In addition to body shape, exposure to EE2 also affected male body coloration. Exposure to 100 ng/L EE2 caused males to develop iridescent lateral stripes, which are

Table 4.1: The effects of EE2 exposure on male body morphology.

	Control Male	**1ng/L EE2 Male**	**N**	**Paired T-Test**	**P-value**	**Control Male**	**100ng/L EE2 Male**	**N**	**Paired T-Test**	**P-value**
Pre BD:Length Ratio	0.039 ± 0.002	0.038 ± 0.002	9	$\|t\|_{1,8} = 0.82$	0.38	0.042 ± 0.001	0.042 ± 0.001	10	$\|t\|_{1,9} = 0.189$	0.85
Post BD:Length Ratio	0.038 ± 0.004	0.041 ± 0.003	9	$\|t\|_{1,8} = 3.06$	0.01	0.042 ± 0.001	0.050 ± 0.001	10	$\|t\|_{1,9} = 28.28$	0.0003
Pre Max/Mean CI	2.33 ± 0.14	2.37 ± 1.20	11	$\|t\|_{1,10} = 0.13$	0.9	2.56 ± 0.21	2.47 ± 0.24	10	$\|t\|_{1,8} = 0.25$	0.78
Post Max/Mean CI	2.01 ± 0.15	2.68 ± 0.35	11	$\|t\|_{1,10} = 2.01$	0.07	2.30 ± 0.19	3.28 ± 0.28	10	$\|t\|_{1,8} = 2.88$	0.02

Body depth (BD):length was calculated by dividing maximum body depth by standard length. Maximum/mean color intensity (CI) is the maximum color intensity divided by the mean color intensity of a 1.3 cm long X 0.2 cm high area along the lateral side of the males where the iridescent stripes develop. Paired t-test were used to compare BD:length and maximum/mean CI between control and experimental males before (Pre) and after exposure (Post). All data are reported as mean ± S.E of untransformed data.

normally only found in female pipefish (Figure 4.1). Some of the 1 ng/L exposed males also developed these female-like iridescent stripes, although the expression was not as pronounced as in males exposed to the higher concentration of EE2 (Figure 4.1). In order to quantify these changes, we examined the maximum/mean color intensity (see methods) along the lateral side of control and exposed males, where the iridescent bands develop, both prior to and after exposure. We found that the maximum/mean color intensity along the banding area was significantly different between control and males exposed to 100 ng/L EE2 after exposure (Table 4.1). In addition, while no significant difference in maximum/mean color intensity was observed between control and 1ng/L males after exposure, we did observe a strong trend with 1ng/L exposed males tending to have areas within the banding area that were brighter than control males (Table 4.1).

Given that EE2 exposure affects male morphology, the next question was whether or not such changes potentially impact mating dynamics in this species. Based upon our results it appears that male attractiveness is affected even by low doses of EE2, whereas female mating behavior and attractiveness is largely unaltered. In the mate choice experiments with a focal female choosing between an exposed and an unexposed male, we found that focal females spent more time actively courting (posing and dancing; Table 4.2) and performed more twitches (Table 4.2) toward control males compared to males exposed to either 1ng/L or 100 ng/L of EE2. Females did not differ in the proportion of time they spent in their color display between control and treatment males (Table 4.2). However, female color display is likely a general indicator of female receptivity, and not indicative of choice, since females often invoke their color display

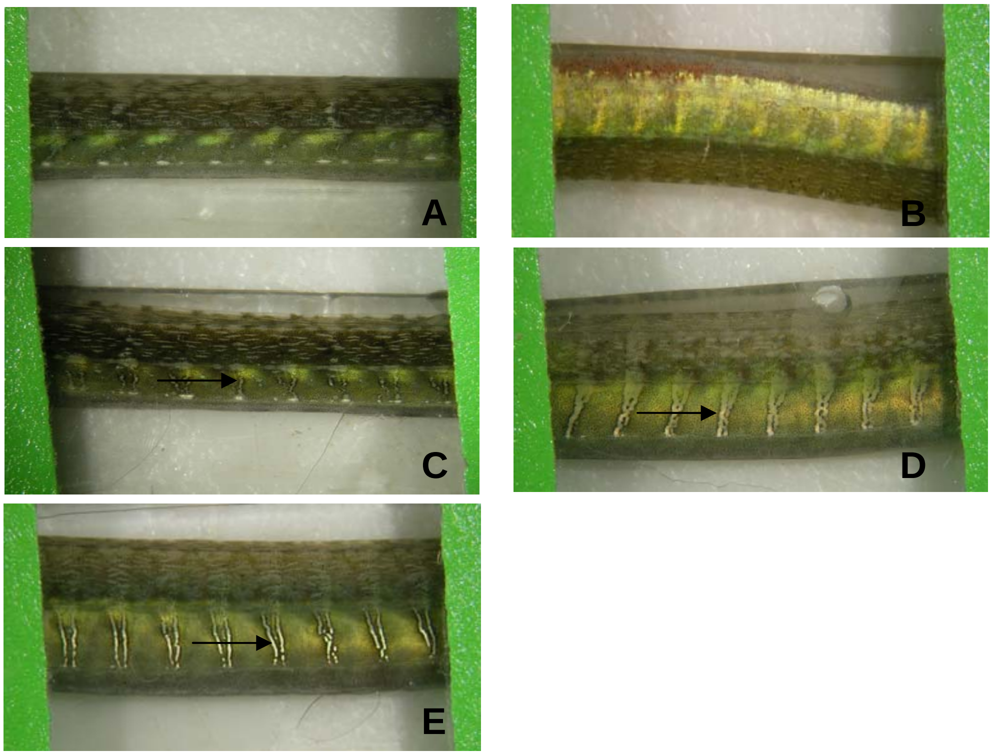


Figure 4.1: Induction of secondary sexual traits in male pipefish after exposure to EE2. (A) Control male prior to exposure, (B) Control male after 10 days of exposure to 0 ng/L EE2, (C) Treatment male after 10 days of exposure to 1ng/L EE2, (D) Treatment male after 10 days of exposure to 100 ng/L EE2, (E) Non-exposed female. The black arrows point to the iridescent stripes, a secondary sex trait normally confined to females. The green bars at the ends of each photograph are separated from one another by 1.5 cm. This figure shows that exposure of males to EE2, especially the 100 ng/L concentration, induces the development of female-like secondary sexual traits. Thus, EE2 feminizes the males with respect to external morphology.

Table 4.2: Effects of EE2 exposure on female mate choice.

	Control vs 1 ng/L EE$_2$ Male					**Control vs 100 ng/L EE$_2$ Male**				
Female Behavior	*Control Male*	*1ng/L Male*	*N*	*Paired T-test*	*P-value*	*Control Male*	*100ng/L Male*	*N*	*Paired T-Test*	*P-value*
Prop of Time w/ Male	0.58 ± 0.06	0.42 ± 0.06	11	$\|t\|_{1,10} = 1.38$	0.2	0.63 ± 0.06	0.365 ± 0.06	12	$\|t\|_{1,11}= 2.02$	0.07
Rel Prop of Time in Color Display	0.57 ± 0.06	0.43 ± 0.06	11	$\|t\|_{1,10} = 1.19$	0.26	0.60 ± 0.08	0.40 ± 0.08	12	$\|t\|_{1,11} = 1.38$	0.2
Rel Prop of Time Active Court Male	0.65 ± 0.06	0.35 ± 0.06	11	$\|t\|_{1,10} = 2.49$	0.03	0.80 ± 0.05	0.20 ± 0..05	12	$\|t\|_{1,11} = 5.04$	0.0004
Number of Twitches to Males	12.1 ± 3.1	3.7 ± 1.2	11	$\|t\|_{1,10} = 3.03$	0.01	7.4 ± 1.7	1.3 ± 0.5	12	$\|t\|_{1,11} = 4.46$	0.001

The experiment consisted of a female choosing between two males, one of which had been exposed to EE2 and one of which had not. Female behaviors included the proportion of time female spent on the same side of the tank as each male, relative proportion of time female was in color display in front of each male, relative proportion of time female actively courted each male and the number of twitches females performed to each male. All data are reported as mean ± S.E. of untransformed data.

even in the absence of males. Thus based upon these results, EE2 exposure clearly affects male mating opportunity by altering their attractiveness to females, as might be expected from the feminizing effects of this compound.

In contrast to our results for males, we found no detectable effects of EE2 exposure on female attractiveness. Male pipefish did not significantly differ in their response between control females and EE2 exposed females from either the 1ng/L or 100 ng/L EE2 treatments (Table 4.3). Likewise, female mating behavior did not differ significantly between the two treatments (Table 4.3). In male mate choice tests with non-exposed females the factors that best predicted relative male response time were relative female active courtship and the interaction between female active courtship behavior and the number of twitches performed by a female (See Chapter III). Since female behavior appears to be the major factor influencing male response time, the lack of an effect of EE2 on female courtship behavior likely explains why exposure did not affect male mate preference.

In our final experiment, we addressed the question of whether or not exposure to EE2 affects mating ability and male pregnancy. When females were given no choice (see methods), they mated as successfully with males exposed to 1ng/L EE2 ($N = 18$; proportion impregnated = 0.50) as with control males ($N = 33$, proportion impregnated = 0.58). However, at some point exposure to EE2 limits the efficacy of mating, as we found that males exposed to 100 ng/L of EE2 were significantly less likely to become impregnated (N = 19; proportion impregnated = 0.21) than control males (for all three treatments, contingency-$\chi^2 = 6.66$; p = 0.04). Of the 100 ng/L EE2 males that mated,

Table 4.3: The effects of EE2 exposure on male mate choice and female courtship behavior.

	Control vs 1 ng/L EE_2 Female					**Control vs 100 ng/L EE_2 Female**				
Male Behavior	*Control Female*	*1 ng/L Female*	*N*	*Paired Test*	*P-value*	*Control Female*	*100ng/L Female*	*N*	*Paired Test*	*P-value*
Prop of Time w/ Female	0.48 ± 0.12	0.52 ± 0.12	10	$\lvert t\rvert_{1,9} = 0.27$	0.8	0.40 ± 0.09	0.60 ± 0.09	9	$\lvert t\rvert_{1,8} = 0.98$	0.36
Rel Prop. of Time Respond to Female	0.51 ± 0.13	0.49 ± 0.13	10	$\lvert t\rvert_{1,9} = 0.11$	0.92	0.49 ± 0.14	0.51 ± 0.14	8	$\lvert t\rvert_{1,7} = 0.03$	0.98
Number of Twitches to Female	1.3 ± 0.6	4.3 ± 1.4	7	$\lvert t\rvert_{1,7} = 1.36$	0.22	6.1 ± 3.2	1.2 ± 0.7	6	$\lvert t\rvert_{1,5} = 1.05$	0.33
Female Behavior										
Prop of Time in Color Display	0.22 ± 0.12	0.34 ± 0.12	8	$\lvert t\rvert_{1,7} = 0.65$	0.54	0.25 ± 0.09	0.11± 0.06	9	$\lvert t\rvert_{1,8} = 1.04$	0.33
Prop of Time Actively Courting	0.06 ± 0.09	0.15 ± 0.13	8	$\lvert t\rvert_{1,7} = 1.20$	0.27	0.05 ± .03	0.01± 0.009	9	$\lvert t\rvert_{1,8} = 0.86$	0.41
Number of Female Twitches	2.0 ± 1.4	6.2 ± 3.6	9	$\lvert z\rvert = 0.841$	0.4	6.6 ± 3.7	1.3 ± 1.2	9	$\lvert z\rvert = 1.08$	0.28

These data are from an experiment in which a single focal male could choose between two females, one of which had been exposed to EE2 and one of which had not. We measured both male and female behavior in these experiments. Male behavior included the proportion of time males spent on the same side of the tank as female, the relative proportion of time males spent responding to each female and the number of twitches males preformed to each female. Female courtship behaviors included the proportion of time females were in color display and the proportion of time females actively courted males. Transformation of the number of twitches performed by females was unable to satisfy the assumption of normality, so a Wilcoxon two-sampled paired signed rank test was performed. All data are reported as mean ± S.E. of untransformed data.

these mating events did not occur until at least four days after removal from the exposure tanks, suggesting that there may be a lag time after removal from treated water during which males are not able to mate. For those males that became pregnant, exposure to EE2 did not affect the number of offspring in their broods (Control: 13.9 ± 4.4 [mean ± S.E.], n = 8; 1ng/L: 16.2 ± 3.8, n =8; 100ng/L: 16.3 ± 5.2, n = 4, $F_{2,19} = 0.10$, p = 0.9). Development of the offspring also appeared to be unaffected, as males that mated within 10 days after removal from treated water produced successful broods with completely developed juveniles, regardless of treatment. It is important to note, however, that during the period of brood development all males were housed in EE2-free water.

Discussion

Overall, our data clearly show that short-term exposure to even low levels of EE2 can significantly impact male reproductive fitness. Male attractiveness was significantly undermined by exposure to 1ng/L EE2, even though these males could still become pregnant and successfully carry broods to term. The results were even more pronounced when males were exposed to a higher dose of EE2. For example, males exposed to 100ng/L of EE2 were not only less attractive than control males, but they also experienced difficulty becoming pregnant. These effects occurred after only a 10-day exposure to EE2, and some of the morphological changes persisted well after the males were removed from the contaminated water (at least 19 days, Figure 4.2). In natural populations, exposure periods will typically be much longer, so the effects could be even greater than those we observed. How these changes in mating behavior are likely to

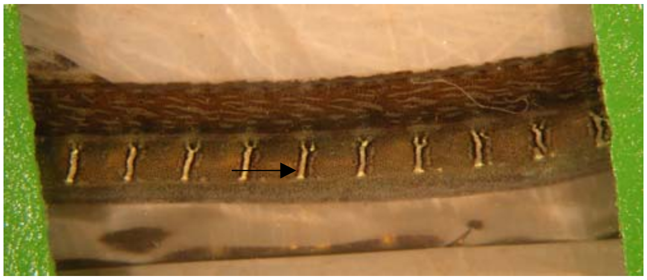


Figure 4.2: A male pipefish with iridescent stripes 19 days after removal from treated water. The arrow indicates the iridescent stripes, and the space between the green bars on either end of the photograph is 1.5 cm. This image shows that the feminization of males by EE2 persists after the males are moved to clean water. These traits persist at least several weeks, as they were still present when the males were sacrificed at the end of the experiment (2-3 weeks post-exposure). They may persist much longer, but this question was beyond the scope of our study.

impact natural populations calls for additional work, but it seems clear that the potential exists for some males to become sufficiently unattractive or female-like to be perceived as unacceptable for mating by some females, a situation that could impact short-term population viability. In addition, the disruption of normal mating dynamics imposed by EE2 contamination has the potential to alter the dynamics of sexual selection in Gulf pipefish populations, potentially altering long-term evolutionary trajectories.

Why does EE2 decrease male attractiveness and reproductive potential? One possible hypothesis is that changes in the body morphology of males may mimic female-specific visual cues, causing females to incorrectly identify exposed males as other females. Alternatively, EE2 exposure may cause males to release chemicals that contain components that typically are released only by females. However, female pipefish do not readily discriminate between males and females based on chemical cues alone (Ratterman et al. 2009), suggesting that our results are more likely related to visual cues. Males exposed to high doses of EE2 could experience high mating failure rates for two main reasons. First, as in the case of the males exposed to 1 ng/L EE2, females could be avoiding males exposed to 100 ng/L as mates. This effect should be even more pronounced for the high EE2 exposure treatments, in which exposed males are morphologically very similar to females. Second, exposure to high levels of EE2 also appears to affect the male's brood pouch, where eggs are received, fertilized and develop. Boisseau (1967) found that the brood pouch of male seahorses is under testicular control and that male castration significantly retards pouch development. This effect can be reversed by testosterone injections. The brood pouches of male Gulf pipefish, which are close relatives of seahorses, are likely under similar hormonal regulation. Males exposed to high concentrations of EE2 had brood pouches that appeared thin and resembled the pouches of non-reproductive males. Since testosterone is known to be involved in maintaining the brood pouch, it is likely that EE2 exposure interferes with testosterone levels, preventing the male brood pouch from remaining in a reproductive state. After the male is removed from treated water, it may take a few days to completely eliminate the EE2 from the body and allow the brood pouch to become functional again, possibly explaining why males in the high EE2 exposure treatment were unable to mate for four days after removal from the exposure treatment.

Our observations regarding the effects of a xenoestrogen on mating dynamics and reproduction at environmentally relevant concentrations, even after a short period of exposure, are significant with regard to the short- and long-term factors affecting populations. With respect to short-term effects, the disruption of mating patterns in pipefish has the potential to reduce intrinsic population growth rates. Even though the effects seem subtle at one level, as it seems likely at lower exposure concentrations that most males will eventually mate, reduced male attractiveness will be one among many factors negatively impacting populations at contaminated sites. In addition, EE2-affected males may experience reduced survivorship or reproductive success due to costs associated with increased time spent searching for mates, mating with suboptimal females or an inability to find any suitable mate. In pipefish, most of the variance in relative fitness in female pipefish due to sexual selection is driven by differential mating success (Partridge et al. 2009). Thus, anything that substantially affects mating patterns has the potential to have a large impact on individual fitness.

One troubling implication of our observations is that the effects of this compound may not be restricted to contaminated sites. Our study suggests that some of the female secondary sexual characteristics that are induced in males upon exposure are maintained for some time after removal from treated waters. We found that males continue to express the iridescent bars that are normally found on females for at least 19 days after removal from treated water (Figure 4.2). After this point males were sacrificed, so the amount of time exposed males continue to express altered traits is currently unknown. Thus, males migrating from contaminated sites to relatively pristine locales have the potential to carry the phenotypic effects, and presumably the effects on mating dynamics, of EE2 with them to the new site.

In addition to short-term effects on population health, our results suggest that endocrine disruptors may have long-term effects on important evolutionary processes. In the Gulf pipefish, males are the limiting sex, and sexual selection tends to act more strongly on females. Any factor that has a major impact on mating patterns, as EE2 has the potential to do, could disrupt sexual selection mechanisms. The exact effects are difficult to predict and would be worthy subjects of future research. On the one hand, modest levels of EE2 contamination might increase the strength of sexual selection on females, because females would be competing for a smaller pool of males that would be perceived as potential mates. In extreme cases, some males would be unable to mate altogether due to suppression of their pouches. On the other hand, EE2 contamination could decrease the strength of sexual selection, as males made artificially unattractive by EE2 exposure may be discriminated against by attractive females, forcing them to mate with less attractive females, which would normally be excluded from the mating pool due to strong female competition. Regardless, changes in the intensity of sexual selection in some populations could affect a number of key evolutionary processes. For example, increased competition for mates could reduce the effective population size, resulting in a loss of variation due to genetic drift. A decrease in sexual selection intensity could result in reduced population viability, as EE2-affected males are forced to mate with sub-par females potentially resulting in fewer direct or indirect benefits of mate choice and reduced offspring fitness. If populations differ with respect to levels of EE2 contamination, the differences in mating systems imposed by EE2 could even decrease population connectivity in the long term (Zeh and Zeh 2000). The disruption of an evolutionary mechanism as important as sexual selection may have many other, unanticipated effects in addition to the ones we have mentioned here. Overall, these considerations lead to the conclusion that the population-level effects of the EE2-induced behavioral changes observed in the present study represent an area of critical need for future research.

Our study's call for additional research on population-level effects of anthropogenic contaminants that disrupt sexual selection is bolstered by other recent studies. For example, changes in reproductive hierarchies and sexual selection were observed by Coe et al. (2008) in zebrafish after exposure to EE2. The proportion of offspring sired by males that were dominant in the hierarchy prior to exposure was significantly suppressed after exposure to EE2, allowing subordinate males to increase their relative reproductive fitness. A similar effect was observed in the sand goby, where exposure to EE2 decreased the strength of sexual selection on male size, allowing smaller males to gain mating opportunities (Saarisota et al. 2009). In addition, exposure of pregnant female amarillo fish to methyl parathion, an insecticide, suppressed the expression of secondary sexual traits in male offspring (Arellano-Aguilar and Garcia 2008). Similar to our study, this effect resulted in the exposed male offspring being less preferred by females during choice trials. These results, along with our study suggest that solely examining the effect of these compounds on reproductive output may not be enough to determine how they are affecting populations, especially considering that processes involved in pre-copulatory sexual selection may be more sensitive to endocrine disruptors than general reproductive success. Thus in order to understand how endocrine disruptors are affecting ecosystems, it is important to be aware that these compounds can impact these long-term selection processes.

In summary, we found that even low levels of exposure to the endocrine disruptor EE2 in Gulf pipefish are sufficient to disrupt mating dynamics. Males exposed to EE2 become more female-like with respect to external morphology and develop secondary sexual traits that normally appear only in females. In addition, these altered traits may persist for long periods of time post-exposure. Females prefer non-exposed males compared to exposed males, and in some cases exposed males have a reduced capacity to become pregnant. Thus, EE2 exposure decreases male mating opportunities, potentially affecting population-level mating dynamics. Overall, our study provides evidence for a role of endocrine disruptors in disrupting social interactions in natural populations, a topic that has remained largely unstudied. Our study clearly shows that the effects of endocrine disruptors on other aspects of reproductive fitness, especially pre-copulatory mate choice mechanisms, could have profound impacts on natural populations with respect to short-term population viability and long-term evolutionary processes. The effects of these behavioral changes in populations occupying contaminated environments should be a major focus of future research.

CHAPTER V

POPULATION STRUCTURE OF THE GULF PIPEFISH, *SYNGNATHUS SCOVELLI,* IN MOBILE BAY AND THE GULF OF MEXICO

Introduction

Understanding and identifying the mechanisms leading to population divergence is one of the greatest challenges in evolutionary biology. This challenge becomes even more complex when population divergence occurs in species where a high potential for gene flow between populations exists, such as between sympatric or parapatric populations. One reason for genetic divergence between these populations is that they may inhabit different environmental niches or environments and local selection pressure may differ enough to drive speciation (Schluter 2000, 2001; reviewed in Rundle and Nosil 2005). Speciation associated with populations inhabiting different ecological niches, termed ecological speciation, has been shown to be a major evolutionary factor and is one of the driving components in the adaptive radiation of a number of species, including African cichlids (Wagner and McCune 2009), Darwin's finches (reviewed in Grant and Grant 2008) and Anolis lizards (reviewed in Losos 2009).

Recently it has been proposed that ecological speciation is likely one of the main driving forces causing population divergence between coastal and marine populations (Beheregaray and Sunnucks 2001). One specific model, the "divergence-with-gene-flow model" of speciation suggests that populations with no barriers to gene flow can diverge when strong divergent selection acts on sub-populations that are separated geographically (Endler 1977; Rice and Hostert 1993). In the case of most marine species, high levels of gene flow occur among populations. These populations tend to be continuously distributed, maintain a large effective population size and, in the case of pelagic spawners, produce larvae that can disperse large geographic distances, all of which aid in suppressing genetic divergence. However, when groups colonize new environments, such as estuarine habitats, differences in the selective pressure between marine and estuarine environments may drive speciation. In support of this model, recent studies have shown that colonization of a marine species into an estuarine habitat has lead to genetic divergence in the marine silverside (Beheregaray and Sunnucks 2001). Physiological, behavioral and reproductive differences can also occur between these populations and it has been suggested that these colonized estuarine populations may actually be insipient species (Beheregaray and Sunnucks 2001). Consequently, studying these recently established populations may provide information concerning factors that are influencing present day speciation events.

Euryhaline species are one of the most appropriate groups in which to examine divergence-with-gene-flow models of speciation. In many cases these groups can migrate between marine, estuarine and freshwater environments. However, because of the difference in selective pressure among these ecosystems, populations inhabiting these three environments may diverge even though migration occurs. This is especially true if developmental constraints limit reproductive success when individuals migrate to new environments. Acclimation to environmental salinity during development may impact the ability of euryhaline organisms to fully adjust to new environmental salinities once adulthood is reached (Lee and Peterson 2003). This limit on salinity tolerance due to developmental acclimation may aid in driving divergence between marine, euryhaline and freshwater populations. For example, adults that developed in a freshwater environment may not be able to fully adapt to a marine system after migration, which may decrease their relative reproductive success. This decrease in fitness of migratory individuals would strengthen genetic isolation between these two groups, because while migration may occur between populations, individuals migrating to environments that are significantly different from the one in which they developed would have lower relative fitness values compared to local individuals. Thus, while some gene flow would be occurring between populations, migrant individuals may not contribute significantly to the gene pool of their new environment.

The Gulf pipefish, *S. scovelli*, is an excellent model system in which to examine how local habitats may be influencing population structure. *S. scovelli* is a member of the family Syngnathidae, which includes seahorses and pipefish. This family is characterized by male pregnancy, where females either glue eggs to the ventral surface of the male or deposit eggs into the brood pouches of males, depending upon the species (Wilson et al. 2001). The males then brood the embryos until they are juveniles after which they are released into the environment and no other paternal care occurs. *S. scovelli* is one of the most common pipefish in the Gulf of Mexico. This species has a broad geographic range that extends from the Georgia coast to Central America (Amos and Amos 1985) and while this species is relatively abundant along the entire Gulf of Mexico coast, very little is known about its population structure. Pipefish are found in submerged aquatic vegetation beds, and they are thought to migrate little during the summer breeding season. However, during the winter months, once the shallow-water vegetation dies, it is thought that they may move into deeper waters (Hamilton 1942; Lazzari and Able 1990; Franzoi et al. 1993; Teixeira 1995; Power and Attrill 2003; Bolland and Boettcher 2005), where individual groups that were segregated during the breeding period may intermix. In addition, unlike other North American pipefish species, *S. scovelli* can regularly inhabit freshwater environments as well as brackish and marine habitats (Hoese and Moore 1977) and multiple freshwater populations have been observed along the southeastern United States (McLane 1955; Whatley 1969; Targett 1984; Viola 1992). Because juvenile dispersal in this species is probably limited and local selective pressures between coastal, estuarine and freshwater populations likely differ, populations may show some degree of genetic structure between environments. However, since it has been suggested that these individuals may intermix during the winter months and *Sygnathus spp*. are not known to exhibit any type of site fidelity (Vincent et al. 1995), it is possible that these populations may be panmictic.

The purpose of this study is two-fold. First we used microsatellite data to assess the genetic structure of *S. scovelli* populations in and around Mobile Bay and the Gulf of Mexico. Three of these populations were located in two freshwater rivers that run into Weeks Bay, a sub-estuary of Mobile Bay, one population was located in the mid-upper part of Mobile Bay, and three populations were located throughout the Gulf of Mexico. Second, we wanted to determine whether local habitat influenced genetic structure in this species by determining whether coastal populations were genetically distinct from populations within Mobile Bay.

Methods and Materials

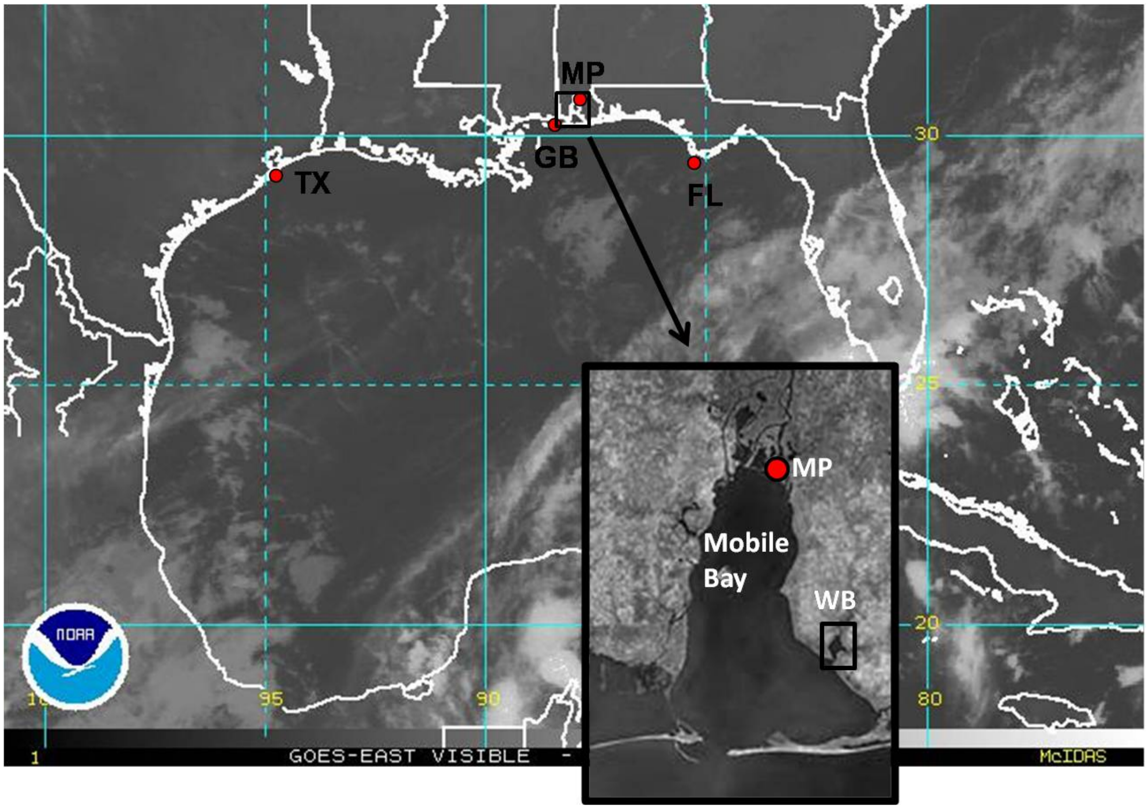
Population Structure

Pipefish Collection. Male and female pipefish were collected using a seine net in shallow grass beds from a total of seven sites within Mobile Bay and throughout the Gulf of Mexico (Table 5.1). Three of these sites were located in coastal areas along the Gulf of Mexico and included one within the Grand Bay National Estuary Research Reserve, MS (GB), one at St. Joseph Bay, FL (FL), and one at Aransas Pass, TX (TX) (Figure 5.1). One site was located within the middle/upper part of Mobile Bay at Meaher State Park, AL (MP). The remaining three sites were located within freshwater rivers that are part of the Weeks Bay watershed, a sub-estuary of Mobile Bay, two of which were located in Fish River (WBS1 and WBS2) and the third site was located in Magnolia River (WBS4) (Figure 5.2). Sites were chosen to ensure a broad geographic distribution and to obtain individuals from differing ecological habitats. After collection, pipefish were anesthetized using MS222 and sacrificed. Individuals were preserved in 95% EtOH for genetic analysis.

DNA Extraction and Microsatellite Analysis. Total DNA was extracted using a Gentra Puregene® Cell Tissue Kit (Quiagen, Valencia, CA). Individuals were genotyped at six microsatellite markers. These markers included previously described

Table 5.1: Sites, site abbreviations (Abb), number of individuals collected (N) and GPS coordinates of Gulf pipefish, *S. scovelli*, sampling locations.

Site	Abb	N	GPS Coordinates (N, W)
Weeks Bay - Turkey Branch, AL	WBS1	30	30°42978, 87°32378
Weeks Bay - Mouth of Barner, AL	WBS2	39	30°45894, 87°80418
Weeks Bay - Magnolia River, AL	WBS4	15	30°45783, 87°80242
Meaher Park, AL	MP	44	30°66624, 87°92731
Grand Bay, MS	GB	32	30°36164, 88°39776
St. Joseph Bay, FL	FL	24	29°4776', 85°1824'
Aransas Pass, TX	TX	54	27°8806, 97°1019'



http://

Figure 5.1: Location of *S. scovelli* collection sites within Mobile Bay and the Gulf of Mexico. St. Joseph Bay, FL (FL); Weeks Bay watershed, AL (WB); Meaher Park, AL (MP); Grand Bay Research Reserve, MS (GB); Port Aransas, TX (TX).

Figure 5.2: Google earth image of sample locations of sites throughout the Weeks Bay watershed. Collection sites WBS1 and WBS2 are located in Fish River and collection site WBS4 is located in Magnolia River.

markers, *Micro 11.1*, *Micro 22.3*, *Micro 25.1*, and *Micro 25.22* (Jones and Avise 1997b), and one newly developed marker, *SS07-25* (*SS07-25 forward* : TTGCAGAAAGGAAATTACTGAGG; *SS07-25* reverse: GCAGTAGCAATCATGGAGAGG), all of which were developed for *S. scovelli.* In addition, one previously described microsatellite developed for *S. typhle*, *Typh12*, was used (Jones et al. 1999). Microsatellites were amplified using polymerase chain reaction (PCR). The thermal cycling included an initial denaturation step of 2 min at 95ºC followed by 35 cycles of the following: 1 min at 95°C, 1 min at the annealing temperature (*Micro 11.1*: 50ºC, *Micro 22.3*: 58ºC, *Micro 25.1*: 57ºC, *Micro 25.22*: 58ºC, *SS07-25*: 53ºC, and *Typh 12*: 54ºC), and 1 min at 72ºC. After cycling, an elongation step of 4 min at 72ºC was performed. All fragment analysis was performed on an ABI Prism® 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) and was analyzed using ABI Prism® PeakScanner™ software (Applied Biosystems, Foster City, CA). All microsatellite loci were binned using the program Flexibin (Amos et al. 2007) and then manually checked for error.

Population Structure Analysis. Population data from microsatellite loci were analyzed using GENEPOP version 4.0 (Rousset 2008). The observed heterzoygosity (H_o), expected heterozygosity (H_e), mean number of alleles per locus (N_a) and inbreeding coefficient (F_{IS}) were calculated for each locus at each site. All loci for each site were tested for deviations from Hardy-Weinberg equilibrium (HWE) using exact tests in GENEPOP. *SS07-25* was found to have an excess of homozygotes for four of the seven populations, thus this locus was removed from further analysis. In order to determine the genetic divergence among populations, global and population pair-wise F_{ST} and R_{ST} values were estimated and significant values were inferred based on a G based exact test (Goudet et al. 1996). All significant levels were adjusted based on a Bonferroni correction when appropriate.

Pair-wise genetic identity and genetic distance values (Nei 1978) were calculated using POPGENE version 1.31 (Yeh et al. 1999). Because previous studies have suggested that estuarine populations are distinct from coastal populations, we grouped populations into a coastal group (GB, TX and FL) and an estuarine/freshwater group (WBS1, WBS2, WBS4, and MP) and utilized an analysis of molecular variance (AMOVA) using the program ARLEQUIN version 2.0 (Schneider et al. 2000) to determine how genetic variability was partitioned within and among these groups. A Bayesian clustering approach was used to estimate the number of populations (K) of the Gulf pipefish within the Gulf of Mexico and Mobile Bay using the program STRUCTURE version 2.3 (Pritchard et al. 2000). A Markov chain Monte Carlo (MCMC) method was utilized and the simulation was run for 10^6 iterations after a burn-in period of 10^5 iterations for 1-8 populations. Each simulation was run 10 times and this model assumed an admixture ancestry, correlated allele frequencies and included a prior location information. The most likely value of K was then determined using the method described by Evanno et al. (2005).

Results

All microsatellite loci were found to be highly polymorphic and the number of alleles per loci across all populations ranged from 29 (*Micro 22.3*) – 52 (*Micro 25.1* and *Typh12*). In general, loci satisfied the assumptions of HWE for most populations. One exception to this was the *SS07-25* locus which was found to be out of HWE for 4 out of 7 of the populations analyzed and was removed from further analysis (Table 5.2).

Microsatellite data suggests that there is moderate genetic structure among some populations of S. *scovelli* within Mobile Bay and the Gulf of Mexico (global F_{ST} = 0.025). Pair-wise comparisons of populations from Texas, Florida, and Mississippi produced significant F_{ST} values (Table 5.3). Also, all coastal populations were found to be genetically distinct from the population at Meaher Park and all populations within Weeks Bay. However, pair-wise F_{ST} values were not significant between any of the populations within Weeks Bay, suggesting that there is no genetic difference between populations from the Fish (WBS1 and WBS2) and Magnolia Rivers (WBS4) within Weeks Bay (Table 5.3). Interestingly, both populations within the Fish River (WBS1 and WBS2) were found to be genetically distinct from the Meaher Park population; however, the Magnolia River (WBS4) population was not distinct from the population at Meaher Park.

Pair-wise comparisons of Nei's genetic distance values showed that populations within Weeks Bay and Mobile Bay were the most closely related among the sampled populations (Table 5.4; Figure 5.3). In addition, while most of the genetic variation was explained by within population effects, AMOVA showed a weak but significant pattern

Table 5.2: Number of individuals (N), allele number (N_a), expected and observed heterozygosity (H_e, H_o) and inbreeding coefficient (F_{IS}) (Weir and Cockerham 1984) for microsatellite loci from 7 populations of *S. scovelli.* Bold values indicate loci that deviated significantly from Hardy-Weinberg equilibrium.

Locus	Samples						
	WBS1	WBS2	WBS4	MP	GB	FL	TX
Micro 25.1							
N	30	38	15	44	32	24	52
N_a	28	30	12	34	24	23	40
H_E	0.908	0.797	0.805	0.859	0.776	0.914	0.94
H_O	0.933	0.842	0.733	0.795	0.719	0.833	0.865
F_{IS}	-0.0278	-0.0571	0.091	0.0744	**0.0746**	0.09	**0.08**
Micro 25.22							
N	28	36	12	41	32	23	50
N_a	16	18	12	26	20	20	32
H_E	0.897	0.866	0.902	0.936	0.904	0.954	0.941
H_O	0.964	0.861	1	0.951	0.813	0.957	0.96
F_{IS}	-0.076	0.006	-0.114	-0.0169	0.1029	-0.0031	-0.0202
Micro 11.1							
N	29	37	12	36	27	23	51
N_a	17	20	15	27	23	23	28
H_E	0.8494	0.868	0.942	0.903	0.957	0.956	0.942
H_O	0.9655	0.891	0.917	0.806	0.963	1	0.882
F_{IS}	-0.14	-0.0281	0.028	**0.1096**	-0.0067	-0.0476	**0.064**
Micro 22.3							
N	29	33	15	39	28	23	51
N_a	19	22	17	22	20	20	23
H_E	0.935	0.953	0.947	0.938	0.944	0.942	0.937
H_O	0.966	0.97	1	0.974	1	0.957	0.902
F_{IS}	-0.0329	-0.017	-0.058	-0.039	-0.0603	-0.0157	0.0383
SS07-25							
N	29	37	15	33	25	23	53
N_a	20	20	14	21	29	21	41
H_E	0.945	0.9411	0.92	0.936	0.973	0.962	0.973
H_O	0.897	0.8649	0.8	0.97	0.68	0.6087	0.811
F_{IS}	0.0521	**0.0821**	0.134	-0.0364	**0.3055**	**0.5988**	**0.1677**
Typh 12							
N	25	33	9	44	32	22	47
N_a	20	22	8	27	30	27	38
H_E	0.926	0.887	0.869	0.887	0.954	0.975	0.974
H_O	0.8	0.879	0.889	0.812	0.938	1	0.851
F_{IS}	0.138	0.0096	-0.024	**0.078**	0.0174	-0.0267	**0.127**

Table 5.3: Pair-wise F_{ST} and R_{ST} values among population of *S. scovelli* in the Gulf of Mexico and Mobile Bay. F_{ST} values are above the diagonal and R_{ST} values are below the diagonal. Bold values indicate significant F_{ST} values after Bonferroni corrections.

	WBS1	WBS2	WBS4	MP	FL	GB	TX
WBS1	0	0.0009	0.0016	**0.0169**	**0.0305**	**0.0323**	**0.0292**
WBS2	-0.0035	0	0.0013	**0.0158**	**0.0389**	**0.0254**	**0.0375**
WBS4	0.0167	-0.0028	0	0.0045	**0.0238**	**0.019**	**0.0279**
MP	0.0024	-0.002	-0.0085	0	**0.0245**	**0.0174**	**0.0279**
FL	0.0932	0.0518	0.0486	0.0174	0	**0.0129**	**0.0138**
GB	0.0673	0.0301	0.0168	0.0109	-0.0108	0	**0.0302**
TX	0.0713	0.1067	0.167	0.0948	0.198	0.182	0

Table 5.4: Pair-wise estimates of Nei's (1978) genetic identity and distance values among populations of *S. scovelli*. Genetic identity values are above the diagonal and genetic distance values are below the diagonal.

	WBS1	**WBS2**	**WBS3**	**MP**	**FL**	**GB**	**TX**
WBS1	0	0.9502	0.8905	0.7937	0.555	0.6468	0.6285
WBS2	0.0511	0	0.8941	0.8406	0.5744	0.7576	0.6349
WBS3	0.116	0.112	0	0.8521	0.6211	0.7271	0.5798
MP	0.2311	0.1736	0.1601	0	0.6229	0.7832	0.6298
FL	0.5887	0.5545	0.4763	0.4734	0	0.7577	0.5567
GB	0.4358	0.2776	0.3261	0.2444	0.2775	0	0.5713
TX	0.4644	0.4543	0.545	0.4623	0.5858	0.5598	0

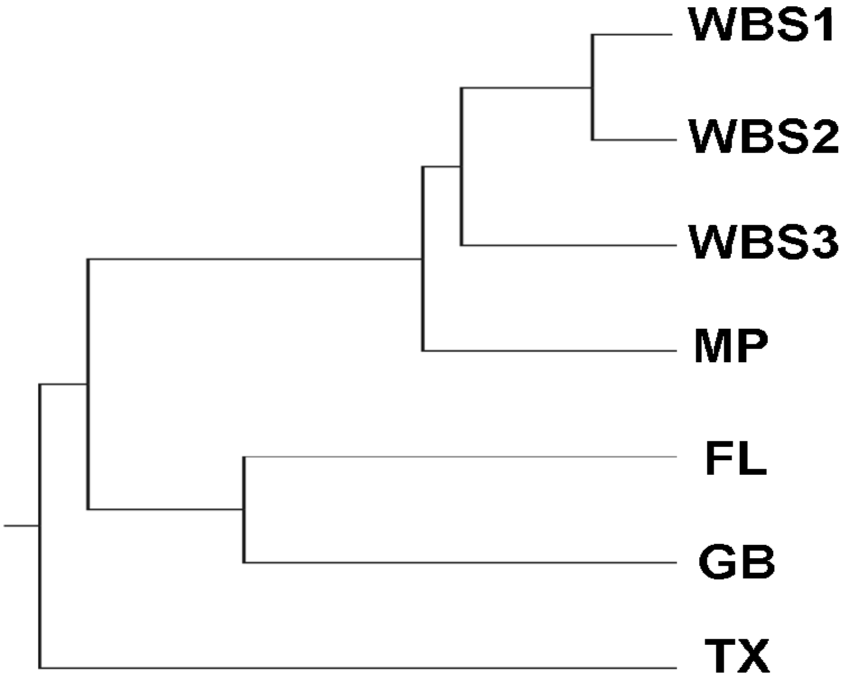


Figure 5.3: UPGMA dendrogram based upon Nei's (1978) genetic distance values showing the genetic relatedness of 7 pipefish populations throughout the Gulf of Mexico and Weeks Bay. Sample locations include 3 populations within Weeks Bay watershed, two of which were located in the Fish River (WBS1 and WBS2) and the third located within the Magnolia River (WBS3), one population within Meaher State Park (MP), one population within the Grand Bay Estuarine Research Reserve (GB), a population from St. Joseph's Bay, FL (FL) and a population from Port Aransas, TX (TX).

of genetic structure between the estuarine/freshwater group (WBS1, WBS2, WBS4, MP) and the coastal group (FL, GB, TX) (Table 5.5).

Bayesian cluster analysis estimated 2 parental populations (K=2) of Gulf pipefish within the Gulf of Mexico and Mobile Bay (Figure 5.4). Texas and Florida populations showed little admixture with over 90% of individuals being assigned to Cluster 2 (Table 5.6). All other populations contained some degree of admixture with a proportion of individuals being placed into both clusters. However, for all three Weeks Bay populations, the majority of individuals were assigned to Cluster 1, while the majority of individuals from Grand Bay, MS were assigned to Cluster 2. Meaher Park contained approximately the same proportion of individuals assigned to Clusters 1 and 2 (Table 5.6).

Discussion

This study had two specific goals. The first goal was to determine whether populations of Gulf pipefish within the Gulf of Mexico and Mobile Bay were genetically distinct. Based on microsatellite data, we found that moderate levels of genetic structure occur in the Gulf of Mexico. All coastal populations were genetically distinct from one another and from populations within Mobile and Weeks Bay. Populations within Weeks Bay were not genetically distinct from one another and the Magnolia River population within Weeks Bay (WBS4) was not genetically distinct from the Meaher Park population, located in middle/upper Mobile Bay (Table 5.3). However, pipefish populations within Fish River, which drains into Weeks Bay, were found to be

Table 5.5: Analysis of molecular variance (AMOVA) among 7 populations of *S.scovelli* group into either an estuarine/freshwater group (WBS1, WBS2, WBS3 and MP) or a coastal group (FL, MS, and TX).

Source of variation	df	Variance components	% variation	Fixation Indices	P-value
Among groups	1	0.029	1.45	0.015	0.026
Among pops. within groups	5	0.021	1.06	0.011	<0.0001
Within populations	469	1.963	97.49	0.025	<0.0001

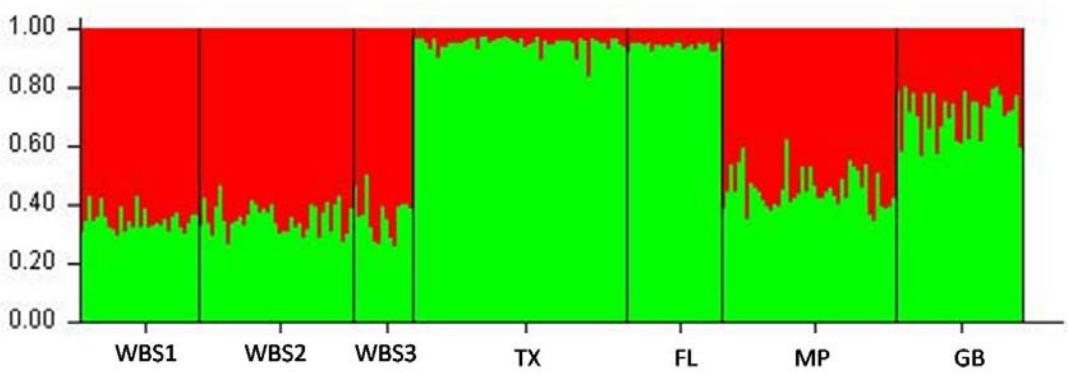


Figure 5.4: Results from Bayesian cluster analysis using the program STRUCTURE indicating the presence of 2 (K=2) population clusters of *S. scovelli*, a costal population (green) and a Weeks Bay populations (red). Each line represents an individual within a population and populations are grouped together on the x-axis. On the y-axis are probabilities of individual assignment to either cluster.

Table 5.6: Proportion of individuals assigned to Cluster 1 (Weeks Bay cluster) and Cluster 2 (coastal cluster) after Bayesian cluster analysis using the program STRUCTURE.

Population	No. of individuals	Cluster 1	Cluster 2
Weeks Bay Site 1	30	0.651	0.349
Weeks Bay Site 2	39	0.644	0.356
Weeks Bay Site 4	15	0.636	0.364
Meaher Park, AL	44	0.546	0.454
Grand Bay, MS	32	0.289	0.711
St. Joe Bay, FL	24	0.058	0.942
Port Aransas, TX	54	0.047	0.953

moderately distinct from the Meaher Park population (Table 5.3). While significant genetic structure exists between many of the populations sampled, our data show that these populations probably maintain some level of gene flow, since F_{ST} values were relatively low.

Our second goal was to examine whether habitat may be contributing to genetic differentiation. To examine if habitat differences are related to genetic structure, our freshwater and estuarine populations (WBS1, WBS2, WBS4 and MP) were grouped together to form an estuarine/freshwater group, while coastal populations (FL, MS and TX) were grouped together to form the coastal group. We then examined the proportion of genetic variance explained by variation among groups, variation among populations within groups and variation within populations. Much of the variation was explained by within population variance; however, a significant amount of variation was also explained by the variation among groups, suggesting that some genetic structure exists between estuarine/freshwater and coastal populations (Table 5.5). In addition, populations within groups showed significant genetic structure, which is not surprising since F_{ST} values indicated that all coastal populations were genetically distinct from one another (Table 5.3).

Another way in which to analyze this data set would be to separate out estuarine and freshwater populations and then examine the degree of genetic structure for all three ecosystems. However, we felt that grouping the Meaher Park and Weeks Bay populations together for the estuarine/freshwater group was justified for at least two reasons. First, no genetic structure was found between the Meaher Park population and the population within the Magnolia River in Weeks Bay (WBS4). Since the Magnolia River population is also connected to Fish River populations within Weeks Bay (WBS1 and WBS2), we felt justified in grouping all four populations together. Second, the physical environment at Meaher Park is very similar to that of a true freshwater environment. Meaher Park is located within the middle-upper part of Mobile Bay and is heavily influenced by freshwater tributaries that enter into the Bay. The salinity at this site normally ranges between 0-3 ppt and the submerged aquatic vegetation consists of freshwater plants (Bolland and Boettcher 2005). In addition, most of the other fish collected at Meaher Park are freshwater species. Because of the openness of Mobile Bay to the Gulf of Mexico, saltwater can encroach into this area, especially during drought conditions or storm events. However, during most of the breeding season Meaher Park tends to resemble a freshwater system (Boettcher and Bolland 2005) and thus the selective pressure on this pipefish population most likely resembles the selection pressure observed in other freshwater systems.

The result that coastal Gulf pipefish populations are genetically distinct from one another is not too surprising considering similar results have been found for populations of the dusky pipefish, *S. floridae*, in which Texas and Florida populations were shown to have moderate levels of genetic structure (Mobley 2007). As suggested for *S. floridae*, limited swimming ability of adults and lack of a pelagic larval stage limit gene flow between these populations and are likely to account for much of the genetic divergence (Mobley, 2007). Although these coastal populations were genetically distinct, there also appears to be moderate levels of gene flow between them. Pipefish migration has never fully been assessed; however, it has been suggested that once submerged vegetative beds die during the winter, pipefish migrate to deeper waters where they may intermix with other populations (Hamilton 1942; Lazzari and Able 1990; Franzoi et al. 1993; Teixeira 1995; Power and Attrill 2003; Bolland and Boettcher 2005). Once waters begin to warm and the vegetation returns, pipefish then return to these shallow grass beds; however, whether pipefish display some form of site fidelity or whether they return to a different areas each season is unknown. Site fidelity has only been studied in a handful of syngnathid species. It has been shown that at least two species of pipefish, *Nerophis lumbriciformis* (Monteiro et al. 2005) and *Corythoichthys haematopterus* (Matsumoto and Yanagisawa 2001), exhibit some site fidelity; however, this type of behavior has not been observed for any *Syngnathus* spp (Vincent et al. 1995). While it is unlikely that pipefish move over long distances to new populations during this period, due to their limited swimming ability, they may move between neighboring populations. Also, it has been suggested by Dawson (1982) and Mobley (2007) that while this Family does not have a pelagic larval phase, juvenile dispersal may occur between populations. Juvenile pipefish are often found within floating aquatic vegetation, which can be transported by oceanic currents over several hundred kilometers to neighboring pipefish populations. This passive migration of juveniles would promote modest levels of gene flow between these populations.

Our data also suggests that moderate levels of gene flow are occurring between coastal populations and those within Mobile and Weeks Bay. Similar to coastal populations, gene flow between these two areas could be the result of migrants moving between the Bay and the Gulf of Mexico or due to passive migration of juveniles floating in aquatic vegetation out of the Bay. Additionally, the Gulf Coast region is known to be prone to severe weather, such as tropical depressions, tropical storms and hurricanes. When these events occur within the Gulf of Mexico, storm surges can push large amounts of saltwater from the Gulf of Mexico into Mobile Bay and surrounding areas. In 2004 and 2005, Hurricanes Ivan and Katrina produced storm surges of up to 10-12 feet around Mobile Bay, pushing with it not only large amount of saltwater but also marine aquatic life. In addition, water influx from the Gulf of Mexico is not solely limited to major hurricane events as smaller tropical systems can also push modest levels of saltwater from the Gulf into the Bay. In Meaher Park, saltwater pipefish, such as *Syngnathus floridae*, can commonly be found after this type of event. In addition, depending upon the location of the storm, winds can also transport water out of the Bay into the Gulf of Mexico, thus carrying estuarine and freshwater species into the Gulf. While freshwater fish would not tolerate the change in salinity, most euryhaline organisms would be able to acclimate and thus be moved to coastal populations. It is therefore likely that these storm events are at least in part contributing to gene flow between the coastal populations and populations within Mobile and Weeks Bay.

According to Bayesian cluster analysis, two “parental” populations of Gulf pipefish occur within the Gulf of Mexico and Mobile Bay. One population appears to include the majority of coastal individuals (Coastal cluster), while the other population appears to consist mainly of those within Weeks Bay (Weeks Bay cluster). However, the individuals from Meaher Park were assigned in almost even proportions to both clusters.

These observations, along with AMOVA data, suggest that populations along coastal areas are distinct from some populations within the Bay, particularly those located in Weeks Bay. This distinction between populations within Mobile Bay and the Gulf of Mexico has also been found in other estuarine fish. Populations of the Gulf killifish within Mobile Bay form a unique genetic cluster that is genetically distinct from two other population clusters within the Gulf of Mexico (Williams et al. 2008). Interestingly, all populations sampled contained individuals that were assigned to the predominantly coastal cluster (Cluster 2), while only 5-6% of individuals from the Texas and Florida populations were assigned to the predominantly Weeks Bay cluster (Cluster 1). Meaher Park and Grand Bay populations contained moderate proportions of individuals assigned to both the coastal and Weeks Bay cluster. This suggests that coastal individuals are migrating, either due to normal migration patterns or due to storm events, into Mobile and Weeks Bay. However, there is little contribution from the Weeks Bay cluster to Texas and Florida population. Individuals from Grand Bay were mostly assigned to the coastal cluster, but 30% of these individuals were assigned to the Weeks Bay cluster. This indicates that some individuals from the Weeks Bay cluster were able to enter the Gulf and become established in coastal populations that are located close to the Bay.

With moderate levels of gene flow and the likelihood of coastal individuals being pushed into the Bay during tropical events, which normally occur annually, the question then becomes, what is maintaining the significant genetic structure between coastal populations and populations within Mobile and Weeks Bay? First, migration rates between these populations may not be large enough to limit genetic differentiation. This is especially true for the river systems within Weeks Bay. Weeks Bay proper has limited submerged aquatic vegetation and in order for pipefish populations to migrate out of the river systems and into Mobile Bay they would have to traverse this open area. In this case, potential predation within Weeks Bay proper may be high enough to limit the number of migrants that move in and out of Weeks Bay. The potential for migration between Meaher Park and coastal populations is higher due to the openness of the Bay and continued submerged aquatic vegetation beds along the shores. However, as with the coastal populations, limited swimming ability and no larval dispersal may limit migration between these areas. Second, it has been suggested that divergent selective pressure between these environments may be driving population divergence even when the potential for gene flow exists (Beheregaray and Sunnucks 2001). As has been suggested for other euryhaline organisms, development acclimation may negatively impact individuals migrating between areas where salinity significantly differs (Lee and Peterson 2003). Currently, there are no data assessing the effects of developmental acclimation on pipefish performance or offspring quality. However, if these migrants are more stressed relative to local individuals, then this may limit their ability to compete over access to resources or mates and thus limit the amount they contribute to the gene pool. In addition, during embryonic development, the osmotic environment of the brood pouch, where offspring develop, is maintained by the male. Structural differences of mitochondria-rich cells, which aid in osmoregulation, within the brood pouch occur between males from low salinity and saltwater environments (Partridge et al. 2007).

Whether these structural differences are plastic and can change when migrants enter environments with significantly different salinities is not known. However, if migration to areas of differing environmental salinities constrains their ability to utilize the pouch as an osmoregulatory organ, then this may affect offspring viability. Thus, offspring produced from native individuals would be more viable relative to those produced from migrants.

Our study suggests that coastal and estuarine/freshwater populations are genetically distinct even though moderate levels of gene flow occur between these populations. The presence of individuals within Weeks Bay that were assigned to the coastal cluster suggests that contemporary gene flow of coastal individuals into the Bay is occurring. Additionally, gene flow out of Weeks Bay to coastal regions also appears to occur since moderate numbers of individuals from Grand Bay were assigned to this cluster; however, these individuals do not appear to significantly contribute to populations within Florida and Texas. Weeks Bay is approximately 6,000 years old and throughout this time has consistently been connected to Mobile Bay. Therefore, past geographic isolation with secondary contact between these estuarine/freshwater and costal populations is unlikely to explain this pattern of genetic differentiation. We therefore suggest that the differentiation between these populations most resembles the divergence-with-gene-flow models. Whether this pattern is mainly due to limited migration between pipefish populations from Weeks Bay to the Gulf of Mexico, or whether selective pressures between these two populations are contributing significantly to this divergence should continue to be assessed. There has been little research partitioning out the relative contribution of various selective process on the genetic differentiation of coastal and estuarine populations. However, this should be an active area of research since it will provide vital information concerning how selective mechanisms promote genetic differentiation when gene flow still occurs between populations.

CHAPTER VI

DISTRIBUTION OF MERCURY AMONG PIPEFISH POPULATIONS WITHIN THE WEEKS BAY WATERSHED, AL

Introduction

Population and genetic diversity have long been associated with environmental health. Loss of genetic diversity can be a natural process due to inbreeding or genetic drift, particularly in small populations. However, it has also been suggested that levels of genetic diversity may be influenced by anthropogenic stressors, such as pollutants (Strittholt et al. 1988; Foré et al. 1995a; Foré et al. 1995b; Gillespie and Guttman 1993; Benton et al. 2002). Until recently, it has been largely assumed that exposure to toxic compounds leads to a decrease in the genetic diversity of natural populations due to population bottlenecks or contamination-induced selection (Theodorakis 2003). However, recent studies suggest that populations in contaminated areas may be just as diverse, if not more so, than those in areas that are not impacted by pollution (Eeva et al. 2006; Theodorakis et al. 2006). In contaminated areas, initial exposure to mutagenic toxins may cause a loss of genetic diversity due to selective mortality of maladapted individuals (Guttman 1994; Theodorakis 2003). However, due to the high mutation rates that contaminated populations incur, studies are now showing that the diversity of these populations can be greater than that of non-contaminated populations (Thedorakis et al. 2006). However, this increase in genetic diversity does not necessarily translate into increased population health, since accumulation of deleterious mutations could affect the average fitness of the population, potentially leading to extinction due to mutational meltdown (Gabriel and Bürger 1994; Theodorakis 2003).

One of the most concerning environment pollutants is mercury. Mercury is a naturally occurring heavy metal that is commonly found in natural ecosystems at low concentrations (Bargagli 2000). However, the burning of coal, waste incineration and other industrial processes can produce large amounts of mercury, which are deposited into the ecosystem through particulate aerial deposition or precipitation (Lovett et al. 2009). Mercury is particularly concerning for a number of reasons. First, once it enters freshwater environments it can become methylated, which aids in its ability to bioaccumulate up aquatic food webs (Kidd et al. 1995). This is significant considering most commercially important species for human consumption are predatory fish and would contain the highest levels of mercury. Second, the health effects of mercury exposure in humans are numerous. This compound most commonly affects neurological and renal systems (reviewed in Florea and Büsselberg 2006). Specifically, the lipid solubility of mercury allows it to be easily transported to the brain where it can affect neuromotor, behavior and cognitive function (Ratcliffe et al. 1996; Sanfeliu et al. 2003; reviewed in Florea and Büsselberg 2006). Mercury has also been shown to be a genotoxin and can severely damage DNA (Sarafian 1999; Grotto et al. 2009). In addition to its negative effects on humans, mercury can significantly impact natural wildlife populations. Exposure to mercury has been shown to disrupt organismal development, gonadal development, reproduction and some neurological functions, such as predator avoidance behaviors (Webber and Haines 2003; reviewed in Lovett et al.

2009) for a number of different systems. Because of the deleterious effects environmental exposure to mercury can induce, in both human and wildlife populations, identifying areas of potential contamination and understanding how these compounds impact population viability is vital.

Studies evaluating the effects of heavy metal contamination on genetic diversity in natural populations have been mixed. Some studies have shown genetic diversity decreases with heavy metal exposure (Ross et al. 2002), while others have shown the exact opposite effect (Theodorakis et al. 2006). Decreases in genetic variation can be the result of selective mortality (Guttman 1994; Theodorakis 2003), by either limiting the reproductive success or increasing the mortality rates of more susceptible individuals (Guttman 1994). On the other hand, mercury has one of the highest genotoxic potentials of the heavy metals and in marine mussels, it has been shown to cause more single-strand breaks in DNA than either copper or cadmium (Bolognesi et al. 1999). In addition, mercury exposure can inhibit DNA repair systems within cells, allowing damaged DNA to be maintained within the genome (Christie et al. 1985; Au et al. 2003; Cebulska-Wasilewska et al. 2005). These two factors together would likely increase mutation rates within affected populations. While the effect of this compound on genetic diversity is still unclear, either the loss of genetic variation or an increase in deleterious mutations can be detrimental to population viability.

The level of gene flow that occurs between populations can influence how severely environmental contaminants impact genetic diversity and population viability. If populations exposed to environmental contaminants are small and isolated, then the effect of these pollutants may be extremely detrimental because the negative effects of environmental contaminants on genetic diversity will be compounded by the populations' susceptibility to inbreeding and genetic drift. However, if these populations maintain moderate levels of gene flow with non-contaminated populations, then genetic diversity could be reconstituted through the influx of migrants. Euryhaline migratory fish provide excellent models to examine how migration rates may influence genetic diversity in contaminated areas. Euryhaline fish can migrate between marine, estuarine and freshwater environments. However, differing selective pressures among these ecosystems has been shown to cause estuarine populations to diverge from their coastal counterparts (Beheregaray and Sunnucks 2001; Pampoulie et al 2004). If these populations are distinct, the effect of environmental contamination on these smaller estuarine populations may be more detrimental than previously assumed. Therefore, in order to gain a better understanding as to how environmental contaminants are impacting genetic diversity and population viability, the genetic structure of populations in and around contaminated areas should be examined.

One area where mercury contamination is particularly concerning is the Weeks Bay watershed. Weeks Bay is a sub-estuary within Mobile Bay, AL and is a key breeding ground for many important commercial species along the Gulf coast. Weeks Bay watershed encompasses two main river systems, the Fish River and the Magnolia River, which feed into Weeks Bay proper. Portions of Fish River have historically shown elevated levels of mercury contamination (Novoveska 2005) and the Fish River is currently listed as an impaired water source according to guidelines established by the

Clean Water Act due to presence of elevated mercury concentrations (ADEM 2008). The Magnolia River has never been extensively assayed for mercury; however, there is a concern that populations within this system are also being impacted.

In order to examine whether mercury is present within Week Bay and infer how these compounds may be impacting the genetic diversity of exposed populations, we used the Gulf pipefish as a model system. The Gulf pipefish, *Syngnathus scovelli*, is the most common pipefish found within the Gulf of Mexico and Mobile Bay. It is a euryhaline pipefish and the only North American pipefish that can inhabit freshwater. Populations within Weeks Bay are genetically distinct from population within the Gulf of Mexico; however they maintain some level of gene flow with their coastal counterparts (see Chapter V). By comparing populations from potentially contaminated areas within Weeks Bay to non-exposed populations, we can begin to (1) assess the level of contamination within Weeks Bay and (2) assess the effect of mercury on the genetic diversity of exposed populations. In addition, since the genetic structure of these populations is known, we can begin to understand how this structure may be influencing the effect of mercury exposure on these populations.

Methods and Materials

Mercury Analysis

Water and Sediment Analysis. Water samples were collected from a total of 7 sites in and around Mobile Bay (Table 6.1). Water was collected from just below the surface at 5 sites within Weeks Bay (Figure 6.1). Three of these sites are located within Fish River

Table 6.1: Sites, site abbreviations (Abb), number of individuals collected (N) and GPS coordinates of Gulf pipefish, *S. scovelli*, sampling locations.

Site	Abb	GPS Coordinates (N, W)
Weeks Bay - Turkey Branch, AL	WBS1	30°42978, 87°32378
Weeks Bay - Mouth of Barner, AL	WBS2	30°45894, 87°80418
Weeks Bay - Barner Branch, AL	WBS3	30°45783, 87°80242
Weeks Bay - Magnolia River, AL	WBS4	30°45783, 87°80242
Weeks Bay - Magnolia River, AL	WBS5	30°39642, 87°78063
Meaher Park, AL	MP	30°66624, 87°92731
Grand Bay, MS	GB	30°36164, 88°39776

Figure 6.1: Google earth image of sample locations of sites throughout the Weeks Bay watershed. Three of these sites were located within the Fish River (WBS1, WBS2 and WBS3), while two sites were located within the Magnolia River (WBS4 and WBS5). Water samples were taken from all five sites. Fish were only sampled from WBS1, WBS2 and WBS4 because no fish were found at WBS3 or WBS5 during the collection period.

(WBS1, WBS2, and WBS3) and the remaining two are located within the Magnolia River (WBS4 and WBS5). Water samples were also collected from Meaher State Park (MP), located within the middle/upper part of Mobile Bay, and from one site along the coast of Mississippi within the Grand Bay Estuarine Research Reserve, MS (GB) (Figure 5.1). Water samples were collected in cleaned brown amber bottles using the protocol for "Ultra-Clean Collection of Water and Wastewater Samples for the Analysis of Low Level Mercury" (modified form of EPA method 1669). Samples were stored on ice until returned to the lab. Once in the lab, water was filtered through a 0.8μm nitrocellulose filter and preserved with concentrated HCl.

In December 2007, two sediment samples were collected from two sites within Fish River (WBS2 and WBS3). These two sites were chosen because WBS3 has historically been shown to contain high mercury levels, which was not observed in our water data, and WBS2 contains a pipefish population just downstream from WBS3. Samples from the upper 2 cm of the sediment substrate were taken from these two sites and individually stored in zip-lock bags. Samples were immediately placed on ice and then frozen upon return to the laboratory until analysis.

Mercury analysis for both water and sediment were performed by cold vapor generation and sector field inductively coupled plasma mass spectrometry (CV-ICP-MS) by the Trace Elements Analysis Core Facility at Dartmouth College. The detection limit of this method for total mercury was 1.0 ng/L.

Fish Collection. Fish were collected using a hand seine from shallow grass beds from a total of five sites. Three sites were located within Weeks Bay watershed, of which two of these sites were located within Fish River (WBS1 and WBS2) and the third site was located within Magnolia River (WBS4). During the sampling periods, no fish were found at WBS3 within Fish River or at WBS5 within the Magnolia River. In addition, fish were also collected from Meaher State Park, AL (MP) and Grand Bay National Estuarine Research Reserve, MS (GB). Fish were returned to the lab, anesthetized using MS222 and sacrificed. Tail tissue samples from pipefish were then preserved in 95% ethanol for genetic analysis. The remaining tissue was frozen for mercury analysis. In addition to pipefish, representatives of other fish species were collected from each site during the sampling period. These individuals were separately stored in zip-lock bags and frozen prior to mercury analysis.

Fish Mercury Tissue Analysis**.** Total mercury concentrations in fish tissue were assessed using cold vapor atomic absorption spectrophotometry as outlined in "Guidelines for Assessing Chemical Contaminant Data for Use in Fish Advisories" (EPA 823-B-00-007). All fish were sent to and processed by David Evans at the NOAA laboratory in North Carolina for total mercury analysis. Data for mercury concentrations are reported as parts per million (ppm) dry weight. Significant differences in mercury levels between populations were determined using a Kruskal-Wallis one-way analysis of variance. Pair-wise differences were assessed using Tukey's post hoc test.

Population Structure Analysis. As described in Chapter V, population data from 5 microsatellite loci were analyzed using GENEPOP version 4.0 (Rousset 2008). In order to determine the genetic divergence among populations, population pair-wise F_{ST} and R_{ST} values were estimated and significant values were inferred based on a G based exact test (Goudet et al. 1996). All significance levels were adjusted based on a Bonferroni correction when appropriate. The program ARLEQUIN version 2.2 (Pritchard et al. 2000) was used to carry out an analysis of molecular variance (AMOVA) to estimate the levels of population divergence between exposed populations and non-contaminated populations. In addition, the average genetic diversity of each population was assessed.

Results

Mercury Analysis

Water and Sediment. Surface water samples from all sites contained mercury levels well within background levels (Table 6.2). Sediment from WBS3, located within Barner Branch of Fish River, contained total mercury levels between the probable effects level (PEL) and upper effects threshold (UET) (509.2 ppb). However, sediment from WBS2, which is located where Barner Branch joins the Fish River, showed mercury levels within background levels (11.2 ppb).

Fish Tissue. Pipefish and other species of fish collected at WBS2 in the Fish River and at WBS4 in Magnolia River, the two main rivers that feed into Weeks Bay proper, contained fish with significantly higher concentrations of mercury than fish collected

Table 6.2: Total mercury levels of surface water from sites within the Weeks Bay watershed, Meaher State Park, AL and Grand Bay Research Reserve, MS.

Collection Site	Month of Collection	Total Mercury (ng/L)
Grand Bay	July	0.92
Meaher Park	July	1.77
Weeks Bay- WBS1	July	1.31
Weeks Bay –WBS2	July	0.93
Weeks Bay – WBS4	July	1.94
Weeks Bay – WBS1	September	1.92
Weeks Bay – WBS2	September	3.26
Weeks Bay – WBS3	September	4.78
Weeks Bay – WBS4	September	2.3
Weeks Bay – WBS5	September	2.18
Weeks Bay – WBS1	December	4.3
Weeks Bay – WBS2	December	2.56
Weeks Bay – WBS3	December	5.56
Weeks Bay – WBS4	December	4.32
Weeks Bay – WBS5	December	5.03

from all other sites (Figure 6.2 and 6.3). The appendix contains a list of all species of fish collected, sample sizes and their mean total mercury concentrations.

Population Genetic and Genetic Diversity

As reported for Chapter V, populations within Weeks Bay were not significantly distinct from one another (Table 6.3). Populations within Fish River (WBS1 and WBS2) were genetically distinct from the population at Meaher Park, while the population within Magnolia River (WBS4) was not. All populations within Weeks Bay and the population at Meaher Park were genetically distinct from the Grand Bay population (Table 6.3).

Exposed groups (WBS2 and WBS4) and non-contaminated groups (WBS1, MP and GB) were not genetically distinct from one another and most of the genetic variation found within this data set was explained by the variation within populations and among populations (Table 6.4). Additionally, levels of genetic diversity were similar between all populations sampled, regardless of the mercury levels found within these populations (Table 6.5).

Discussion

Our data from this study suggest that sites within the main rivers that feed into Weeks Bay contain significant levels of mercury contamination. While mercury concentrations of surface waters from these rivers were within background levels, total mercury within the sediment of Barner Branch (WBS3) in the Fish River showed levels that were within

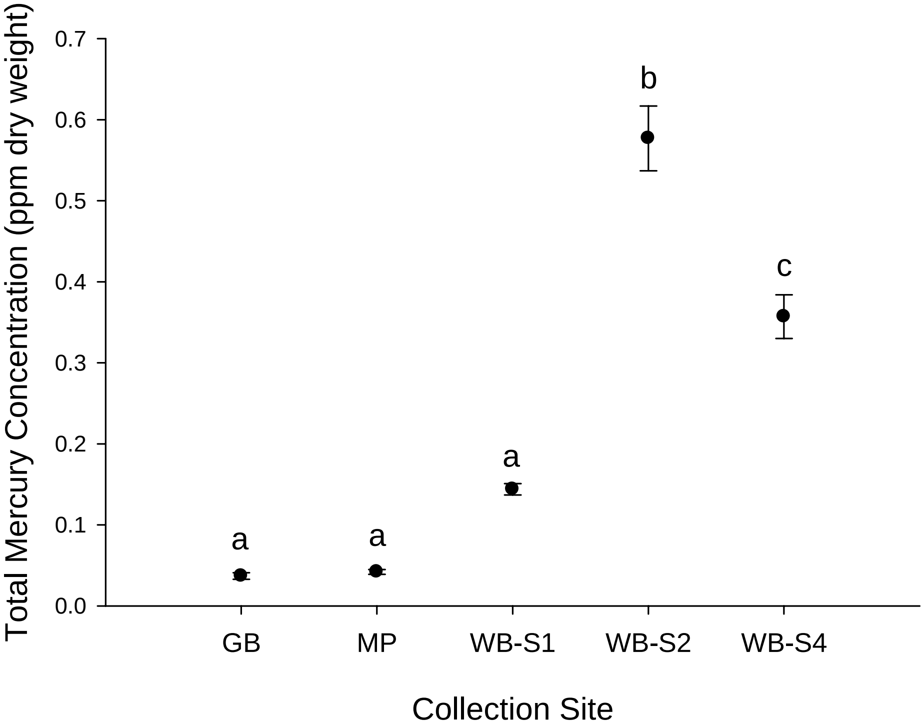


Figure 6.2: Average total mercury concentration of *S. scovelli* from Grand Bay, MS (GB), Meaher Park, AL (MP) and three sites within the Weeks Bay watershed, two of which were from Fish River (WBS1 and WBS2) and one within the Magnolia River (WBS3). Vertical bars on means indicate standard error. Treatment results with the same letter above error bar are not significantly different at $p < 0.05$.

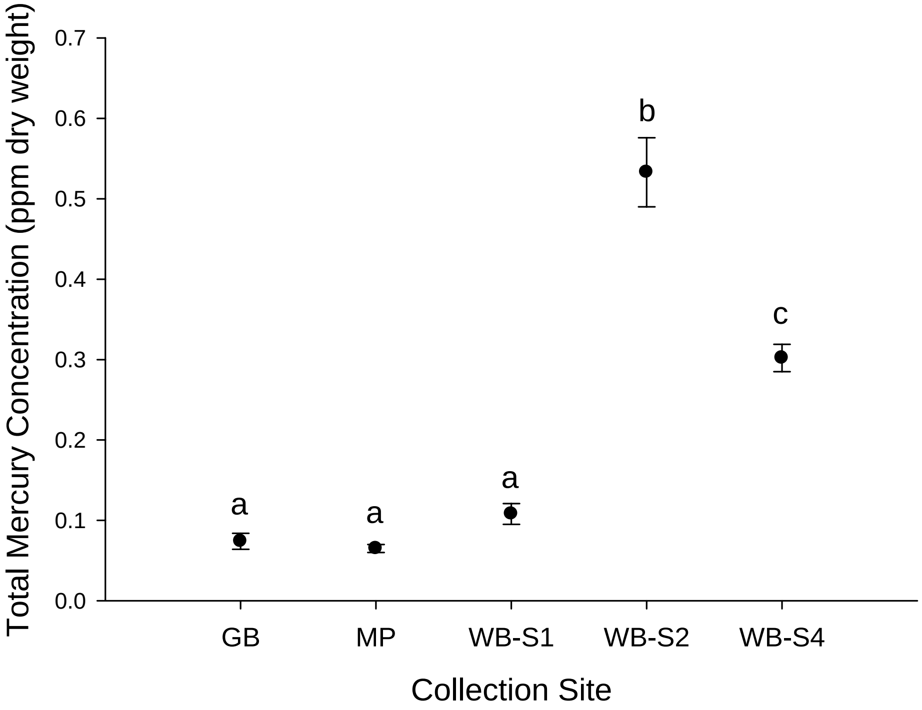


Figure 6.3: Average total mercury concentration of other fish species collected from Grand Bay, MS (GB), Meaher Park, AL (MP) and from three sites within the Weeks Bay watershed, two of which were from Fish River (WBS1 and WBS2) and one within Magnolia River (WBS3). Vertical bars on means indicate standard error. Treatment results with the same letter above error bar are not significantly different at $p < 0.05$.

Table 6.3: F_{ST} and R_{ST} values among population of *S. scovelli* in Weeks Bay, Mobile Bay and Grand Bay, MS. F_{ST} values are above the diagonal and R_{ST} values are below the diagonal. Bold values indicate significant F_{ST} values after Bonferroni corrections.

	WBS1	**WBS2**	**WBS4**	**MP**	**GB**
WBS1	0	0.0009	0.0016	**0.0169**	**0.0323**
WBS2	-0.0035	0	0.0013	**0.0158**	**0.0254**
WBS4	0.0167	-0.0028	0	0.0045	**0.019**
MP	0.0024	-0.002	-0.0085	0	**0.0174**
GB	0.0673	0.0301	0.0168	0.0109	0

Table 6.4: AMOVA of 5 populations of *S. scovelli* that were grouped into contaminated (Weeks Bay-S2 and Weeks Bay-S4) and non-contaminated (Weeks Bay-S1, Meaher Park, and Grand Bay) groups.

Source of variation	**df**	**Variance components**	**% variation**	**Fixation Indices**	**P-value**
Among groups	1	-0.011	-0.57	-0.006	0.912
Among pops. within groups	3	0.238	1.24	0.0123	<0.001
Within populations	315	1.903	99.32	0.007	<0.0001

Table 6.5: Average genetic diversity of exposed populations (Weeks Bay-S2 and Weeks Bay-S4) and non-contaminated populations (Weeks Bay-S1, Meaher Park and Grand Bay) of the Gulf pipefish, *S. scovelli*.

Sample location	**No. of individuals**	**No. of gene copies**	**Avg. genetic diversity**
Weeks Bay Site 1	30	60	0.799 ± 0.449
Weeks Bay Site 2	39	78	0.718 ± 0.408
Weeks Bay Site 4	15	30	0.646 ± 0.381
Meaher Park	44	88	0.779 ± 0.467
Grand Bay, MS	32	64	0.807± 0.452

the probable to upper effects level. Additionally, all fish collected from WBS2 within the Fish River and WBS4 within the Magnolia River showed mercury levels that were significantly higher than those outside of the Weeks Bay watershed (Figure 6.3 and 6.4). One major concern from this study is that most of the fish caught from these sites were small, <1g, low-level planktivores. Because mercury bioaccumulates up the food web, this suggests that larger predatory fish, notably those fish most often caught for human consumption within Weeks Bay, would be expected to contain even higher levels of mercury.

The other major concern that this study identified is that single parameter measurements, such as sampling only water or sediment, do not accurately describe what is occurring within the system. While fish and sediment samples from sites within the Weeks Bay watershed contained elevated levels of mercury, surface waters for all sites were consistently within background levels (Table 6.2). Barner Branch (WBS3) is particularly concerning because of the high levels of mercury found in the sediment. However, mercury levels of sediment from the area where Barner Branch joins the Fish River (WBS2) were within background levels, while fish at this site showed significantly elevated levels. One possible reason for the difference in mercury concentrations between these two sites is the difference in their substrate. The substrate of Barner Branch (WBS3) is mainly composed of mud and silt, while downstream from Barner Branch, where it connects to the Fish River (WBS2), the substrate is mainly composed of sand. Because clay particles readily bind mercury, it is likely that much of the mercury within the watershed may be sequestered within the substrate. However, since fish collected directly down-stream from Barner Branch (WBS2) contain significantly higher levels of mercury than any other site sampled, it appears that not all of the mercury is sequestered and some of it is entering the trophic system. Based upon these observations, we feel that it is imperative that future work evaluates both sediment and biological samples in order to assess the level of mercury contamination within this system.

Interestingly, while pipefish collected from the three sites within Weeks Bay are not genetically distinct from one another, mercury levels in pipefish at these sites differed significantly. Fish collected from both WBS2, within the Fish River, and WBS4, within the Magnolia River, contained mercury levels that were significantly higher than those of fish from WBS1, Meaher State Park and Grand Bay Research Reserve. It is also interesting that fish within WBS1 did not show significantly elevated levels of mercury, especially considering the close proximity of this site to WBS2 and the fact that populations within Weeks Bay are not genetically distinct, which suggests significant levels of migration between these groups. Mercury accumulates over time and while the reports concerning the retention rate of mercury vary, it is generally considered to be maintained in the body for at least 2-3 months (van Walleghem et al. 2007). The lack of individuals with significantly elevated levels of mercury at WBS1 suggests that migration among these sites may be limited during the breeding months. If high rates of migration were continuously occurring, individuals from WBS1 would likely contain moderate levels of mercury since they would be receiving individuals from WBS2 and WBS4. More than likely, the lack of genetic structure observed among populations within Weeks Bay is due to pipefish from both river systems intermixing once individuals move to other locations during the winter and, since site fidelity has not been observed in any *Syngnathus* sp. (Vincent et al. 1995), these individuals are likely to move to different grass beds within the Bay each breeding season. Also, if individuals move to non-contaminated areas between breeding seasons, the amount of time spent at these new locations may be such that exposed individuals can eliminate most of the mercury from their system prior to the next breeding season. This could potentially explain why the mercury levels of individuals from WBS1 were significantly lower than other sites within Weeks Bay, yet no significant genetic structure was detected among these populations.

While mercury levels have never been addressed for fish within the Magnolia River, our data suggests that it may also contain a mercury source. The presence of fish with higher levels of mercury within the Magnolia River can be explained by three different hypotheses. First, it is possible that the Magnolia River also contains high levels of mercury, similar to those of Fish River. Second, it is possible that fish exposed to mercury from the Fish River are migrating to the Magnolia River. However, as explained above, because fish migrating between WBS2 and WBS4 would have to pass through WBS1 in order to enter the Magnolia River, we would expect pipefish from WBS1 to have mercury levels that were intermediate or not significantly different from fish collected from the other sites. Additionally, it is not likely that mercury levels are lower at WBS1 because of the presence of new non-exposed migrants from Mobile Bay, since the population within the Magnolia River (WBS4) was the only population not genetically distinct from the Mobile Bay population (MP). Third, it is possible that the location where pipefish move during the winter months may be a site of contamination. If this is the case, all pipefish populations would initially have higher levels of mercury during the beginning of the breeding season and those that bred in areas that are not contaminated would eventually eliminate mercury over the course of the breeding season. However, this is not the case since mercury levels of pipefish within WBS4 did not significantly change between our July and September sampling period (July: 0.394 ± 0.05 (mean ± S.E.), n = 17; September: 0.394 ± 0.022, n = 13; Mann-Whitney U test: $\chi^2_{0.05,1} = 1.738$, p = 0.187). This all suggests that pipefish with high levels of mercury within the Magnolia River are not recent migrants from contaminated sites within the Fish River but it is likely there is another mercury source within the Magnolia River.

While, studies have shown that exposure to environmental contaminants can significantly affect genetic diversity, we found no difference among populations that were exposed and those with no known history of contamination. Exposed groups and non-contaminated groups were not genetically distinct from one another. In addition, the amount of genetic diversity within populations that contained individuals with high mercury levels appear similar to populations with no history of mercury contamination, such the population from Grand Bay National Research Reserve, MS (GB). Thus, mercury exposure has not affected levels of genetic diversity within these populations.

Traditionally, it would be assumed that mercury exposure did not impact levels of genetic diversity of exposed populations for one of three reasons: (1) Selective mortality is not occurring within these exposed populations; (2) Mutation rates are not significantly higher in exposed populations, or (3) Selective mortality and increased levels of mutation are balancing each other. However, another reason could be that migrant influxes from non-contaminated populations could be helping to reestablish genetic loss in exposed populations. In Weeks Bay, individuals at contaminated areas may be affected, but since migrants from non-contaminated sites immigrate at least every breeding season, the effects of exposure on overall population viability not be as severe. However, if the majority of this immigration is coming solely from populations within Weeks Bay, then this may not be the case. Since both of the main river systems that feed into Weeks Bay contained fish with significantly elevated levels of mercury, it is possible that much of this system is contaminated. Therefore, if most of the migration is solely occurring from other populations within Weeks Bay, it is likely that a significant number of these migrants have been previously exposed at some point and therefore their general health may already be affected.

The moderate level of gene flow from non-contaminated areas outside of Weeks Bay may also be influencing the effect of mercury exposure on population viability. Since coastal and estuarine migrants are likely to enter Weeks Bay, due to normal migration patterns or storm events, it is possible that any potential decrease in genetic diversity caused by mercury exposure may be counteracted by these migrants. In addition, since there is gene flow between coastal populations, the amount of genetic diversity that is reestablished through coastal migrants with each storm event may be enough to counteract the effect of mercury exposure on genetic variability and help maintain populations within Weeks Bay.

Overall, our data show that mercury contamination is a cause of concern within the Weeks Bay watershed. Pipefish within the Fish and Magnolia River, the main rivers that feed into Weeks Bay, contained levels of mercury that were significantly higher than pipefish from other populations. In addition, all other fish collected showed patterns of contamination similar to that of our pipefish samples. As stated previously, these results are particularly concerning because all fish sampled were low level planktivores and mercury tends to bioaccumulate up the food web. This suggests that larger predatory fish within the Bay, which are typically caught for human consumption, may be a health concern. This study also shows that a variety of monitoring tools, including water, sediment and biological, are needed to establish the full extent of mercury contamination within this area. Finally we show that levels of genetic diversity were not different between exposed population and non-contaminated populations of pipefish and suggest that moderate levels of gene flow from non-contaminated sites may be a factor in maintaining genetic diversity.

CHAPTER VII

CONCLUSIONS

Sexual selection is a powerful mechanism that has led to the evolution of some of the most elaborate traits in nature (reviewed in Andersson 1994) and can aid in driving population divergence (Lande 1981; Nagel and Schluter 1998; Jones et al. 2003; Boake 2005). Understanding the factors that contribute and influence sexual selection is an important step in understanding how this process contributes to evolutionary change. Disrupting sexual selection mechanisms can have long-term effects on population fitness and viability and recent studies have shown that environmental disturbances are impacting both natural selection and sexual selection processes (Hayes 2002, 2003; Hill and Janz 2003; reviewed in Reish et al. 2006; Arellano-Aguilar and Garcia 2008; Coe et al. 2008, Saarisota et al. 2009). Of these disturbances, one of the most disruptive may be the impact of environmental contaminates on natural populations (Schwarzenbach et al. 2006). Comprising mainly of synthetic compounds, pollutants have been shown to disrupt development, reproduction, risk associated behavior, mating behavior, mating dynamics and increase morality rates (Hayes et al. 2002, 2003; Hill and Janz 2003; reviewed in Reish et al. 2006; Menza et al. 2007; Kidd et al. 2007; Maunder et al. 2007; Peters et al. 2007, Schäfers et al. 2007, Arellano-Aguilar and Garcia 2008; Cevasco et al. 2008; Coe et al. 2008; Haxton and Findlay 2008, Rippey et al. 2008; Brunelli et al. 2009; Guimaraes et al. 2009, Haeba et al. 2009; Saarisota et al. 2009; Xu et al. 2009). While much of the research has focused on the effects these compounds have on survivorship and reproduction, how these compounds influence sexual selection processes is still vague and should be explored further. The purpose of this dissertation was to examine how environmental contaminants impact sexual selection processes is a sex-role reversed pipefish and address how population structure may influence the impact of these contaminants on population viability.

Before assessing the impact of environmental contaminants on sexual selection processes, factors that contribute to the opportunity for sexual selection in pipefish were determined. CHAPTER II evaluated the influence of post-copulatory choice mechanisms, particularly male cryptic choice, on offspring survival and examined how both pre- and post-copulatory behaviors contribute to the opportunity for selection in the broad-nosed pipefish, *S. typhle*. It was determined that male cryptic choice based on perceived parasite load contributed very little to offspring survivorship. However, other post-copulatory processes appear to be important. First, females mating with empty males appear to have an advantage over females mating with males that have partially filled brood pouches since these females were able to deposit significantly more eggs per copulation. This difference in the number of eggs that first females are able to contribute suggests that a “first female advantage” may exist in this system. Also, the data suggest that female size and/or egg size may play an important role in offspring survivorship, since female size and the proportion of offspring surviving were significantly correlated. Variance in relative fitness values for females was also found to be influenced by both pre- and post-copulatory mechanisms. Behaviors contributing the most to the variance in relative female fitness were female mating success, the number of eggs transferred per copulation and egg survivorship, suggesting these factors can significantly influence the direction of sexual selection.

Most of the recent research on sexual selection in pipefish has been addressed using *S. typhle* as the model species, while these processes in many other syngnathid species are relatively unexplored. One particularly interesting species is the Gulf pipefish, *Syngnathus scovelli*. *S. scovelli* is a polyandrous pipefish and this species shows one of the largest discrepancies between the variance in male and female mating success of any polyandrous species to date (Jones et al. 2001). In this species, variance in female mating success is likely to be one of the main factors impacting the opportunity for selection. While the influence of the number of eggs transferred per copulation did contribute to the variance in relative female fitness in *S. typhle*, this factor may contribute to a much less degree in *S. scovelli* since most females will completely fill a male during one mating attempt (personal observation). As a result, variance in mating success among females is probably much larger than the variance in the number of eggs transferred per copulation. Thus, because of the strong contribution mating success has toward relative fitness and sexual selection, CHAPTER III identified the physical and behavioral traits that influence mate choice in both male and female Gulf pipefish, *S. scovelli*. In order to assess the traits that influence male and female mating preference, we evaluated the proportion of time focal males responded to stimulus females of differing sizes and the proportion of time focal females actively courted stimulus males of differing sizes. The behavior of the stimulus females was also examined in order to determine how female behavior influenced male choice. The amount of time males responded to females was most significantly influenced by female behavior. However, female size may also contribute to male preference. On the other hand, female mate preference was not found to be influenced by male size, yet the influence of male behavior on female choice should continued to be addressed. I also determined that the interaction between female courtship behavior and male response time was primarily driven by changes in female behavior, but that male behavior may be providing a form of positive reinforcement for females.

CHAPTER IV examined whether exposure to a synthetic estrogen impacted mating dynamics in *S. scovelli*. The expression of secondary sexual traits in many organisms can be directly related to mating success (reviewed in Andersson 1994). These traits are normally under the control of sex steroids and it has been suggested that exposure to endocrine disrupting compounds may affect their expression (Ueda et al. 2005). In this chapter, two specific questions were addressed: (1) Does exposure to 17α-ethinylestradiol (EE2), a synthetic estrogen, affect the expression of secondary sex traits in males, and (2) What effect do changes in male trait expression have on mating dynamics and sexual selection processes in a sex-role reversed pipefish. To assess these questions, male and female *S. scovelli* were paired based on size and sex. One individual of the pair was exposed to a control, while the other individual was exposed to either 1ng/L or 100ng/L EE2 for 10 days and the effect on body morphology, mate choice, female courtship behavior, male mating success and male reproductive success were addressed. Exposure to EE2 did not significantly impact female mating opportunity given that males did not discriminate against exposed females in choice trials. The reason for this lack of an effect may be because neither concentration of EE2 significantly impacted female courtship behavior, which was shown in Chapter III to be the major factor influencing male response. However, exposure to as little as 1ng/L EE2 for only 10 days significantly affected both male body morphology and mating opportunity. Exposed males had significantly larger body depth to length ratios than their control partners and exposure to 100 ng/L EE2 induced the expression of iridescent lateral body stripes, a trait normally only found in females. Additionally, exposure to both concentrations caused exposed males to be discriminated against in female choice tests. Exposure to 100 ng/L EE2 also significantly decreased male mating success; although, if males became impregnated, previous exposure did not impact male reproductive success.

Once it is known whether environmental contaminants significantly impact individual fitness, the next question becomes how these effects translate into population health. One important component that can contribute to the way environmental pollutants affect population viability is how exposed populations are connected to non-contaminated populations (Theodorakis 2003). In the case of endocrine disruptors, populations in freshwater and estuarine habitats are more likely to be exposed to these compounds than coastal populations due to their location near wastewater treatment plants or due to agricultural run-off into these systems. If these exposed populations are genetically isolated from other populations, the impact of these compounds could be particularly detrimental since the effects of the pollutants are confounded by inbreeding and genetic drift. However, if these populations maintain high levels of gene flow with noncontaminanted populations, it may be able to counteract these affects. Thus, by understanding how populations within and around contaminated areas are genetically structured, the impact of environmental contaminants on genetic diversity and population health can be more accurately assessed. In CHAPTER V, the population structure of the Gulf pipefish throughout the Gulf of Mexico and within Mobile Bay was addressed using microsatellite data. Coastal populations throughout the Gulf of Mexico (Texas, Mississippi and Florida) were found to be genetically distinct from one another and from populations within Mobile Bay. Bayesian cluster analysis also suggested that pipefish populations within the Gulf of Mexico and Mobile Bay are derived from two parental populations, one coastal population and one estuarine/freshwater population. However, moderate levels of gene flow appear to be maintained among all populations sampled.

Many of the pipefish collected for this dissertation were collected from areas in and around Mobile Bay, AL. Because this area is heavily involved in the fishing industry one environmental pollutant that is particularly concerning for this region is mercury. Mercury is a heavy metal that has been shown to impact the health of both human and wildlife populations (Webber and Haines 2003; reviewed in Florea & Büsselberg 2006; reviewed in Lovett et al. 2009). Once mercury becomes methylated, it can bioaccumulate up the aquatic food chain, where it becomes concentrated in larger predatory fish that are most commonly used for human consumption (Kidd et al. 1995). Within the Weeks Bay watershed, a sub-estuary of Mobile Bay, mercury levels in fish have been shown to be above the limits deemed safe by the U.S. Food and Drug

Administration (ADEM 2001). In CHAPTER VI, the level of mercury contamination within the Weeks Bay watershed was examined. In order to assess these levels, water samples and fish were collected from locations inside the Weeks Bay watershed, in the middle/upper part of Mobile Bay, and within the Grand Bay Research Reserve, MS and assayed for total mercury. In addition, the effect of mercury exposure on the genetic diversity of exposed populations was examined. Populations within both of the main rivers that feed into Weeks Bay proper were found to contain significantly elevated levels of mercury when compared to pristine sites, such as the Grand Bay Estuarine Research Reserve. However, exposure did not significantly impact the genetic diversity of these populations as they were found to be just as diverse as non-contaminated populations. It is possible that moderate levels of gene flow between exposed and non-contaminated populations may be helping to maintain genetic diversity of these populations. However, whether this influx of migrants can sustain these populations should be addressed further.

The ultimate goal of this research was to understand how environmental contaminants may be affecting sexual selection processes and examine how these effects may impact long-term population viability. Overall, these data show that environmental contaminants, particularly endocrine disruptors, can significantly impact mating dynamics and sexual selection processes by impacting mating opportunity, one of the main factors contributing to the opportunity for sexual selection in pipefish. In the case of endocrine disruptors, not only do these effects impact individual fitness, they could also impact long-term evolutionary processes and population viability. If males that are sensitive to exposure are not able to obtain mates due to female discrimination, then this could limit the effective population size and lead to decreased genetic diversity. On the other hand, if exposure decreases the strength of sexual selection, by allowing non-preferred females the opportunity to increase their reproductive fitness by mating with exposed males, then this could lead to a decrease in overall population fitness. Also, if the induction of female secondary sex traits in males is long-term, the moderate levels of gene flow that occur between pipefish populations would allow exposed males to carry this phenotype, and its likely impacts on mating dynamics and sexual selection processes, to non-exposed populations. While the movement of exposed individuals to new populations may negatively impact non-contaminated populations, migrants moving from non-contaminated populations to exposed population may have the reverse effect. In the case of mercury, exposed populations within Weeks Bay may be able to maintain genetic diversity through the influx of migrants from non-contaminated populations outside of Weeks Bay. However, if mercury levels begin to impact individual health to the point that mortality rates in these populations became higher than immigration rates into these sites, then it is likely that these areas would simply become population sinks for migrant individuals. Overall, this study provides significant insight as to how environmental contaminants can affect sexual selection mechanism and suggests how population structure may play a role in determining the impact of these compounds on population health and viability.

REFERENCES

Amundsen T, Forsgren E (2001) Male mate choice selects for female coloration in a fish. Proc Natl Acad Sci USA 98: 13155-13160.

Ahnesjö I (1992a) Consequences of male brood care – weight and number of newborn in a sex-role reversed pipefish. Funct Ecol 6: 274-281.

Ahnesjö I (1992b) Fewer newborn result in superior juveniles in the paternally brooding pipefish *Syngnathus typhle* L. J Fish Biol 41b: 53-63.

Ahnesjö I (1996) Apparent resource competition among embryos in the brood pouch of a male pipefish. Behav Ecol Sociobiol 38**:** 167-172.

Ahnesjö I, Kvarnemo C, Merilaita (2001) Using potential reproductive rates to predict mating competition among individuals qualified to mate. Behav Ecol 12: 397-401.

Ahtiainen JJ, Alatalo RV, Kortet R, Rantala M (2001) Sexual advertisement and immune function in an arachnid species (Lycosidae). Behav Ecol 15: 602-606.

Alabama Department of Environmental Management (ADEM) (2008) Integrated water quality monitoring & assessment report. 303(d) lists. www.adem.state.al.us/ waterdivision/WQuality/305b/WQ305bReport.htm.

Allen Y, Scott A, Matthiessen P, Hawthorn S, Thain J, Feist S (1999) Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effects on gonadal development of the flounder *Platichthyes flesus*. Environ Toxicol Chem 18: 1793 – 1800.

Amos WH, Amos SH (1985) Atlantic and Gulf Coasts. The Audubon Society Nature Guides. Alfred A Knopf, New York.

Amos W, Hoffman JI, Frodsham A, Zhang L, Best S, Hill AVS (2007) Automated binning for microsatellite alleles: problems and solutions. Mol Ecol Notes 7: 10-14.

Andersson M (1982) Female choice selects for extreme tail length in a widowbird. Nature 229: 818-820.

Andersson M (1994) Sexual Selection. Princeton University Press, New Jersey.

Arellano-Aguilar O, Garcia, CM (2008) Exposure to pesticides impairs the expression of fish ornaments reducing the availability of attractive males. Pro R Soc Ser B 275: 1343–1350.

Arnold SJ, Wade MJ (1984) On the measure of natural and sexual selection: theory. Evolution 38:709–719.

Au WW, Salama SA, Sierra-Torres CH (2003) Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. Environ Health Perspect 111: 1843-1850.

Backwell PRY, Jennions MD, Christy JH, Schober U (1995) Pillar building in the fiddler crab *Uca beebei* – Evidence for a condition dependent ornament. Behav Ecol Sociobiol 36: 185-192.

Baird TA, Hranitz JM, Timanus DK, Schwartz AM (2007) Behavioral attributes influence annual mating success more than morphological traits in male collared lizards. Behav Ecol 18: 1146-1154.

Balch GC, Mackenzie CA, Metcalfe CD (2004) Alterations to gonadal development and reproductive success in Japanese medaka (*Oryzias latipes*) exposed to 17 alpha-ethinylestradiol. Environ. Toxicol Chem 23: 782-791.

Bargagli R (2000) Trace metals in Antarctica related to climate change and increasing human impact. Rev Environ Comtam Toxicol 166: 129-173.

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. Heredity. 2: 349-368.

Beheregaray LB, Sunnucks P (2001) Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odontesthes argentinensis*. Mol Ecol 10: 2849-2866.

Bell AM (2001) Effects of an endocrine disrupter on courtship and aggressive behavior of male three-spined stickleback, *Gasterosteaus aculeatus.* Anim Behav 62: 775-780.

Benton MJ, Malott ML, Trybula J, Dean DM, Guttman SI (2002) Genetic effects of mercury contamination on aquatic snail populations: allozyme genotypes and DNA strand breakage. Environ Toxicol Chem 21: 584-589.

Berglund A (1995) Many mates make male pipefish choosy. Behavior 132: 213-218.

Berglund A, Rosenqvist G (1993) Selective males and ardent females in pipefishes. Behav Ecol Sociobiol 34: 331-336.

Berglund A, Rosenqvist G (2001) Male pipefish prefer dominant over attractive females Behav Ecol 12: 402-406.

Berglund A, Rosenqvist G (2003) Sex role reversal in pipefish. Adv Study Behav 32: 131-167.

Berglund A, Rosenqvist G, Bernet P (1997) Ornamentation predicts reproductive success in female pipefish. Behav Ecol Sociobiol 40: 145-150.

Berglund A, Rosenqvist G, Svensson I (1986a) Reversed sex role and parental energy investment in zygotes of two pipefish (Syngnathidae) species**.** Mar Ecol Prog Ser 29: 209-215**.**

Berglund A, Rosenqvist G, Svensson I (1986b) Mate choice, fecundity and sexual dimorphism in two pipefish species (Syngnathidae). Behav Ecol Sociobiol 19: 301-307.

Berglund A, Rosenqvist G, Svensson I (1988) Multiple mating and paternal brood care in the pipefish, *Syngnathus typhle*. Oikos 51**:** 184-188.

Berglund A, Rosenqvist G, Svensson I (1989) Reproductive success of female limited by males in two pipefish species. Am Nat 133: 506-516.

Berglund A, Widemo MS, Rosenqvist G (2005) Sex-role reversal revisited: choosy females and ornamentation, competitive males in a pipefish. Behav Ecol 16: 649-655.

Birkhead TR (2000) Defining and demonstrating postcopulatory female choice – again. Evolution 54: 1057-1060.

Birkhead TR, Møller AP (1998) Sperm competition and sexual selection. Academic Press, San Diego, California.

Birkhead TR, Pizzari T (2002) Postcopulatory sexual selection. Nature Rev Genet 3: 262-273.

Bjerselius R, Lundstedt-Enkel K, Olsen H, Mayer I, Dimberg K (2001) Male goldfish reproductive behaviour and physiology are severely affected by exogenous exposure to 17β-estradiol. Aquat Toxicol 53: 139-152.

Boake, CRB (2005) Sexual selection and speciation in Hawiian fruitflies. Behav Genet 35: 297-303.

Boake CRB, Hoikkala A (1995) Courtship behavior and mating success of wild-caught *Drosophila silvestris* males. Anim Behav 49: 1303-1313.

Boisseau, J (1967) Les regulation hormonales de l'incubation chez un Vertébré male: recherches sur la reproduction de l'Hippocampe. Dissertation, University of Bordeaux.

Bolland J, Boettcher AA (2005) Population structure and reproductive characteristics of the Gulf pipefish, *Syngnathus scovelli* in Mobile Bay, Alabama. Estuaries. 28: 957-965.

Bolognesi C, Landini E, Roggieri P, Fabbri R, Viarengo A (1999) Genotoxicity biomarkers in the assessment of heavy metal effects in mussels: experimental studies. Environ Mol Mutagen 33: 287-292.

Borgia G, Egeth M, Uy JA, Patricelli GL (2004) Juvenile infection and male display: testing the bright male hypothesis across individual life histories. Behav Ecol 15: 722-728

Brown KH, Schultz IR, Cloud JG, Nagler JJ (2008) Aneuploid sperm formation in rainbow trout exposed to the environmental estrogen 17 alpha-ethynylestradiol. P Natl Acad Sci USA 50: 19786-19791.

Brunelli E, Bernabo I, Berg C, Lundstedt-Enkel K, Bonacci A, Tripepi S (2009) Environmentally relevant concentrations of endosulfan impair development, metamorphosis and behaviour in *Bufo bufo* tadpoles. Aquat Toxicol 91: 135-142.

Bussière LF, Hunt J, Jennions M, Brooks R (2006) Sexual conflict and cryptic female choice in the black field cricket, *Teleogryllus commodus*. Evolution 60: 782-800.

Carcupino M, Baldacci A, Mazzini M, Franzoi P (2002) Functional significance of the male brood pouch in the reproductive strategies of pipefishes and seahorses: a morphological and ultrastructural comparative study on three anatomically different pouches. J Fish Biol 61:1465-1480.

Cebulska-Wasilewska I, Panek A, Zabinski Z, Moszczynski P, Au WW (2005) Occupational exposure to mercury vapor on genotoxicity and DNA repair. Mutat Res 586: 102-114.

Cevasco A, Urbatzka R, Bottero S, Massari A, Pedemonte F, Kloas W, Mandich A (2008) Endocrine disrupting chemical (EDC) with (anti)estrogenic and (anti)androgenic modes of action affecting reproductive biology of *Xenopus laevis*: II. Effects on gonad histomorphology. Comp Biochem Physiol C 147: 241-251.

Christie N, Cantoni O, Sugiyama M, Cattabeni F, Costa M (1985) Differences in the effect of Hg(II) on DNA repair induced in Chinese hamster ovary by ultraviolet or X-ray. Mol Pharmacol 24: 173-178.

Clauzot L, Marrot B, Doumenq P, Roche N (2008) 17α-Ethinylestradiol: an endocrine disrupter of great concern. Analytical methods and removal processes applied to water purification. A review. Environ Prog 27: 383-396.

Clutton-Brock T (2007) Sexual selection in males and females. Science 318 : 1882-1885.

Clutton-Brock T, Parker GA (1992) Potential reproductive rates and the operation of sexual selection. Quart Rev Biol 67 : 437-456.

Clutton-Brock T, Vincent ACJ (1991) Sexual selection and the potential reproductive rates of males and females. Nature 351: 58-60.

Coe TS, Hamilton PB, Hodgson D, Paull GC, Stevens JB, Sumner K, Tyler CR (2008) An environmental estrogen alters reproductive hierarchies, disrupting sexual selection in group spawning fish. Environ Sci Technol 42: 5020-5025.

Crews D, Gore AC, Hsu TS Dangleben NL, Spinetta M, Schallert T, Anway MD, Skinner MK (2007) Transgenerational epigenetic imprints on mate preference. Proc Natl Acad Sci USA 104: 5942-5946.

Crump KL, Trudeau VL (2009) Mercury-induced reproductive impairment in fish. Environ Toxicol Chem 28: 895-907.

Cumming JM (1994) Sexual selection and the evolution of dance fly mating systems (Diptera: Empididae; Emidinae). Can Entomol 126: 907-920.

Cunningham EJA, Russell AF (2000) Egg investment is influenced by male attractiveness in the mallard. Nature 404: 74-77.

Darwin C (1859) The origin of the species. Random House, New York.

Darwin C (1871) The decent of man and selection in relation to sex. John Murray, London.

Dawson CE (1982) Fishes of the Western North Atlantic, Part 8, Order Gasterosteiformes, Suborder Syngnathoidei. Sears Foundation for Marine Research. Yale University, New Haven, CT.

Dawson CE (1985) Indo-Pacific pipefishes: Red Sea to the Americas. Allen Press, Lawrence, Kansas.

Delaney K, Roberts JA, Uetz GW (2007) Male signaling behavior and sexual selection in a wolf spider (Araneae: Lycosidae): a test for dual function. Behav Ecol Sociobiol 62: 67-75.

Demary K, Michaelidis CI, Lewis SM (2006) Firefly courtship: behavioral and morphological predictors of male mating success in *Photinus greeni*. Ethology 112: 485-492.

Drickamer LC, Gowaty PA, Wagner DM (2003) Free mutual mate preference in house mice affect reproductive success and offspring performance. Anim Behav 65: 105-114.

Eberhard WG (1996) Female Control: Sexual selection by cryptic female choice. Princeton Univ Press, Princeton, New Jersey.

Eberhard WG (2000) Criteria for demonstrating postcopulatory female choice. Evolution 54: 1047-1050.

Eberhard WG (2004) Why study spider sex: special traits of spiders facilitate studies of sperm competition and cryptic female choice. J Arachnol 32: 545-556.

Edvardsson M, Arnqvist G (2000) Copulatory courtship and cryptic female choice in red flour beetles *Tribolium castaneum*. Proc R Soc Lond Ser B 267: 559-563.

Eeva T, Belskii E, Kuranov B (2006) Environmental pollution affects genetic diversity in wild bird populations. Mutat Res 601: 8-15.

Emlen ST, Oring LW (1977) Ecology, sexual selection and the evolution of mating systems. Science 197: 215-223.

Endler JA (1977) Geographic variation, speciation, and clines. Princeton University Press, Princeton, New Jersey.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620.

Evans JP, Zane L, Francescato S, Pilastro A (2003) Directional postcopulatory sexual selection revealed by artificial insemination. Nature 421: 360-363.

Fedigan, LM (1983) Dominance and reproductive success in primates. Yearb Phys Anthropol 26: 91-129.

Florea A, Büsselberg D (2006) Occurrence, use and potential toxic effects of metal and metal compounds. BioMetals 19: 419-427.

Folmar LC, Denslow ND, Rao V, Chow M, Crain DA, Enblom J, Marcino J, Guillette Jr. LJ (1996) Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (*Cyprinus carpio*) captured near a major metropolitan sewage treatment plant. Environ Health Persp 104: 1096-1101.

Folstad I, Karter AJ (1992) Parasites, bright males, and the immunocompetence handicap. Am Nat 139: 603-622.

Foré SA, Guttman SI, Bailer AJ, Altfeter DJ, Counts BV (1995a) Potential use of genetic diversity as an indicator of water quality. Part I. *Pimephales notatus* as a model. Ecotoxicol Environ Saf 30: 24-35.

Foré SA, Guttman SI, Bailer AJ, Altfeter DJ, Counts BV (1995b) Potential use of genetic diversity as an indicator of water quality. Part II. *Campostoma anomalum* as a model. Ecotoxicol Environ Saf 30: 36-46.

Franzoi P, MacCagnani R, Rossi R, Ceccherelli VU (1993) Life cycles and feeding habits of *Syngnathus taenionotus* and *S. abaster* (Pices, Syngnathidae) in a brackish bay of the Po River Delta (Adriatic Sea). Mar Ecol Prog Ser 97: 71-81.

Frith CB, Frith DW (2004) The Bowerbirds. Oxford University Press, New York.

Gabriel W, Bürger R (1994) Extinction risk by mutational meltdown: synergistic effects between populations regulation and genetic drift. In : Loeschcke V, Tomiuk J, Jain SK (eds), Conservation Genetics. Birkhäuser Verlag, Basel, Switzerland, pp 69-86.

Gagliano M, McCormick M (2007) Maternal condition influences phenotypic selection on offspring. J Anim Ecol 76: 174-182.

Gillespie RB, Guttman SI (1993) Allozyme frequency analysis of aquatic populations as an indicator of contaminant induced impacts. In: Gorsuch JW et al. (eds) Environmental toxicology and risk assessment, ASTM STP 1173, American Society for Testing and Materials, Philadelphia 2, pp134-145.

Goudet J, Raymond M, Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. Genetics 144: 1933-1940.

Gowaty PA (1997) Sexual dialectics, sexual selection, and variation in mating behavior. In: Gowaty PA (ed) Feminism and evolutionary biology. Chapman, New York, pp 351-384.

Gowaty PA, Drickamer, LC, Schmid-Holmes S (2003) Male house mice produce few offspring with lower viability and poorer performance when mated to females they do not prefer. Anim Behav 65: 95-103.

Grant PR, Grant BR (2008) How and why species multiply: The radiation of Darwin's finches. Princeton Univ. Press, Princeton, New Jersey.

Griggio M, Valera F, Casas A, Pilatro A (2005) Males prefer ornamented females: a field experiment of male choice in the rock sparrow. Anim Behav 69: 1243-1250.

Gronlund CJ, Deangelis MD, Pruett-Jones S, Ward PS, and Coyne JA (2002) Mate grasping in *Drosophila pegasa*. Behaviour 139: 545-572.

Grotto D, Barcelos GRM, Valentini J, Antunes LMG, Angeli JPF, Garcia SC, Barbosa Jr. F (2009) Low levels of methylmercury induce DNA damage in rats: protective effects of selenium. Arch Toxicol 28: 249-254.

Guimaraes L, Gravato C, Santos J, Monteiro L, Guilhermino L (2009) Yellow eel (*Anguilla anguilla*) development in NW Portuguese estuaries with differential contamination levels. Ecotoxicology 18: 385-402.

Guttman S (1994) Population genetic structure and ecotoxicology. Environ Health Perspect 102: 97-100.

Haeba HM, Hilscherova K, Mazurova E, Blaha L (2009) Selected endocrine disrupting compounds (vinclozolin, flutamide, ketoconazole and dicofol): effects on survival, occurrence of males, growth, molting and reproduction of *Daphnia magna*. Environ Sci Pollut Res Int 15: 222-227.

Hamilton WJ (1942) Habitats of *Syngnathus scovelli* (Evermann and Kendall). Copeia 3: 188.

Haresign TW, Shumway SE (1981) Permeablility of the marsupium of the pipefish *Syngnathus fuscus* to [^{14}C]-alpha amino isobutyric acid. Comp Biochem Physiol A 69: 603-604.

Harries JE, Sheahan A, Jobling S, Matthiessen P, Neall P, Sumpter JP, Tylor T, Zama N (1997) Estrogenic activity in five United Kingdom rivers detected by measurement of Vitellogenesis in caged male trout. Environ Toxicol Chem 16: 534-542.

Haxton TJ, Findlay, CS (2008) Variation in lake sturgeon (*Acipenser fulvescens*) abundance and growth among river reaches in a large regulated river. Can J Fish Aquat Sci 65: 645-657.

Hayes H, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A (2002) Feminization of male frogs in the wild. Nature 419: 895-895.

Hayes H, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A (2003) Atrizine-induced hermaphrodism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. Environ Health Perspect 111: 568-575.

Hill R, Janz D (2003) Developmental estrogenic exposure in zebrafish (*Danio rerio*): I. Effects on sex ratio and breeding success. Aquat Toxicol 63: 417-429.

Hoese HD, Moore RH (1977) Fishes of the Gulf of Mexico: Texas, Louisiana, and adjacent waters. Texas A&M University Press, College Station, TX.

Hoikkala A, Suvanto L (1999) Male courtship song frequency as an indicator of male mating success in *Drosophila montana*. J Insect Behav 12: 599-609.

Hotchkiss AK, Rider CV, Blystone CR, Wilson VS, Hartig PC, Ankley GT, Foster PM, Gray CL, Gray LE (2008) Fifteen years after "Wingspread" – Environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. Toxicol Sci 105: 235-259.

Hout, A (1902) Recherches sue les poissons lophobranches. Ann Sci Nat14: 19-88.

Jones AG (2002) The evolution of alternative cryptic female choice strategies in age-structured populations. Evolution 56: 2530-2536.

Jones AG, Avise JC (1997a) Polygynandry in the dusky pipefish *Syngnathus floridae* revealed by microsatellite DNA markers. Evolution 51: 1611-1622.

Jones AG, Avise JC (1997b) Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role reversal. Mol Ecol 6: 203-213.

Jones AG, Avise JC (2003) Male pregnancy. Curr Biol 13: R791-R791.

Jones AG, Ratterman NL (2009) Mate choice and sexual selection: what have we learned since Darwin? Proc Natl Acad Sci USA 106: 10001-10008.

Jones AG, Walker D, Avise JC (2001) Genetic evidence for extreme polyandry and extraordinary sex-role reversal in a pipefish. Proc R Soc Lond Ser B 268: 2531–2535.

Jones AG, Rosenqvist G, Berglund A, Avise JC (1999) The genetic mating system of a sex-role reversed pipefish (*Syngnathus typhle*): a molecular inquiry. Behav Ecol Sociobiol 46: 357–365.

Jones AG, Rosenqvist G, Berglund A, Avise JC (2005) The measurement of sexual selection using Bateman's Principles: an experimental test in the sex-role reversed pipefish *Syngnathus typhle*. Integr Comp Bio 45: 874-884.

Jones AG, Moore GI, Kvarnemo C, Walker D, Avise JC (2003) Sympatric speciation as a consequence of male pregnancy in seahorses. Proc Natl Acad Sci USA. 100: 6598-6603.

Jorgenson JL (2001) Aldrin and Dieldrin: A review of research on their production, environmental deposition and fate, bioaccumulation, toxicology and epidemiology in the United States. Environ Health Perspect 109: 113-139.

Kempenaers B, Foerster K, Questiau S, Robertson BC, Vermeirssen ELM (2000) Distinguishing between female sperm choice verses male sperm competition: a comment on Birkhead. Evolution 54: 1050-1052.

Kidd KA, Hesslein RH, Fudge RJP, Hallard KA (1995) The influence of trophic level as measured by $\delta^{15}N$ on mercury concentrations in fresh-water organisms. Water Air Soil Pollut 80: 1011-1015.

Kidd K, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, Flick RW (2007) Collapse of a fish population after exposure to a synthetic estrogen. Proc Natl Acad Sci USA 104: 8897-8901.

Kjorsvik E, Hoehne-Reitan K, Reitan KI (2003) Egg and larval quality criteria as predictive measures for juvenile production in turbot (*Scophthalmus maximus* L.) Aquaculture 227: 9-20.

Kokko H, Monaghan P (2001) Predicting the direction of sexual selection. Ecol Lett 2: 247-255.

Kokko H, Johnstone RA (2002) Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signaling. Philos Trans R Soc Lond B Bio Sci 357: 319-330.

Kolm N (2001) Females produce larger eggs for large males in a paternal mouthbrooding fish. Proc R Soc Lond Ser B 268: 2229-2234.

Kolpin D, Furlong E, Meyer M, Thurman E, Zaugg S, Barber L, Buxton H (2002) Pharmaceuticals, hormones and other organic wastewater contaminants in US streams, 1999 – 2000: a national reconnaissance. Environ Sci Technol 36: 1202-1211.

Kotiaho J (2002) Sexual selection and condition dependence of courtship display in three species of horned dung beetles. Behav Ecol 13: 791-799.

Kotiaho J, Alatalo RV, Mappes J, Parri S (1996) Sexual selection in a wolf spider: male drumming activity, body size, and viability. Evolution 50: 1977-1981.

Kvarnemo C, Ahnesjö I (1996) The dynamics of operational sex ratios and competition for mates. Trends Ecol Evol 11: 404-408.

Kvarnemo C, Moore G, Jones AG (2007) Sexually selected females in the monogamous Western Australian seahorse. Proc R Soc Lond Ser B 274: 521-525.

Lande R (1981) Models of speciation by sexual selection on polygenic traits. Proc Natl Acad Sci USA 78: 3721-3725.

Larsen M, Hansen K, Henriksen P, Baatrup E (2008) Male zebrafish (*Danio rerio*) courtship behavior resists the feminizing effects of 17α-ethinyloestradiol-morphological sexual characteristics do not. Aquat Toxicol 87: 234-244.

Lazzari MA, Able KW (1990) Northern pipefish, *Syngnathus fuscus*, occurrences over the Mid-Atlantic Bight continental shelf: evidence of seasonal migration. Environ Biol Fishes 27: 177-185.

LeBas NR, Marshall NJ (2000) The role of colour signalling and mate choice in the agamid lizard *Ctenophorus ornatus*. Proc R Soc Lond Ser B 267: 445-452.

Lee CE, Peterson CH (2003) Effects of developmental acclimation on adult salinity tolerance in the freshwater-invading copepod *Eurytemora affinis*. Phys Biochem Zool 76: 296-301.

Leiner, V. M. 1934. Der Osmostisches Druch in den Bruttaschen der Syngnathiden. Zoo Anz 108: 278–298.

Lintelmann J, Katayama A, Kurihara N, Shore L, Wenzel A (2003) Endocrine disruptors in the environment: IUPAC Technical Report. Pure Appl Chem 75: 631-681.

Linton JR, Soloff BL (1964) The physiology of the brood pouch of the male seahorse (*Hippocampus erectus*). Bull Mar Sci Gulf Car 14: 45-61.

Losos, JB (2009) Lizards in an evolutionary tree: ecology and adaptive radiation of anoles. Univ. California Press, Berkeley, California.

Lovett GM, Tear TH, Evers DC, Findlay SEG, Cosby BJ, Dunscomb JK, Driscoll CT, Weathers KC (2009) Effects of air pollution on ecosystems and biological diversity in the eastern United States. The year in ecology and conservation biology. Ann NY Acad Sci 1162: 99-135.

Lutz I, Kloas W (1999) Amphibians as a model to study endocrine disruptors: 1. environmental pollution and estrogen receptor binding. Sci Tot Environ 225: 49-57.

McGraw KJ, Correa SM, Adkins-Regan E (2006) Testosterone upregulates lipoprotein status to control sexual attractiveness in a colorful songbird. Behav Ecol Sociobiol 60: 117-122.

McLane WM (1955) The fishes of the St. Johns River system. Dissertation, University of Florida.

Matsumoto K, Yanagisawa Y (2001) Monogamy and sex role reversal in the pipefish *Corythoichthys haematopterus*. Anim Behav 61: 163-170.

Maunder R, Matthiessen P, Sumpter J, Pottinger TG (2007) Impaired reproduction in three-spined sticklebacks exposed to ethinyl estradiol as juveniles. Biol Reprod 77: 999-1006.

Mazzi D (2004) Parasites make male pipefish careless. J Evol Biol 17: 519-527.

Menza C, Kendal M, Rogers C, Miller J (2007) A deep reef in trouble. Cont Shelf Res 27: 2224-2230.

Miller L, Kapuschinski A (1996) Notes: microsatellite DNA markers reveal new levels of genetic variance in Northern Pike. Trans Am Fish Soc 124: 971–977.

Mobley KB (2007) Geographic and temporal variation in the genetic mating systems of pipefish. Dissertation, Texas A&M University.

Moncaut N, Nostro F, Maggese M (2003) Vitellogenin detection in surface mucus of South American cichlid fish *Sichlasoma dimerus* (Heckel, 1840) induced by estraditol-17β. Effects on liver and gonads. Aquat Toxicol 63: 127–137.

Monteiro NM, Vieira MDN, Almada V (2005) Homing behavior and individual identification of the pipefish *Nerophis lumbriciformes* (Pisces: Syngnathidae): a true intertidal resident. Estuar Coast Shelf Sci 63: 93-99.

Munkittrick KR, Van Der Kraak GJ, Smith IR, McMaster ME, Portt CB, van den Heuvel MR, Servos MR (1994) Survey of receiving water environmental impacts associated with discharges from pulp mills. 2. Gonad size, liver size, hepatic EROD activity and plasma sex steroid levels in white sucker. Environ Toxicol Chem 13: 1089-1101.

Murai M, Backwell PRY (2006) A conspicuous courtship signal in the fiddler crab *Uca perplexa*: female choice based on display structure. Behav Ecol Sociobiol 60: 736-741.

Nagel L, Schluter D (1998) Body size, natural selection and speciation in sticklebacks. Evolution 52: 209-218.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Neri M, Ugolini D, Bonassi S, Fucic A, Holland N, Knudsen LE, Sram RJ, Ceppi M, Bocchini V, Merlo D (2006) Children's exposure to environmental pollutants and biomarkers of genetic damage II. Results of a comprehensive literature search and meta-analysis. Mut Res 612: 14-39.

Nicoletto PF, Kodrick-Brown A (1999) The relationship among swimming performance, courtship behavior, and carotenoid pigmentation of guppies in four rivers in Trinidad. Environ Biol Fishes 55: 227-235.

Novoveska L (2005) Benthic algal community structure and bioaccumulation of mercury in a coastal watershed. Thesis, Eastern Illinois University.

Oshima Y, Kang IJ, Kobayahsi M, Nakayama K, Imada N, Honjo T (2003) Suppression of sexual behavior in male Japanese medaka (*Oryzias latipes*). Chemosphere 50: 429-436.

Otronen M (1990) Mating behavior and sperm competition in the fly, *Dryomiza anilis*. Behav Ecol Sociobiol 26:349-356.

Palace VP, Wautier KG, Evans RE, Blanchfield PJ, Mills KH, Chalanchuk SM, Godard D, McMaster ME, Tetreault GR, Peters LE, Vandenbyllaardt L, Kidd KA (2006) Biochemical and histopathological effects in pearl dace (*Margariscus margarita*) chronically exposed to a synthetic estrogen in a whole lake experiment. Environ Toxicol Chem 25: 1114-1125.

Pampoulie C, Gysels ES, Maes GE, Hellemans B, Leentjes V, Jones AG, Volckaert FAM (2004) Evidence for fine-scale genetic structure and estuarine colonization in a potential high gene flow marine goby (*Pomatoschistus minutes*) Heredity 92: 434-445.

Park BJ, Kidd K (2005) Effect of the synthetic estrogen ethinylestradiol on early life stages of mink frogs and green frogs in the wild and *in situ*. Environ Toxicol Chem 24: 2027-2036.

Parker TH, Knapp R, Rosenfeld JA (2002) Social mediation of sexually selected ornamentation and steroid hormone levels in male junglefowl. Anim Behav 64: 291-298.

Partridge CG, Shardo J, Boettcher A (2007) Osmoregulatory role of the brood pouch in the euryhaline Gulf pipefish, *Syngnathus scovelli*. Comp Bioch Physiol A 147: 556-561.

Partridge CG, Kvarnemo C, Ahnesjö I, Mobley K, Berglund A, Jones AG (2009).The effect of perceived female parasite load on post-copulatory mate choice in a sex-role reversed pipefish. Behav Ecol Sociobiol 63**:** 345-354.

Peters R, Courtenay SC, Cagampan S, Hewitt ML, MacLatchy DL (2007) Effects on reproductive potential and endocrine status in the mummichog (*Fundulus heteroclitus*) after exposure to 17α-ethynylestradiol in a short-term reproductive bioassay. Aquat Toxicol 85: 154-166.

Pettersson I, Arukwe A, Lundsted-Enkel K, Mortensen A, Berg C (2006) Persistent sex-reversal and oviducal agenesis in adult *Xenopus* (Silurana) *tropicalis* frogs following larval exposure to the environmental pollutant ethynylestradiol. Aquat Toxicol 79: 356-365.

Pilastro A, Simonato M, Bisazza A, Evans JP (2004) Cryptic female preference for colorful males in guppies. Evolution 58: 665-669.

Pilastro A, Evans JP, Sartorelli S, Bisazza A (2002) Male phenotype predicts insemination success in guppies. Proc R Soc Lond Ser B 269: 1325-1330.

Pitnick S, Brown WD (2000) Criteria for demonstrating female sperm choice. Evolution 54: 1052-1056.

Pizzari T, Birkhead TR (2000) Female feral fowl eject sperm of subdominant males. Nature 405: 787-789.

Pojana G, Gomiero A, Jonkers N, Marcomini A (2007) Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. Environ Int 33: 929-936.

Power M, Attrill MJ (2003) Long-term trends in the estuarine abundance of Nilsson's pipefish (*Syngnathus rostellatus* Nilsson). Estuar Coast Shelf Sci 57: 325-333.

Pritchard JK, Stephens M, Donnely P (2000) Inference of population structure using multilocus genotype data. Genetics 139: 375-385.

Quast WD, Howe NR (1980) The osmotic role of the brood pouch in the pipefish *Syngnathus scovelli*. Comp Biochem Physiol A 67: 675-678.

Rangel AM (2000) Detection of xenoestrogens, utilizing caged sheepshead minnows, *Cyprinodon variegates*, in the Mobile River and Mobile Bay ecosystem. Thesis, University of South Alabama.

Ratcliffe HE, Swanson MG, Fisher LJ (1996) Human exposure to mercury: a critical assessment of the evidence of adverse health effects. J Toxicol Environ Health 49: 221-270.

Ratterman NL, Rosenthal GG, Jones AG (2009) Sex recognition via chemical cues in the sex-role reversed Gulf pipefish (*Syngnathus scovelli*). Ethology 115: 339-346.

Reish DJ, Oshida PS, Mearns AJ, Ginn T, Buchman M (2006) Effects of marine pollutants on marine organisms. Water Environ Res 78: 2733-2819.

Rice WR, Hostert, EE (1993) Laboratory experiments on speciation – What have we learned in 40 years. Evolution. 47: 1637-1653.

Ripley J, Foran C (2009) Direct evidence for embryonic uptake of paternally-derived nutrients in two pipefish (Syngnathuidae: *Syngnathus* spp.). J Comp Physiol B 179: 325-333.

Rippey B, Rose N, Yang HD, Harrad S, Robson M, Travers S (2008) An assessment of toxicity in profundal lake sediment due to deposition of heavy metals and persistent organic pollutants from the atmosphere. Environ Int 34: 345-356.

Robinson CD, Brown E, Craft JA, Davies IM, Moffat CF, Pirie D, Robertson F, Stagg RM, Struthers S (2003) Effects of sewage effluent and ethynyl oestradiol upon molecular markers of oestrogenic exposure, maturation and reproductive success in the sand goby (*Pomatoschistus minutes*, Pallas). Aquat Toxicol 62: 119-134.

Rosenqvist G, Johansson K (1995) Male avoidance of parasitized females explained by direct benefits in a pipefish. Anim Behav 49: 1039-1045.

Ross K, Cooper N, Bidwell J, Elder J (2002) Genetic diversity and metal tolerance of two marine species: a comparison between populations from contaminated and reference sites. Mar Pollut Bull 44: 671-679.

Rousset F (2008) GENEPOP '007: A complete reimplementation of the GENEPOP for Windows and Linux. Mol Ecol Resour 8: 103-106.

Rundle HD, Nosil P (2005) Ecological speciation. Ecol Lett 8: 336-352.

Saarisota M, Craft JA, Lehtonen KK, Bjork H, Lindstrom K (2009) Disruption of sexual selection in sand gobies (*Pomatoschistus minutus*) by 17α-ethinylestradiol, an endocrine disruptor. Horm Behav 55: 530-537.

Sandvik M, Rosenqvist G, Berglund, A (2000) Male and female mate choice affects offspring quality in a sex-role reversed pipefish. Proc R Soc Lond Ser B 267: 2151-2155.

Sanfeliu C, Sebastia J, Cristofol R, Rodriguez-Farre E (2003) Neurotoxicity of organomercurial compounds. Neurotox Res 5: 283-305.

Sarafian, TA (1999) Methylmercury-induced generation of free radical: biological implications. Met Ions Biol Syst 36: 321-336.

Schäfers C, Teigeler M, Wenzel A (2007) Concentration- and time-dependent effects of synthetic estrogen, 17α-ethinylestradiol, on reproductive capabilities of the zebrafish, *Danio rerio*. J Toxicol Environ Health Part A 70: 768-779.

Schluter D (2000) The ecology of adaptive radiation. Oxford University Press, Oxford.

Schluter D (2001) Ecology and origin of speices. Trends Ecol Evol 16: 372-380.

Schneider S, Roessli D, Excoffier,L (2000) Arlequin ver. 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory. University of Geneva, Switzerland.

Schoenfuss HL, Baftell SE, Bistodeau TB, Cediel RA, Grove KJ, Zintek L, Lee KE, Barber, LB (2008) Impairment of the reproductive potential of male fathead minnows by environmentally relevant exposures to 4-nonylphenolf. Aquat Toxicol 86: 91-98.

Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, von Gunten U, Wehrli B (2006) The challenge of micropollutants in aquatic systems. Science 313: 1072-1077.

Sheldon B (2000) Differential allocation: tests, mechanism and implications. Trends Ecol Evol 15: 397-402.

Silk JB, Clark-Wheatley CB, Rodman PS, Samuels A (1981) Differential reproductive success and facultative adjustment of sex ratios among captive female bonnet macaques (*Macaca radiate*). Anim Behav 29: 1106-1120.

Silva K, Vieira MN, Almadas VC, Monteiro NM (2007) The effect of temperature on mate preferences and female-female interactions in *Syngnathus abaster*. Anim Behav 74: 1525-1533.

Simmons LW, Kvarnemo C (2006) Costs of breeding and their effects on the direction of sexual selection. Proc R Soc Biol Sci Ser B 273: 465-470.

Snook RR (2005) Sperm in competition: not playing by the numbers. Trends Ecol Evol 20: 46-53.

Sogabe A, Yanagisawa Y (2007) Sex-role reversal of a monogamous pipefish without higher potential reproductive rate in females. Proc R Soc Biol Sci Ser B 274: 2959-2963.

Stölting KN, Wilson AB (2007) Male pregnancy in seahorses and pipefish: beyond the mammalian model. BioEssays 29: 884-896.

Strittholt JR, Guttman SI, Wissing TE (1988) Genetic variability of yellow perch, *Perca flavescens* (Mitchell) in Lake Erie and selected impoundments. In: Downhower J (ed)The biogeography of the island region of western Lake Erie, Ohio State University, Columbus, Ohio, pp 246-257.

Sumpter JP (1995) Feminized responses in fish to environmental estrogens. Toxicol Lett 82: 737-742.

Sumpter JP, Jobling S (1995) Vitellogin as a biomarker for estrogenic contamination of the aquatic environment. Environ Health Perspect 103: 9-15.

Targett TE (1984) A breeding population of Gulf pipefish (*Syngnathus scovelli*) in a Georgia estuary. Contrib Marine Sci 27: 169-174.

Tallamy DW, Powell BE, McClafferty JA (2002) Male traits under cryptic female choice in the spotted cucumber beetle (Coleoptera: Chrysomelidae). Behav Ecol 12: 511-518.

Teixeira RL (1995) Reproductive and feeding biology of selected syngnathids (Pisces: Teleostei) of the western Atlantic. Dissertation, The College of William and Mary.

Telford SR, Jennions MD (1998) Establishing cryptic female choice in animals. Trends Ecol Evol 13: 216-218.

Theodorakis, CW (2003) Establishing causality between population genetic alterations and environmental contamination in aquatic organisms. Hum Ecol Risk Assess 9: 37-58.

Theodorakis CW, Lee KL, Adams SM, Law CB (2006) Evidence of altered gene flow, mutation rate and genetic diversity in red-breast sunfish from a pulp-mill contaminated river. Environ Sci Technol 40: 377-386.

Toft G, Baatrup E (2003) Altered sexual characteristics in guppies (Poecilia reticulate) exposed to 17 beta-estradiol and 4-tert-octylphenol during sexual development. Ecotoxicol Environ Safety 56: 228-237.

Torres R, Velando A (2005) Male preference for female foot colour in the socially monogamous blue-footed booby, *Sula nebouxii*. Anim Behav 69: 59-65.

Trivers RL (1972) Parental investment and sexual selection. In Campbell B (ed) Sexual selection and the decent of man, 1871 – 1971, Heinimann, London, pp 136-179.

Ueda N, Partridge C, Bolland J, Hemming J, Sherman T, Boettcher A (2005) Effects of an environmental estrogen on male gulf pipefish, *Syngnathus scovelli* (Evermann and Kendall) a male brooding teleost. Bull Environ Contam Tox 74: 1207-1212.

Vajda AM, Barber LB, Gray JL, Lopez EM, Woodling JD, Norris DO (2008) Reproductive disruption in fish downstream from an estrogenic wastewater effluent. Environ Sci Technol 42: 3407-3414.

Van Walleghem JLA, Blanchfield PJ, Hintelmann H (2007) Elimination of mercury by yellow perch in the wild. Environ Sci Technol 41: 5895-5901.

Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM (2009) Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruptors. Endocr Rev 30: 75-95.

Vandenbergh G, Adriaens D, Verslycke T, Janssen C (2003) Effects of 17α-ethinylestradiol on sexual development of the amphibod *Hyalella azteca*. Ecotox Environ Safe 54: 216-222.

Vincent A, Berglund A, Ahnesjo I (1995) Reproductive ecology of five pipefish species in one eelgrass bed. Environ Biol Fish 44: 347-36.

Vincent A, Ahnesjö I, Berglund A, Rosenqvist G (1992) Pipefish and seahorses: are they all sex role reversed? Trends Ecol Evol 7: 237-241.

Vincent A, Ahnesjö I, Berglund A (1994) Operational sex ratios and behavioural sex differences in a pipefish population. Behav Ecol Sociobiol 34: 435-442.

Viola TL (1992) Occurrence of Gulf pipefish, *Syngnathus scovelli*, in a fresh-water Texas reservoir. Tex J Sci 44: 361-361.

Wade MJ, Arnold SJ (1980) The intensity of sexual selection in relation to male sexual behavior, female choice and sperm precedence. Anim Behav 28: 446-461.

Wagner CE, McCune AR (2009) Contrasting patterns of spatial genetic structure in sympatric rock-dwelling cichlid fishes. Evolution 63: 1312-1326.

Ward PI (2000) Cryptic female choice in the yellow dung fly, *Scathophaga stercoraria* (L.). Evolution 54: 1680-1686.

Watanabe S, Kaneko T, Watanabe Y (1999) Immunocytochemical detection of mitochondria-rich cells in the brood pouch epithelium of the pipefish, *Syngnathus schelegeli*: structural comparison with mitochondria-rich cells in the gills and larval epidermis. Cell Tissue Res 295: 141-149.

Weatherhead, PJ and Robertson RJ (1979) Offspring quality and the polygyny threshold: "The sexy son hypothesis." Am Nat 113: 201-208,

Webber HM, Haines TA (2003) Mercury affects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). Environ Toxicol Chem 22: 1556-1561.

Weber L, Hill R, Janz D (2003) Developmental estrogenic exposure in zebrafish (*Danio rerio*): II. Histological evaluation of gametogenesis and organ toxicity. Aquat Toxicol 63: 431-446.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Wells, KD (2001) The energetic of calling in frogs. In: Ryan MJ (ed) Anuran communication. Institute Press, Washington DC, pp 45-60.

Whatley EC (1969) A study of *Syngnathus scovelli* in freshwaters of Louisiana and salt waters of Mississippi. Gulf Res Rep 2: 437-473.

Williams DA, Brown SD, Crawford DL (2008) Contemporary and historical influences on the genetic structure of the estuarine-dependent Gulf killifish *Fundulus grandis*. Mar Ecol Prog Ser 373: 111-121.

Wilson AB, Vincent A, Ahnesjö I, Meyer A (2001) Male pregnancy in seahorses and pipefishes (Family Syngnathidae): rapid diversification of paternal brood pouch morphology inferred from a molecular phylogeny. J Hered 92**:** 159-166.

Wilson AB, Ahnesjö I, Vincent A, Meyer A (2003) The dynamics of male brooding, mating patterns, and sex roles in pipefishes and seahorses (family Syngnathidae). Evolution 57: 1374-1386.

Wogel H, Abrunhosa PA, Pombal JP (2005) Breeding behavior and mating success of *Phyllomedusa rohdei* (Anura, Hylidae) in south-eastern Brazil. J Nat Hist 39: 2035-2045.

Xu H, Yang J, Wang YX, Jiang Q, Chen H, Song HY (2008) Exposure to 17α-ethinylestradiol impairs reproductive functions of both male and female zebrafish (*Danio rerio*) Aquat Toxicol 88: 1-8.

Xu J, Wang Y, Luo YM, Song J, Ke X (2009) Effects of copper, lead and zinc in soil on egg development and hatching of *Folsomia candida*. Insect Sci 16: 51-55.

Yeh FC, Yang RC, Boyle T (1999) POPGENE: Microsoft Windows-based freeware for population genetic analysis. Release 1.31. University of Alberta, Edmonton.

Zahavi A (1975) Mate selection – a selection for a handicap. J Theor Biol 53: 205-214.

Zeh DW, Zeh JA (2000) Reproductive mode and speciation: the viviparity-driven conflict hypothesis. Bioessays 22: 938-946.

APPENDIX

Total mercury concentration of fish collection from Grand Bay, MS, Meaher Park, AL and three sites within the Weeks Bay watershed. Data is reported as ppm dry weight

Collection Site	Species	Total Mercury (mean ± S.E.)	n
Grand Bay 1	*Chilomycterus atringa*	0.059 ± 0.005	2
Grand Bay 1	*Cynoscion nebulosus*	0.044 ± 0.007	3
Grand Bay 1	*Lagodon rhomboides*	0.069 ± 0.004	7
Grand Bay 1	*Lucania parva*	0.164 ± 0.080	3
Grand Bay 1	*Menidia peninsulae*	0.069 ± 0.001	3
Grand Bay 1	*Oligoplites saurus*	0.093	1
Grand Bay 1	*Sphoeroides parvus*	0.085	1
Grand Bay 1	*Sygnathus scovelli*	0.037 ± 0.004	5
Meaher Park	*Archosargus probatocephalus*	0.047 ± 0.001	2
Meaher Park	*Cynoscion nebulosus*	0.061 ± 0.006	2
Meaher Park	*Drum ?*	0.050 ± 0.003	3
Meaher Park	*Lagodon rhomboides*	0.069 ± 0.011	3
Meaher Park	*Menidia peninsulae*	0.085 ± 0.023	3
Meaher Park	*Micropterus salmoides*	0.065 ± 0.005	3
Meaher Park	*Syngnathus scovelli*	Not processed	29
Weeks Bay 1-Fish River	*Archosargus probatocephalus*	0.106	1
Weeks Bay 1-Fish River	*Lagodon rhomboides*	0.108 ± 0.017	5
Weeks Bay 1-Fish River	*Menidia peninsulae*	Not processed	5
Weeks Bay 1-Fish River	*Sygnathus scovelli*	Not processed	30
Weeks Bay 2-Fish River	*Lepomis macrochirus*	0.585 ± 0.085	2
Weeks Bay 2-Fish River	*Lepomis sp.*	0.359 ± 0.049	2
Weeks Bay 2-Fish River	*Lucania parva*	0.504 ± 0.074	7
Weeks Bay 2-Fish River	*Menidia peninsulae*	0.501 ± 0.067	2
Weeks Bay 2-Fish River	*Micropterus salmoides*	0.690 ± 0.034	2
Weeks Bay 2-Fish River	*Sygnathus scovelli*	0.422 ± 0.034	10
Weeks Bay 4-Magnolia Riv	*Lagodon rhomboides*	0.280 ± 0.017	4
Weeks Bay 4-Magnolia Riv	*Lucania parva*	0.302 ± 0.023	4
Weeks Bay 4-Magnolia Riv	*Micropterus salmoides*	0.360	1
Weeks Bay 4-Magnolia Riv	*Sygnathus scovelli*	0.332 ± 0.030	20

VITA

Name: Charlyn Gwen Partridge

Address: 660 S. Euclid
Washington University in St. Louis
School of Medicine
St. Louis, MO

Email Address: cpartridge@mail.bio.tamu.edu

Education: B.S., Biology, Nicholls State University, 1998
M.S., Biology, University of South Alabama, 2003
Ph.D., Biology, Texas A&M University, 2009

Publications: Partridge C, Ahnesjö I, Kvarnemo C, Mobley K, Berglund A, Jones A (2009) The effect of perceived female parasite load on post-copulatory male choice in a sex-role reversed pipefish. Behav Ecol Sociobiol 63: 345-354.

Partridge C, Shardo J, Boettcher A (2007) Osmoregulatory role of the brood pouch in the euryhaline Gulf pipefish, *Syngnathus scovelli*. Comp Biochem Physiol A. 174: 556-561.

Ueda N, Partridge C, Bolland J, Hemming J, ShermanT, Boettcher A (2005) Effects of an environmental estrogen on male Gulf pipefish, *Syngnathus scovelli*, a male brooding teleost. Bull Environ Contam Toxicol 74: 1207-1212.

Partridge C, Cazalas C, Rozelle J, Hemming J, Boettcher A (2004) Small-scale captive breeding of a euryhaline pipefish. World Aquaculture 35: 51-54.

Dupre T, Granier T, Keife S, Marino R, O'Rourke S, Partridge C, Schultz D, Manhare K, Beck J (1999) Variation of mercury concentration in fish taken from Lake Boeuf, southeastern Louisiana. Microchem J 61: 156-164.

Aucoin J, Blanchard R, Billion C, Partridge C, Schultz D, Mandhare K, Beck M, Beck J (1999) Trace metals in fish and sediments from Lake Boeuf, southeastern, Louisiana. Microchem J 62: 299-307.