# IDENTIFYING THE DIFFERENCES IN SURVIVORSHIP AND GROWTH OF OFFSPRING OF LARGE AND SMALL FEMALES IN THE PIPEFISH SPECIES *SYNGNATHUS SCOVELLI*

An Undergraduate Research Scholars Thesis

by

ANDREA MARTINEZ AGUIRRE

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by
Research Advisor: Dr. Adam G. Jones

May 2015

Major: Microbiology

# TABLE OF CONTENTS


Page

ABSTRACT ........ 1

ACKNOWLEDGEMENTS ........ 3

CHAPTER

I INTRODUCTION ........ 4

II METHODS ........ 7

III RESULTS ........ 9

Female Category and Reproductive Success ........ 9
Offspring Size Across Female Categories ........ 9
Growth and Survivorship of Offspring in Food Treatments ........ 11

IV CONCLUSION ........ 13

REFERENCES ........ 17

# ABSTRACT

Identifying the differences in survivorship and growth of offspring of large and small females in the pipefish species *Syngnathus scovelli*. (May 2015)

Andrea Martinez Aguirre
Department of Biology
Texas A&M University

Research Advisor: Dr. Adam G. Jones
Department of Biology

While maternal investment in many fish species is well studied, the effect of maternal investment on offspring quality in the sex-role-reversed pipefish *Syngnathus scovelli* has not yet been determined. Previous studies in other species of pipefish have looked at egg size and components of an egg's makeup as indicators of offspring quality and have found conflicting results. In this study, we compared growth and survivorship of offspring from females of different sizes by mating a controlled sized male with a large or small female and measuring growth every ten days. Using the number of eggs transferred by each female to her mate as a predictor of female reproductive success, we found that in a single mating there is no difference in the number of eggs or surviving offspring at birth for the two female size categories. The size at birth of the offspring were not significantly different but showed a trend towards larger females having longer offspring at birth. Growth and survivorship of offspring of large and small females was also compared when offspring are put into resource limited environments by having three levels of feeding for offspring from each brood. The results showed that offspring of both female categories had decreased growth and survivorship in the lowest food treatment and had similar growth and survivorship in the control. However, the experiment showed an unexpected result in the low feeding treatment where the offspring of small females grew more and had a higher

survivorship than offspring of large females in the same feeding treatment. We hypothesize that the increased growth in offspring of small females when food is limited is a result of increased female provisioning from the smaller females who are compensating for their lower mating successes compared to larger females. These results may indicate that size of maternal female is not a definite indicator of offspring fitness and there are many interactions and environmental conditions that need to be taken into account in order to predict the future fitness of offspring in Gulf pipefish.

ACKNOWLEDGEMENTS

I would like to thank Dr. Adam Jones for giving me the opportunity to work in his lab and develop skills that would help me in my future projects. I would also like to thank Andrew Anderson and Emily Rose for their helping developing this project as well as their guidance thorough the various steps of the project. Finally I would like to thank the Texas Sea Grant College Program for providing funds for this project.

# CHAPTER I

# INTRODUCTION

Parental investment varies greatly between species. Some animals take care of their young until adulthood, while others stop investment after copulation. The family Syngnathidae contains species of pipefishes and seahorses that carry their young in specialized brood pouches. Some species, like the Gulf pipefish, are characterized by being sex-role-reversed, where the male is the choosy sex. In Gulf pipefish, females must compete for access to mates as a result of limited mating opportunities, resulting in females experiencing greater sexual selection (Berglund and Rosenqvist 2003). Studies across a variety of pipefish species have looked for predictors that will illustrate if the size of the female is an indicator off offspring survivorship as well as development until adulthood. A previous study showed that the mating preferences observed in males and females may be inherited by their offspring, preparing the offspring of dominant parents for better survivorship and mating success during adulthood (Qvarnstrom and Price 2001). In the Qvarnstrom and Price study, there were various perspectives presented regarding mate choice, with the central idea being that preferences have evolved in order to maximize the benefits that the sexes take into account when choosing a mate. A mating between a dominant male or female is believed to increase the fitness of the offspring.

This study focuses on the growth and survivorship of offspring during their first month of life, in the sex-role-reversed Gulf pipefish, *Syngnathus scovelli*, which is a polyandrous pipefish species where females have multiple males. This species was chosen because unlike other pipefish species, the Gulf pipefish males carry eggs from only one female per brood, which allows us to compare maternal effects on offspring survivorship broods from a single mother. As a result of female competition for access to mates I hypothesize that females will greatly invest in eggs when a mating opportunity appears. I will measure and compare female reproductive success by measuring how many eggs are transferred at the time of copulation, as well as how many offspring are born. I will also measure survivorship and growth between offspring of small and large females and determine survivorship and fitness of offspring in a resource limited environment.

In recent studies, it has been shown that Gulf pipefish males prefer to mate with larger females (Paczolt and Jones 2010). While this may suggest that larger females will generate better quality offspring, a study published on a closed related species *Syngnathus typhle* presents a different perspective. Mobley et al. found that when smaller females lay fewer eggs than larger females, their offspring have a higher survivorship (Mobley et al. 2011). This brings us to the question of how much of an effect the environmental conditions, including the resources available at the time of copulation, will have on the growth and survivorship of offspring, and also if maternal size creates an advantage to her offspring. Common diets for pipefish are microscopic copepods or Artemia. Pipefish must consume large numbers of Artemia in order to grow at a normal rate (Clifford and Boehlert 1983). By limiting the amount of food the offspring receive we are creating a resource limited environment.

In this study, I hypothesize that larger females will give more eggs to their mates and their offspring will have greater survivorship and will grow faster, due to increased provisioning by the mother. An alternate hypothesis may be that offspring of smaller females will be larger at time of birth, as a result of a smaller number of eggs being transferred to the male during copulation and more resources given per egg, which will allow a higher survivorship.

# CHAPTER II

# METHODS

Pipefish were collected in Port Aransas, TX, between May and November 2014. After being acclimated for a day, they were put into a flow through system of 26ppt artificial seawater, at Texas A&M University in College Station. Males of only 100mm ± 4mm length were used in the experiment to maintain a constant size of male mates. Females were measured and separated into small and large female categories, with small ranging from 95-108mm and large from 118-130mm. Males were put into groups of four or five in 30L tanks, and a female from a category was introduced during the morning of copulation. Pictures of the female were taken before being introduced to the tank to determine exact size and bands. Fish were given approximately one week to mate and after copulation occurred the impregnated male was taken out of the tank and relocated in a separate 7L tank for 12-14 days until offspring were born. At approximately the 7th day of pregnancy pictures were taken of the brood pouch of the male to estimate the number of eggs transferred by the female.

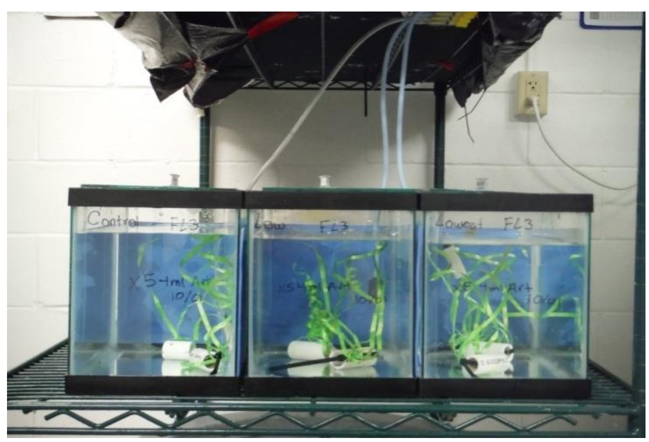


**Figure 1. Set up of feeding categories in each replicate.**

At the time of birth a sample of 15 newborn was separated randomly into three different categories corresponding to the amount of food given. The control category was fed 4ml of Artemia, which corresponds to a normal feeding for offspring of this species and a low category was fed 2ml. The lowest category only received 0.60ml representing an eighty five percent decrease feeding compared to the control tanks. Each category was put in a 7L tank, and contact between fish of different categories was not allowed. Arrangement of the tanks is observed in Figure 1. Fish were fed Artemia twice a day with a P-1000 micropipette, including a morning and an evening feeding. Artemia was hatched every day during the morning, and enrichment was added to increase the size of the brine shrimp when given to the fish.

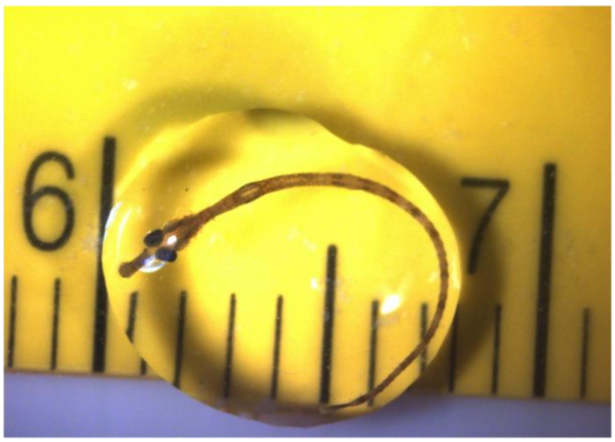


**Figure 2. Picture of newborn taken with a dissecting microscope and scale attached.**

Fish were counted daily to see if any fish had died to measure survivorship and to adjust feeding amounts. If a fish died in any of the tanks, the amount of feeding was adjusted so the fish continue to receive the same percentage of food until the end of the experiment. Pictures were taken every ten days, the first day with a dissecting microscope, and the tenth, twentieth and, thirtieth day photos taken with a waterproof camera to measure growth, a tripod was used when taking pictures to avoid possible errors. Image J was used to measure length of fish and a ruler was used as a scale in every picture as shown in figure 2. The experiment lasted one month to monitor survivorship and growth of the offspring during the most difficult part of their lifespan. After a month, the fish were sacrificed in MS222 and were preserved in ethanol.

# CHAPTER III

# RESULTS

## Female Category and Reproductive Success

Large and small females were significantly different in length and depth (Student's t-test: length: p= 0.0001, depth: p= 0.0094). Average length and depth of large females was 125 mm and 0.662 mm, while average size and depth of small females was 99 mm and 0.433 mm, respectively.

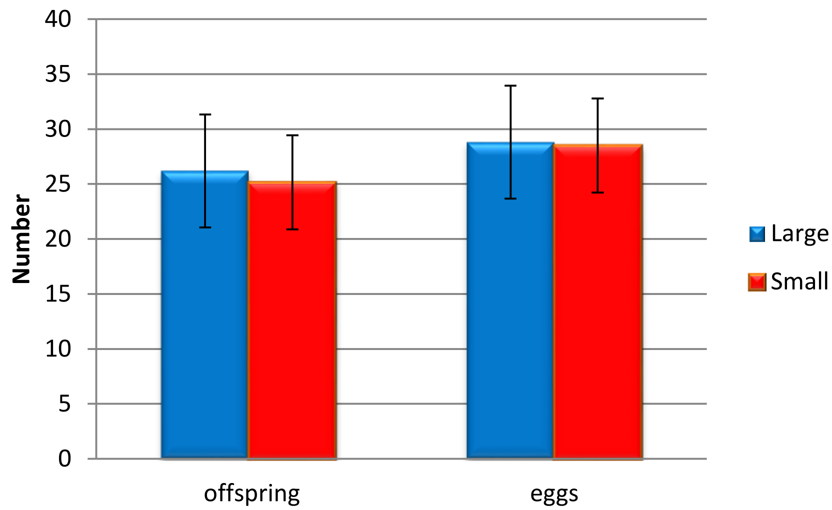


**Figure 3. Number of offspring born and eggs transferred to male from large and small females**

A regression line showed no correlation between length and depth in females from both treatments ($r^2$= 0.001092, p= 0.9231). Numbers of eggs transferred to males and number of surviving newborns at time of birth were factors used to measure female reproductive success. Using a student's t-test, I found no significant difference in number of eggs or newborns as a result of maternal size (number of eggs: p=0.8450, offspring: p= 0.9457), as shown in Figure 3.

## Offspring Size Across Female Categories

When comparing the size of the offspring at birth of each female category, by using a student's-test and combining the offspring regardless of replicates, a significant difference in size between offspring was found (p= 0.0387). The results of the student's t test showed that offspring of large females have a longer length at time of birth. However, to account for the offspring from each replicate not being an independent data point I ran a two-way ANOVA using replicate as a random effect. Although a two-way ANOVA test with replicate as random effect did not show a significant difference in offspring size across the maternal size treatments, a trend was observed ( p= 0.1066). At the end of the experiment, the control offspring from large and small females were also not significantly different from one another (Two-way ANOVA interaction: size*treatment p= 0.9411, post-hoc Tukeys t-test: p= 0.9071).Figure 4. In the lowest feeding category a similar pattern was observed, offspring growth in large and small females were not significantly different (Two-way ANOVA interaction: size*treatment, post-hoc Tukeys t-test: p=0.9997).

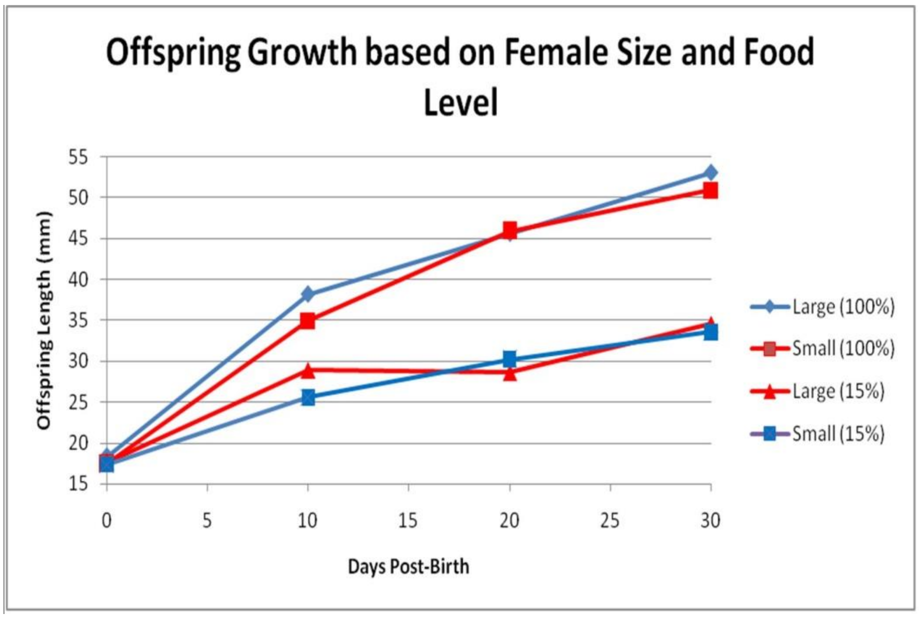


**Figure 4. Growth rates for offspring of the large, in blue, and small, in red, females in the control treatment and lowest treatment**

**Growth and Survivorship of Offspring in Food Treatments**

Using a two-way ANOVA with replicate as random effect, I tested effects of maternal size, feeding treatment, and the interaction of the two treatments on offspring survivorship. The results indicated that size had no effect in survivorship but treatment had a high effect in survivorship (maternal size: p=0.1391, food treatment: p=0.0001). Offspring of the control and low feeding treatments were not significantly different from one another, but both categories where significantly different from the lowest feeding treatment (ANOVA, food treatment: p=0.8716, post-hoc Tukeys t-test: control vs. lowest p=0.0001, low vs. lowest p=0.0001).Although not significant, survivorship of small female in the low feeding category showed a trend of higher survivorship than offspring of large females (Two way ANOVA size*treatment: post-hoc Tukeys t-test: p=0.9008). Survivorship of offspring from all feeding categories throughout the experiment is reported in table 1.

**Table 1. Table of percent survivorship for three feeding categories at two time points for each small and large female replicate.**

| Percent survivorship | | | | | | |
|---|---|---|---|---|---|---|
| **Feeding level** | **control** | | **Low** | | **lowest** | |
| Days post-birth | 20 | 30 | 20 | 30 | 20 | 30 |
| **large replicates** | | | | | | |
| Female 1 | 80% | 80% | 20% | 20% | 40% | 40% |
| Female 2 | 40% | 40% | 80% | 20% | 60% | 40% |
| Female 3 | 100% | 80% | 80% | 80% | 40% | 20% |
| Female 4 | 100% | 100% | 100% | 100% | 60% | 0% |
| Female 5 | 100% | 100% | 100% | 100% | 60% | 20% |
| **small replicates** | | | | | | |
| Female 1 | 100% | 100% | 100% | 100% | 40% | 40% |
| Female 2 | 100% | 100% | 60% | 60% | 40% | 20% |
| Female 3 | 100% | 100% | 100% | 100% | 80% | 20% |
| Female 4 | 60% | 60% | 100% | 100% | 100% | 60% |
| Female 5 | 100% | 100% | 100% | 80% | 80% | 20% |

The percent growth change, which accounted for total growth over the course of the entire experiment, was compared across the two treatments using a two-way ANOVA. The results showed that female size did not affect growth, but treatment as well as female size with treatment had an effect in offspring growth (female size: p= 0.9493, treatment: p=0.0001, female size*treatment: p=0.0008). All three treatments were significantly different from each other when compared using a Tukey's post-hoc test (control* lowest p=0.0001, low*lowest p=0.0001, control*low p= 0.0017).

When comparing growth, the offspring of the large control category were larger than the large low category, as expected, but the small control

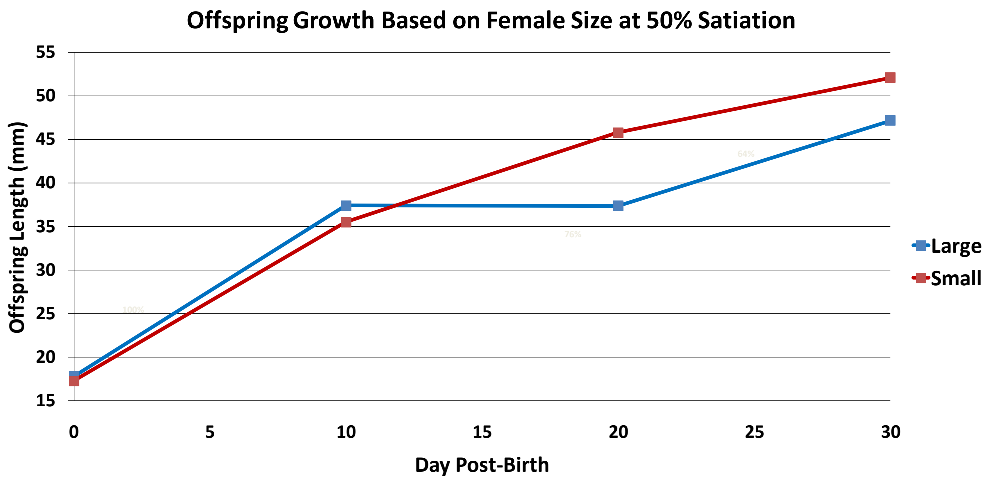


**Figure 5. Growth rate of offspring from large female in blue, and small female in red of the low feeding category.**

category was not much larger than the small low category (Tukey's post-hoc test : large control*large low: p=0.001, small control* small low: p=0.9997).Only the low categories showed a significant difference between them as seen in Figure 5, showing that offspring from small females had an increased growth than offspring from large females (Tukey's post-hoc test small low*large low: p= 0.0439).

# CHAPTER IV

# CONCLUSIONS

One of the goals of this study was to see if varied environmental conditions would have an effect on survivorship and growth of offspring from large and small female Gulf pipefish. While the offspring in the lowest food treatment, regardless of maternal size, showed a higher rate of mortality compared to the control offspring, there was one unexpected result in the low feeding treatment. The results of the low food treatment showed that offspring of small females grew more and had a higher percentage of survivorship than offspring of large females in the same feeding treatment. The results regarding the lowest treatment were as expected and indicated that a drastic decrease in food resulted in offspring with decreased growth rates and survivorship, with no difference between the offspring of the two maternal categories observed. Finally growth and survivorship of offspring at normal conditions showed no apparent difference related to the mother's size. Additional goals of this study were to determine if female Gulf pipefish of varying maternal sizes transferred different number of eggs to their mates and if maternal size affected the growth rates in their offspring. Regarding female reproductive success, the results indicated that there was no difference in number of eggs transferred to the male brood pouch or the number of newborn surviving between large and small females, thus we predict that size of female does not give an advantage regarding female reproductive success for a single mating. Nevertheless, when comparing size at birth we were able to see a trend that showed that offspring of large females were longer in length at time of birth.

In the low food treatment we were able to observe that offspring from smaller females grew more and had a higher percentage of survivorship. The reproductive compensation hypothesis says that when either of the sexes is constrained, the individuals can show flexibility in their physiology, as well as in their behavior in order to increase the survival of their offspring (Gowaty 2008). Paczolt and Jones have previously documented that males in Gulf pipefish prefer to mate with large females, thus, we predict that males will invest more in broods from large females than small females. In accordance with the reproductive compensation hypothesis, small females may compensate for their disadvantage in gaining mates by increasing the nutrients in their eggs to benefit their offspring and increase their chances of survival. Although this phenomenon may not be observed in normal feeding conditions, our experiment provides support that when a decrease in food availability occurs; offspring of small females may utilize their resources to maintain their growth rate and survivorship.  When we compare the small female control group and the small female low group, the growth rate is the same for both groups and survivorship has little variation. When we compare the large female control group with the large female low group, we see that growth rate is significantly different as well as percent survivorship. One possible explanation for the observed results may be that large females gave only the necessary resources required for offspring to survive at optimal conditions while small females increased provisioning to compensate for their possible disadvantage. Mobley et al. also found in another pipefish species, the broad nosed pipefish, that the offspring of small females showed greater fitness than large female's offspring in certain specific conditions. Although our prediction assumes that increased provisioning may give an advantage to the low feeding group, when resources were extremely scarce, offspring of large and small females both showed very high mortality and a significant decrease in growth. The results regarding survivorship obtained in the lowest feeding treatment, showed the expected results of a substantial decrease in survivorship. In previous studies it has been shown that starvation of offspring in the early stages of life in marine species is the factor responsible for high mortality (Legget and Debois 1994). In this the study offspring of pipefish showed a considerable decrease in survivorship when feeding was decreasing significantly.

The present study is considered a pilot study since we were only able to perform five replicates for each category and each replicate contained only five fish. In order to have certainty of the results obtained, more replicates will need to be conducted. Food ration was determined at the beginning of the experiment and feeding was not increased at any point in the experiment, a factor that might have had an influence on survivorship as well as growth. If in a later time more replicates are performed, rations of food will be increased as fish grow to maintain satiation and results will be compared to see if any changes appear. An additional factor that might have affected maternal provisioning may be the amount of time females spend in the lab prior to the beginning of the experiment, since it was observed that females who spend less time in a holding tank after being captured from the wild, had larger and more successful broods than females who had spent more time in holding tanks in the laboratory setting. One possible explanation for the pattern observed may be that pipefish in the wild feed on a variety of organisms, whereas in captivity pipefish are only fed a single diet, in this case enriched Artemia, and it has been documented in a closely related seahorse species *Hippocampus kuda* that fish grown in captivity show a decrease of nutrient compared to wild fish (Lin et al., 2009).

In conclusion, this study showed that even though males of the Gulf pipefish species seem to show a preference to mate with larger females, offspring of small females could have an advantage over offspring of large females in some environmental conditions. These results may indicate that size of maternal female is not a definite indicator of offspring fitness and there are many interactions that need to be taken into account in order to predict the future fitness of offspring in Gulf pipefish. The results found by performing this study provide an indirect approach when looking at maternal investment. In the future, we will look for an experiment with a more direct approach in which eggs' nutrients will be tested after being transferred into the male's brood pouch, to see if in fact there is a difference in nutrients and if a difference in nutrients correlates with the size of the female that was mated with the male pipefish.

.

# REFERENCES


Berglund A, G Rosenqvist. "Sex Role Reversal in Pipefish." Advances in the study of behavior 32 (2003): 131.

Clifford, HR, GW Boehlert. "Feeding Chronology, Daily Ration, and the Effects of Temperature upon Gastric Evacuation in the Pipefish, *Syngnathus fuscus*." Environmental Biolofy of fishes 9.3-4 (1983): 301.

Mobley, KB, Kvarnemo, C, Ahnesjo, I, Partridge, C, Berglund, A, AG Jones. "The Effect of Maternal Body Size on Embryo Survivorship in the Broods of Pregnant Male Pipefish." Behavioral Ecology and Sociobiology 65.6 (2011): 1169.

Paczolt, KA, AG Jones. "Post-Copulatory Sexual Selection and Sexual Conflict in the Evolution of Male Pregnancy." Nature 464 (2010): 401.

Qvarnstrom, Anna-Price D.,Trevor. "Maternal Effects, Paternal Effects and Sexual Selection." Trends in Ecology and Evolution 16.2 (2001): 95.

Gowaty, Patricia Adair. "Reproductive Compensation." Journal of Evolutionary Biology 21.5 (2008): 1189-200.

Legget, W. C., and E. Deblois. "Recruitment in Marine Fishes: Is it Regulated by Starvation and Predation in the Egg and Larval Stages?." Netherlands Journal of Sea Research 32.2 (1994): 119-34.

Lin, Qiang, Junda Lin, and Chao Wang. "Biochemical Composition of the Wild and Cultured Seahorses, Hippocampus Kuda Bleeker and Hippocampus Trimaculatus Leach." Aquaculture Research 40.6 (2009): 710-9.