ANALYSIS OF SPAWNING BEHAVIOR AND GAMETE AVAILABILITY OF THE FLORIDA POMPANO (*Trachinotus carolinus*) IN A RECIRCULATING AQUACULTURE SYSTEM.

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULLFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2010

ACKNOWLEDGMENTS

I would like to thank the chair and the members of my committee for their mentoring and advice, the Mote Marine Laboratory for the use of their facilities and collaboration, and my loving parents, grandparents, husband, and friends who encouraged me and motivated me through this study.

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August 2010

Chair: Colette M. St. Mary
Major: Zoology

Marine finfish recirculating aquaculture systems are a viable way to produce environmentally-sustainable stock. However, one of the biggest challenges production managers face in recirculating systems is that fertilized egg yields are low or inconsistent. An economically valuable fish that is currently in population decline is the Florida pompano (*Trachinotus carolinus*). This is a preliminary study that focuses on the factors that contribute to the low fertilized egg yields of the Florida pompano in the recirculating aquaculture facility at Mote Aquaculture Research Park, a field station of the Mote Marine Laboratory. In this study, we used video analysis of spawns in combination with sampling and fertilized egg yield data to determine A) if pompano will exhibit natural spawning behavior in a recirculating system, B) does pompano gamete maturity remain consistent throughout the entire February-October spawning season, and C) what are the fertilization rates of pompano spawns in recirculating systems, and how does it compare to other captive pompano fertilization rates. From our data, we determined that pompano spawning behavior is not inhibited by recirculating aquaculture systems. In fact, both false (spawning behavior without the release of

gametes) and true spawning behavior (with the release of gametes) occurred and were quantified. We also found that the production of mature gametes in males is highly variable and possibly the true cause of low fertilized egg yield. A relatively high proportion of males had mature gametes at the beginning (53%, February) and end (36%, September) of the spawning season, but in June, only 7% of males had mature gametes at the time of sampling. Female gamete maturity also peaked in February and September (70% and 60% of females had mature gametes in these months, respectively), but female gamete maturity did not plunge significantly like the male gamete maturity did. This pattern in gamete maturity could reflect a need for a 90-day break in the middle of the spawning season so adults can produce more mature gametes. Finally, fertilization rates of 0%-57% were much lower than other captive pompano studies and were also variable across the spawning season. Some causes for these low fertilization rates could be smaller egg sizes, small egg fecundity, and sperm limitation. Strong flow rate in the tanks during spawning could also cause fertilization dysfunction.

CHAPTER 1
MARINE FINFISH AQUACULTURE SYSTEMS AND RELATED REPRODUCTIVE CHALLENGES

The struggle between the demand for commercial fishing and conservation has proved to be a tumultuous battle. In response to this problem and the ever-increasing demand for food fish, the development of recirculating aquaculture systems has been proposed as an environmentally-friendly and sustainable solution to the demands for commercial fish. Over the past century, commercial fishing for finfish has increased exponentially (Watson and Pauly 2001). During the 1980s, overfishing was such a threat to marine species that the government passed the Magnuson-Stevens Fishery Conservation and Management Act in 1996 that required an end to over-exploitation of fish populations and mandatory rebuilding of fish stocks (Rosenberg et al. 2006). Unfortunately, even though there is evidence that biomass in stocks managed under rebuilding plans has the potential to increase, overfishing still occurs in 45% of these stocks (Rosenberg et al. 2006). Perhaps the most vulnerable species to overexploitation are those that predictably spawn in the same area each season (de Mitcheson et al. 2008). For example, seasonal spawning aggregations seen in many shallow-water grouper such as Nassau grouper (*Epinephelus striatus*), Gag Grouper (*Mycteroperca microlepis*), and Scamp Grouper (*Epiniphelus morio*) are often the target of commercial fishing due to their predictability (Coleman et al. 1996; de Mitcheson et al. 2008). The risk of rapid stock decline is increased as a result.

To combat the decline in fish populations due to overfishing and other causes of stock decline (e.g. development, habitat degradation, and pollution), agencies have attempted to increase marine fish yields through restocking natural populations (increase spawning biomass), fisheries enhancement (overcome recruitment limitation),

and sea ranching (put, grow, and take method) (Bell et al. 2008). All of these supplemental additions first require the production of juveniles (Bell et al. 2008). The principle method of producing juveniles for release is through aquaculture.

Aquaculture is by no means a new system for producing fish. Historically, aquaculture involved producing fish in inland ponds or coastal cages (Losordo et al. 1998; Holmer et al. 2003). Unfortunately, aquaculture in inland ponds relies on large amounts of water (as much as 1 million gallons per acre to fill a pond, and an equal amount every year to compensate for evaporation, and seepage; Losordo et al. 1998), a large land area, and its productivity is limited by environmental factors. In addition, the costs of nutrient effluent, shifts in biodiversity, and changes in water chemistry have caused coastal cages to come under scrutiny (Holmer et al. 2003; Alongi et al. 2003; Islam 2005). The necessity for a sustainable system for fish production is especially important when aquaculture is expected to produce over half the total fish consumed by the world population by 2030 (FAO 2000.) In the latter half of the 20th century, aquaculture systems have changed toward environmentally sustainable facilities that have little or no impact on the coastal ecosystems or waterways (Kupren et al. 2008). For many years, scientists have been developing the vision of recirculating aquaculture systems that require less land, have lower water impact, allow environmental control for year-round production, and offer the flexibility to be located near prime markets (Masser et al. 1992).

Recirculating aquaculture systems, or closed-system aquaculture, use a recirculating water system instead of a flow-through design. In a study comparing the energy use, water dependence, and environmental impact of a recirculating and flow-

through trout systems on the same waterway, it was shown that the water use of a recirculating system was 93% less than that of a flow through design (D'Orbcastel et al. 2009). Also, the eutrophication potential of the recirculating system was 26-38% lower than that of the flow-through system because of reduced waste release (D'Orbcastel et al. 2009). Although d'Orbcastel et al. (2009) found that the recirculating systems used 24-40% more energy than traditional aquaculture systems due to aeration and water treatment, it was acknowledged that early recirculating systems could be engineered using biofilters and airlift designs that would reduce their energy use. Airlift is a cost-effective design that provides water movement and aeration (Pfeiffer and Wills 2007). Much work is underway on the exact design of the most effective and cost-efficient biofilters, but in fact, many recirculating systems today already use biofiltration and airlift technology as a means of water treatment (Pfieffer and Wills 2007). The technology of recirculating aquaculture systems is rapidly advancing to limit environmental impact and increase cost-effectiveness, but there are still many concerns about whether recirculating systems can produce sufficient marine finfish to meet the present and future demands.

One of the biggest challenges aquaculture managers are currently facing in recirculating aquaculture systems is the inconsistency of reproductive success (the production of fertilized eggs) (Holt et al. 2007; Cejko et al. 2008; Kucharczyk et al. 2008). The individual factors needed for reproductive success are predictable spawning, the production of viable gametes, spawning participation, and stable fertilization kinetics. Some causes for the reproductive breakdown specifically in recirculating systems may be due to A) the lack of environmental stimuli, such as

temperature, photoperiod, and lunar phase, B) spatial constraints of depth and volume, and C) a lack of spawning stimuli due to group size, unnatural spawning sex ratios, and the absence of hormonal cues.

Substantial research has been done on photoperiod manipulation and spawning success in marine finfish aquaculture. By advancing or delaying the annual photoperiod in relation to the natural changes in the environment, researchers found that they could control the spawning season of marine finfish (Roberts et al. 1978; Campos-Mendoza et al. 2004; Van der Meeren and Ivannikov 2006). In some cases, Atlantic cod were induced to have two spawning seasons each year, instead of only one, by truncating the annual photoperiod (Van der Meeren and Ivannikov 2006). In many marine species, specific changes in temperature can be an indicator of when to spawn and may actually affect egg quality. For example, Atlantic halibut (*Hippoglossus hippoglossus)* that experienced a temperature drop in late December were induced to spawn in the spring and produced more eggs with a higher fertilization and hatch rate than fish that experienced a temperature rise in late December (Brown et al. 2006). These important environmental cues can be tightly controlled and manipulated with today's aquaculture equipment, and thus fish can be induced to spawn via these techniques. Nonetheless, there remain other problems that have not been adequately addressed in recirculating aquaculture.

The problem of poor gamete quality goes deeper to the physiological level. Often females will not ovulate in captivity as they do in their natural habitat. Previous studies have hypothesized that this failure to ovulate is due to a lack of a luteinizing hormone (LH) surge from the pituitary (Zohar and Mylonas 2001). Standard practices of

spawning fish in captivity include artificial injection of hormones that simulate LH-like activity and induce the oocytes to undergo final oocyte maturation and ovulation (Shirashi et al. 2005). In standard aquaculture spawning practices, females undergo cannulation, or oocyte biopsy, to examine the stage of the oocytes. As described by Shiraishi et al. (2008), only females with oocytes predominantly in the late stages of vitellogenesis are selected for hormone injection (Ibarra-Castro and Duncan 2007). In addition to female examination and injection, males are also examined for milt flow, percent sperm motility, and duration of sperm motility. For example, in spotted rose snapper (*Lutianus guttatus)*, males were sampled with haematocrit tubes, the sperm analyzed at 400X magnification, and the sperm was observed for motility (Ibarra-Castro and Duncan 2007).

Two of the most widely used hormones to induce fish ovulation are human chorionic gonadotropin (HCG) and gonadotropin releasing hormone analog (GnRHa) (Shiraishi et al. 2008). Both hormones are injected into the fish intramuscularly. The exact timing of the induced-spawn can be variable. In an experiment done with chub mackerel (*Scomber japonicas)*, ovulation occurred about 33 hours post HCG-injection and 36 hours post GnRH-injection (Shiraishi et al. 2008). However, in red snapper (*Lutjanus campechanus)*, most females ovulated between 24-32 hours post HCG-injection and larval survival rate was highest when females spawned during this time period (Bourque and Phelps 2007).

These hormone techniques are useful to induce mature fish to release gametes, but little is known about the participation of individuals within the group during spawning. Similarly, little is known about the actual fertilization kinetics in recirculating systems.

Historically, mixed-milt fertilization, or fertilization that includes combining eggs and milt from many individuals in a single container (Wedekind et al. 2007), has been widely-used to produce fertilized eggs, with varying success (Hoff et al. 1972; Kloth 1980). However, this method of mixed-milt fertilization has been discouraged due to decreased genetic variation from sperm competition, increased artificial selection, and domestication of the stock population (Wedekind et al. 2007). The restocking of wild fish populations is an important part of management, and both genetic variability and quality of the stock must be maintained through natural spawning.

Due to the high water and land requirements of pond aquaculture and the effects of effluent loading on the environment in coastal cages, the most environmentally sustainable option for producing high quantities of fish remains recirculating aquaculture systems. While research has been done on controlling environmental cues and hormonal levels that facilitate spawning, much research is still needed to increase annual fertilized egg yield. The specific questions for future progress in recirculating systems will be can they provide a suitable environment for natural spawning behavior? And can gamete production be optimized or at least controlled to produce consistent yields of fertilized eggs? Once researchers have consistent production of fertilized eggs, the next step, which has already been undertaken, is to develop cost-effective systems to hatch eggs and rear juveniles (for a review of marine finfish larviculture techniques see Lee and Ostrowski 2001).

CHAPTER 2
SPAWNING BEHAVIOR AND GAMETE AVAILABILITY OF FLORIDA POMPANO

Introduction

Florida pompano is one species that is not recovering as expected under its overexploitation recovery plan. Economically, Florida pompano is one of the most desirable marine fish with a price per pound (Retail market prices in 2005 were around $8.00/lb; Main et al. 2007) that is higher than most other US marine and freshwater fish (Weirich et al. 2005). As a fish with such high market demand, it is not surprising that the pompano stock condition was listed as decreasing due to overfishing for the years 1998-2007, based on catch-rate information (FWRI 2008). To limit the impact of overexploitation and decline of natural populations as well as create an economically lucrative industry, aquaculture techniques for the Florida pompano have been in development for more than 40 years. Unfortunately, a recirculating system that produces reliable yields of fertilized eggs has yet to be developed.

Both HCG and GnRHa can be used to induce spawning in Florida pompano, and it has been documented that spawning occurs about 36 hours post-injection (Weirich et al. 2005). In addition to hormone treatments, temperature shifts from 74 to 78˚F (24 to 26˚C) to 86 to 88˚F (30 to 31˚C) can induce natural spawning when combined with a photoperiod ranging from 11 hours (winter) to 13 hours (summer) (Hoff et al. 1972, 1978a, b; Kloth 1980; Weirich et al. 2005; Main et al. 2007; Weirich and Riley 2007). Unfortunately, even while using these spawning methods, fertilization rates are highly variable (0% to 91%, Main et al. 2007) and remain a large problem when producing pompano on a commercial scale. While pompano can be conditioned to spawn under artificially controlled conditions of photoperiod, temperature and hormone

injection/implantation, new aquaculture techniques must be developed to improve fertilized egg yield.

One possible explanation for low fertilized egg yield in many marine fish, including the Family Carangidae of which pompano are a member of (Graham and Castellanos 2005), is that they naturally spawn in large groups (involving 100s of individuals) and in captivity the group size is small and the quarters too confined. These factors may inhibit natural spawning behavior and hence the production of fertilized eggs. Few observations of pompano group-spawning in the field have been made, but group spawning behavior is often stereotyped across taxa. Graham and Castellanos (2005) described the spawning behavior of two members of the family Carangidae (the permit, *Trachinotus falcatus*, and the yellow jack, *Carangoides bartholomaei*), of which pompano is also a member. In permit and yellow jack, Graham and Castellanos (2005) reported schools of at least 300 individuals aggregating about an hour before sunset. As spawning began, subgroups of the large school broke off as a female swam rapidly toward the surface followed by multiple males and followers. The female would stop approximately 5 m from the surface and vibrated rapidly, releasing a cloud of eggs, the males released their sperm essentially simultaneously and in very close proximity to the female (Graham and Castellanos 2005). These spawning subgroups are very typical in group-spawning species, however data have not been collected to determine if females spawn once and then leave the aggregation, or if they spawn multiple times in the same event. In wrasses, for example, females come to the aggregation, and spawn a single clutch of eggs, and then leave (Colin 2010). Replicating this dramatic spawning

behavior in a tank setting may be an important limitation to high fertilized egg yield in aquaculture.

Past attempts to spawn pompano have involved selecting individuals with mature gametes, injecting them with a hormone, and either strip-spawning or allowing the individuals to spawn in a separate tank (Hoff et al. 1978; Kloth 1980; Weirich and Riley 2007). While these techniques may produce a larger proportion of fertilized eggs, pompano are especially delicate fish, and susceptible to the detrimental effects of excessive handling. To maintain the effects of sexual selection and to limit handling of the fish, natural group spawning behavior was the focus of our study (Forsberg 2008). In this study, the entire group was allowed to participate in the spawn.

In this study, I examined whether pompano exhibit natural spawning behavior in a commercial recirculating aquaculture system. This was done using video analysis of spawning and by comparing the number of participants, spawning rush duration, and spawning behavior to the limited descriptions of natural group spawning in carangids. Video analysis of pompano group spawning behavior has never been conducted, and the characterization of pompano spawning behavior in a recirculating system should give an indication of whether pompano are receiving the necessary cues to promote normal spawning behavior. In addition, the proportions of males and females producing mature gametes vs. those adults that are found without mature gametes was assessed, and from that analysis, a refined spawning schedule is proposed with the focus on developing a schedule that provides consistent yields of fertilized eggs each season.

Materials and Methods

Study Facility and Housing Conditions

The work reported herein was conducted at the Mote Aquaculture Research Park (MAP) in Sarasota, FL, a field station of the Mote Marine Laboratory. This private marine research organization is dedicated to increasing both marine knowledge and education in the community. Although Mote Marine Laboratory was built in 1955, the Mote Aquaculture Park was established in 2001 and primarily serves as a facility to further the development of sustainable marine and freshwater aquaculture technology.

In 2009, Mote Aquaculture Research Park housed 20-30 adult pompano in each of two cylindrical indoor recirculating tanks (Group1 and Group2). Our study focuses only on Group1 because a more complete data set from Group1 was collected. The tanks are each 4.57m (15 ft) diameter 1.52m (5 ft) deep with a volume of about 28,000L. To maintain tank clarity and water quality there was a 0.085 m^3 drop filter (Aquaculture Systems Technologies, L.L.C, New Orleans, La) for solids separation, a 900-L moving bed for biofiltration, and 2 150-W High Output SMART HO UV® units (Emperor Aquatics, Inc®, Pottstown, PA). The tanks were maintained at a temperature of 28 ± 1.0ºC during the spawning season. Salinity was held at 35 ± 0.5ppt, D.O. at a range of ≥4 and ≤9ppm and pH maintained between the ranges of 7.5 and 8.5. Lighting was controlled in each tank using Solar 1000 series dimmer (BlueLine Aquatics, San Antonio, TX), a system designed to simulate solar and lunar events. These events closely followed natural solar and lunar patterns of the pompano spawning season of February-October. Sex ratios began at 19M:10F during the first sampling and changed to 14M:10F in April.

Experimental Procedures

Beginning February 2009 and continuing until October 2009, the pompano were artificially induced to spawn every other month. Handling was restricted to one day before the spawn. The purpose of this handling was to sample the gametes of the fish over the course of the spawning season. Statistical tests (Fisher's exact tests, $\alpha=0.05$ and unpaired t-tests) were performed to analyze any changes in gamete maturity throughout the season. During fish handling, the water level of the tanks was dropped by 60%, lab technicians corralled fish and one-by-one anesthetized the fish using a separate tank filled with water and tricaine methanesulfonate (MS-222) at a concentration of 200mg/L. Once the fish were anesthetized, weight, length and the presence/absence of mature gametes were assessed. The presence of mature oocytes was assessed using a cannulation biopsy. Oocytes recovered from the biopsy were measured and staged. When oocytes were in vitellogenic or post-vitellogenic stages, at least 450 µm in diameter, and the macronucleus had migrated to one side, the oocytes were considered mature. Females with oocytes that were less than 450 µm in diameter were considered to contain no mature gametes. All mature females were injected with a maturation and ovulation-advancing hormone during each handling period. Females were either injected with human chorionic gonadotropin (HCG), Ovaplant (active ingredient Salmon gonadotropin sGnRHa), or Ovaprim (active ingredients Salmon gonadotropin sGnRHa and Domperidone, a dopamine inhibitor), see below for more detail.

The assessment of males involved determining the amount of milt that could be manually expressed as well as measuring sperm motility using a compound microscope. Percent motility as well as motility duration was recorded when a milt

sample could be obtained. Males were considered to have mature gametes when milt was free-flowing when expressed and when sperm was active for at least 15 sec. Males in which a sample was difficult or impossible to obtain, or whose sperm was not motile under a microscope were categorized as having no mature gametes. Males were injected with Ovaprim twice during the season to increase milt production, as detailed below. After handling, the fish were revived by gill ventilation in the original tank.

Hormone Induction of Spawning

Various hormones were used over the course of the breeding season in an attempt to evaluate the different hormones' effects. During the course of the breeding season, mature females were injected intramuscularly at each handling period with HCG, Ovaplant, or Ovaprim. HCG (1000IU/kg body weight) was injected in mature females February, April, and September. In June, all females were injected with Ovaprim (0.5ml/kg body weight for females with mature oocytes at the time of sampling, 0.25ml/kg body weight for females found without mature oocytes at the time of sampling). In August and October, females were injected with Ovaplant based on size (<1500g body weight, 75ug; >1500g body weight, 150ug). In August and October, all males were injected with 0.5ml/kg body weight Ovaprim.

Behavioral Filming Equipment

Three underwater Neuros OSD (Neuros Technology International, LLC., Chicago, IL) cameras were used to record spawning behavior of the pompano during February and April 2009. The cameras were installed near the bottom of the tanks with a slight upward angle during the handling period. They were mounted on pvc pipe and secured to the tank so water flow or fish interference would not dislodge the position

during filming. In February, data were captured on VHS tapes, but in April filming was recorded directly to digital thumb drives. Three cameras were set to record from 1500-1900 on February 12, 2009, and from 1330-1700 on April 8, 2009.

Behavioral and Statistical Analysis

The tapes were analyzed first by identifying possible spawning rushes. During the tape analysis, the duration of the rush, number of participants, relative vertical location at which the rush took place, whether eggs were found shortly after the rush, and period between individual rushes were recorded. A Mann-Whitney U statistical test (α=0.05) was performed to determine if there was a difference between the rush intervals, rush durations, and number of participants between the February and April spawns. Observed spawning rush behavior was categorized into false spawning behavior (that which did not result in the release of eggs) and true spawning behavior (that which did result in the release of eggs.) Differences between false spawning behavior and true spawning behavior were determined by the measurable aspects of the spawning rushes. During each spawning cycle, both fertilized and unfertilized eggs were collected at the surface of the tank using a skimmer bar and deposited into an external egg basket. It is important to note that only fertilized eggs are buoyant (Main et al. 2007; Weirich and Riley 2007), however due to the efficient aeration of the recirculating tank system, unfertilized eggs were also collected using the skimmer bar. The total number of eggs was counted by volumetric estimates post-spawn, and the percent fertilized eggs calculated. While not all fertilized eggs were able to be collected via the skimmer bar due to the filtration system in the tank, the calculated percent fertilized eggs is the maximum fertilization rate possible. Some egg samples were aged under the microscope to estimate the spawning time that produced viable eggs.

Results

Male and Female Gamete Production

Handling data were recorded from February 2009 through October 2009 to characterize variance in gamete production over the 2009 spawning season (Table 2-1). On the first handling date in February 2009, the spawning group had the largest proportion of males with mature gametes across all sampling dates (Figure 2-2). Ten of 19 males had mature gametes at the time of sampling (52%). By the next handling in April 2009, the proportion of males with mature gametes significantly decreased (Fisher's exact test; $p=0.0191$, $\alpha=0.05$, $df=1$). Only 3 males out of 19 were found with mature gametes at the time of sampling (16%). In June 2009, the proportion of males with mature gametes was the lowest of the entire season, with only 1 male out of 14 total males (7%). The number of males with mature gametes increased slightly at the next handling date, August 2009, to 2 males out of 14 total males (14%). By the next sampling date, the proportion of males with mature gametes increased to the second highest value of the season. September 2009, 5 males of 14 were found with mature gametes (36%). The proportion of males with mature gametes on the last sampling date, October 2009, was a moderate 3 males of 14 total males (21%). Thus the proportion of males with mature gametes in the spawning group was highly variable, with two peaks of male gamete maturity in February and September of the spawning season. Although males were injected with Ovaprim in August and October, there was no measurable difference in the proportion of males found with mature gametes in February-June compared to August-October (unpaired t-test: $t=0.09$, $p=0.9326$, $df=4$, $se=0.153$).

Female gamete maturity and fecundity was also recorded for the 2009 spawning season (Table 2-1). Egg maturity was determined in part by egg diameter (Table 2-3). Similar to the males, the highest proportion of females with mature oocytes was found in February (70% females with mature gametes) and September (60% of females). For all months other than February and September, the proportion of females with mature oocytes was below 50% (April, 30%; June, 40%; August, 40%; October, 20%) (Figure 2-2). Fecundity was also estimated by taking the total number of eggs collected and dividing by the number of females with mature oocytes (Table 2-2). The largest number of eggs collected in one spawning cycle was 324,000 eggs in September, 2009. During this cycle, 6 females were found to have mature oocytes, and thus, the average number of eggs per female was 54,000 eggs. The average number of eggs per female across the 2009 season ranged from 32,500 eggs (August 2009) to 74,666 eggs (April 2009) per female with mature oocytes.

Fertilized Egg Yield Across Spawning Cycles

In addition to gamete maturity and hormone injections, the total number of fertilized eggs collected after each spawning cycle was recorded (Table 2-2). The two peak time periods in fertilized egg collection corresponded to the months of peak male gamete maturity. The largest numbers of fertilized eggs were collected in February (112,128 fertilized eggs, 44% total eggs), April (127,904, 57%), and September (139,639, 43%). From February to April, the number of fertilized eggs collected increased slightly (112,128, 44%; 127,904, 57%), even though the numbers of females with mature oocytes dropped by more than half; there were no fertilized eggs collected in June. In August, only 47,970 (37%) fertilized eggs were collected, leading up to the

largest yield in September (139,639 fertilized eggs, 43%). In the final cycle, collected in October, the number of fertilized eggs was very low at 20,893 fertilized eggs (29%).

False Spawning Behavior

Prior to gamete release, the majority of the school swam clockwise against the current. However, rushes, where two to five fish broke out of the school and chased, swam against the current, and stopped mid-swim occurred frequently. False spawning rushes often included a sudden speeding up of a few fish to position themselves with their flanks touching. The fish remained closely positioned for a few seconds, and then the group broke up and the individual fish swam away. The closely swimming group swam either up or down in the water column and often swam in the opposite direction to the rest of the school. Their erratic swimming could be easily seen by flank flashing. None of these rushes included vibrations, often associated with egg release, in any participants.

False spawning rushes were captured April 2009 when recording began several hours prior to the first collection of eggs at 13:30 (Figure 2-1). These false spawning rushes began when one fish paused in the water column with a group of other individuals closely surrounding it, and ended when it began swimming again. During the false spawning rushes, those individuals not directly involved either ignored the rush and kept swimming or followed the false spawning group in the direction of the current. By 14:42, the school still swam against the current, but more often they either stopped swimming or swam in the other direction. The school looked like a disorganized group and individuals milling about in any direction.

The false spawning rushes in April 2009 began at 14:42 and continued until about 14:57. There were at least 6 false spawning rushes that lasted on average 3.6 sec

(SD=1.397 sec) with an average of 3.4 participants (SD=0.976 sec). The average time interval between false spawning rushes was 2 min 59 sec (SD=2.018 min). These events were undoubtedly false spawning rushes because there were still no eggs found at 15:08. The true spawning rushes in April 2009, characterized by the female vibrating during release of gametes, began at 16:07 and lasted until 16:37. No false spawning rushes were observed in February 2009 (Figure 2-1).

True Spawning Behavior

A characteristic true spawning rush involved five or more individuals, with one female in the center of a close group of 4-10 other fish, pausing in the water column. The female then vibrated her whole body for 5-12 sec as she released eggs into the water column. This extended period of female vibration was the most obvious difference between true spawning and false spawning rushes. The group of fish surrounded her and swam slightly behind her while remaining in close contact with her, and the males presumably released their sperm as she released eggs (a milt cloud was difficult to see due to the aeration of the tank). The whole group was either stationary in the water column or swimming very slowly. All true and false spawning rushes occurred between the midlevel and the upper half of the tank (≥0.76m from the tank floor).

The focal breeding group was injected between 10:15 and 12:00 on February 11, 2009, and eggs were found approximately 30 hours later, at 17:06 on February 12, 2009, in the 2-4 egg cell stages (eggs in this stage of cell division are 0-15 min post fertilization). Fourteen true spawning rushes were observed between the time frame of 16:41 and 17:32 (Figure 2-1) despite the fact that only 8 females were found with mature oocytes during handling on February 11, 2009. The average number of participants in each true spawning rush was 8.0 individuals (SD=2.5 fish), and the

average duration of the true spawning rushes was 7.7 sec (SD=2.79 sec). The average time period between true spawning rushes was 3 min 54 sec (SD=2.70 min). From this spawn cycle, a volumetric estimate of 112,128 fertilized eggs was found with an estimated maximum fertilization rate of 44%.

When the focal breeding group was next induced to spawn on April 8, 2009, the fish were injected between 10:15 and 12:00 on April 7, 2009. Eggs were found on April 8, 2009, approximately 30 hours later, at 16:30. Three cameras were set to record from 13:30-17:00 (Figure 2-1). Setting the camera to record earlier provided the opportunity to view false spawning behavior. Beginning 16:07 and lasting until 16:37, while only 4 females were found with mature oocytes during handling on April 7, 2009, there were 8 recorded true spawning rushes that lasted on average 6.3 sec (SD=1.83 sec) with an average of 5.5 participants (SD=1.5 fish). The average time interval between true spawning rushes was 4 min 53 sec (SD=2.49 min). From this spawn cycle, an estimate of 127,904 fertilized eggs was collected with an estimated maximum fertilization rate of 57%.

Comparisons Among True Spawning Rushes.

Mann-Whitney U tests were conducted to compare the February and April spawn cycles. No differences were found between the true spawning rush intervals (Mann-Whitney U test: $U=38$, $p=0.336$, $\alpha=0.05$, $n_1=8$, $n_2=13$) or the true spawning rush durations (Mann-Whitney U test: $U=91.5$, $p=0.072$, $\alpha=0.05$, $n_1=9$, $n_2=14$). However, significantly more fish participated in the true spawning rushes in February than in April (February mean participants=8, April mean participants=5.5; Mann-Whitney U test: $U=102$, $p=0.0064$, $\alpha=0.05$, $n_1=9$, $n_2=14$) which correlates to the larger proportion of adults found with mature gametes, and in particular males with mature gametes, in

February relative to April. On average, spawns included nearly as many males as were sperm producing, suggesting either that males with mature gametes spawned repeatedly or that males without mature gametes were participating in spawning rushes.

Discussion

Natural Spawning Behavior

From this study, we can conclude that natural pompano spawning behavior is not inhibited by closed-system aquaculture. Clear distinctions between false spawning rushes and true spawning rushes were quantified in the video analysis, although no false spawning rushes were recorded for the February spawning event (Figure 2-1). This may have been due to the later start time for recording spawning behavior at 15:00 in February and false spawning rushes were recorded in April at 14:42. The number of participants for each spawning rush, was as expected for group spawning carangids (Graham and Castellanos 2005). Natural observations of spawning groups of a close relative to pompano, the permit, have been observed in which 5 to 9 individuals broke off from the main group and swam toward the surface of the water, stopped about 15m from the surface, and vibrated while releasing a puff of gametes (Graham and Castellanos, 2005). While the average number of participants was different for the February and April spawns, this was not surprising given the differing numbers of males with mature gametes. Also, the group size and sex ratio used in our study appeared to be acceptable for natural pompano spawning behavior.

Interestingly, there is strong evidence that females are participating in multiple spawning rushes during each spawning cycle. In the February spawning cycle, 14 individual spawning events were observed, and out of 10 females in the group, only 7 were found with mature oocytes. Likewise, in the April spawning cycle, 8 spawning

rushes were observed and only 3 females with mature oocytes were identified. Thus it would appear that on average 2 spawning rushes can be expected per fertile female. In addition, the number of participants in each spawn also leads to the conclusion that males or females that are not producing mature gametes are participating in the spawning rushes. For instance, in April, only 3 males and 3 females were producing mature gametes, but the spawning rushes consisted of 4-8 individuals. From this information, it is possible that males with mature gametes are participating in multiple spawning rushes, or that adults without mature gametes are also participating in spawning behavior.

Limited Gamete Quantity

An obvious problem to consider further is the low percentage of individuals with mature gametes. The percentage of males with mature gametes dropped significantly over the 2009 spawning season. Low female fecundity of 0-74,600 eggs/female is also a major consideration in our study. This fecundity is extremely low compared to early natural estimates of 630,000 eggs (Finucane 1969), more conservative recent estimations of 133,400- 205,500 eggs (Muller et al. 2002), and even captive stock estimates of 123,000-772,000 eggs (Weirich and Riley 2007). The cause of this drop of individuals with mature gametes could be the frequency of injection and spawning throughout the season. From natural observations of juveniles, pompano appear to have an extended spawning season that stretches from April to October, with a main spawning peak found in spring (April-June) and another spawning peak in late summer or early fall (July-October) (Finucane 1969). These peaks have been identified based on the documented size of juveniles caught within that time period (Finucane 1969). A supporting study reports young-of-year (YOY) juvenile pompano were collected off the

coast of Florida between April and November, with the majority of YOY collected in May (Solomon and Tremain 2009). In addition, as many as four size categories of juveniles have been collected at one time (Finucane 1969), indicating that pompano spawn over a protracted period and may spawn multiple times during each spawning season. Therefore, it is entirely possible that while natural populations are able to spawn the entire season (April to October), with peaks of spawning in spring and late summer/early fall, that individual fish are spawning less frequently and with some recovery period between spawning activity.

Applying this hypothesis to the aquaculture setting, it may be more effective to induce spawning once or serially in quick succession within the natural spawning peaks, but with an extended resting period between peaks. For instance, instead of inducing pompano to spawn every 60 days from February through October, an experiment where pompano are induced to spawn once a month in March, April, and May, are permitted to recover June and July, and then induced to spawn once a month in August, September, and October may yield higher total eggs as well as a higher fertilization success rate. By scheduling serial spawns within the two natural spawning peaks and a 90-day recovery period between the two peaks, both males and females may produce a larger quantity of sperm and eggs available to be induced with hormone implants. There is evidence that even with serial spawning in a shortened period of time, fecundity, fertilization rates, and spawning activity are not severely decreased when fish are induced with GnRHa. By GnRHa implantation, pompano were induced to spawn six times from July-October, and each spawning cycle lasted 2-6 days with average yields

of 150,000 to 772,000 eggs/female and average percent fertilization (floating eggs) of 81.8 to 96.9% (Weirich and Riley 2007.)

Sperm quantity could also be a limiting factor for egg fertilization when fish are repeatedly induced to spawn over a protracted season. This sperm limitation hypothesis is supported by the dual peaks of males gamete maturity found in our study. The dual peaks of males with mature gametes found in February and September are slightly further apart than the supporting data reflects, but both the data in our study and natural observations indicate that the first spawning cycle of the year is the largest (Finucane 1969). Hence the maximum proportion of males with mature gametes found in February as the first spawn of the season corresponds to the natural first spawn in April. In September, the number of males with mature gametes again spiked, correlating to the natural second spike recorded by Finucane (1969). Between these two peaks, the number of males with mature gametes drops to such low proportions that the sperm produced is insufficient to give an appreciable yield of fertilized eggs.

Another possible explanation for poor gamete maturation in both males and females could be nitrate concentrations in the broodstock tanks. Recirculating aquaculture systems often have a higher nitrate level than natural water systems, and it has been show that elevated levels of nitrate (57 ± 1.52 mg/L) can disrupt endocrine function in female Siberian sturgeon (*Acipenser baeri*) (Hamlin et al. 2008). Measured NO_3 levels in the pompano tanks from August-December 2009 averaged from 55.83 mg/L (Nov. 2, 2009) to 103.64 mg/L (Aug. 13, 2009) with a median of 84.62 mg/L. These high nitrate levels could have contributed not only to the low female fecundity but also to the low proportion of males with mature gametes. Nitrate concentrations are

now being measured and adjusted more frequently to prevent high levels of nitrate in the system.

Hormone treatments have been used to increase gamete production over a spawning season. GnRHa and HCG implants have been shown to increase spermiation in fish such as the European Sea Bass (*Dicentrarchus labrax)* and Japanese eels (*Anguilla japonica)* as well as in a variety of freshwater and marine fish (Rainis et al. 2003; Dou et al. 2007). In Pompano, the use of a GnRHa pellet produces greater egg production, higher fertilization rates, and a spawning period of up to 6 days when compared to HCG (Weirich and Riley 2007). While GnRHa compounds (Ovaplant and Ovaprim) were used in our study, on both males and females, use was limited and there were no indications of increased spermiation or fecundity during the time which it was used. The most likely reason that the proportion of fish with mature gametes did not increase after the treatments is because the product used was not a slow-releasing hormone, and it is sustained release of GnRHa over a period of days to weeks in male fish that results in the most consistent elevation of spermiation (Zohar and Mylonas 2001). To increase gamete maturation, a GnRHa product that is slow-release, such as a pellet, microsphere, or monolithic implant (Zohar and Mylonas 2001), and that is applied consistently over the spawning season should be attempted.

Improving Fertilization Rates

While fertilization rates of field spawning data for pompano and related jacks are unavailable, some studies have focused on another species of group spawners, e.g., the blueheaded wrasse (*Thalassoma bifasciatum*). The mean fertilization success (defined as percent of total eggs that were successfully fertilized) for 304 field collected spawns of *T. bifasciatum* was 76.5% (Petersen et al. 1992). In a captive pompano

spawning event in which one female was induced to spawn with four males, was taken out of the tank, and another female was induced to spawn with the same four males, an estimated total egg yield of 125,000 for each female was collected, with a fertilization rate of 52% (Kloth 1980). More recently, a study by Weirich and Riley (2007) revealed high female fecundity (on average 234,000 eggs per female) and high fertilization rates of over 70% when pompano were injected with a GnRHa slow-release pellet and allowed to spawn in a recirculating system. Maximum fertilization rates in our study were less than 50% in most spawning cycles. One factor that may contribute to low fertilization success is that in our study, is that we considered eggs ≥450 μm in diameter mature. Studies with higher fertilization success categorize eggs ≥500 μm in diameter as mature, and thus only induce spawning in those females with eggs ≥500 μm in diameter (Weirich and Riley 2007). While egg diameter is less reliable than macronucleus placement in the determination of egg maturity, some of the eggs released by our females may not be sufficiently mature to be fertilized.

Another issue that could be hindering fertilization success is the flow rate in the tank. Analysis of natural spawns of *T. bifasciatum* revealed that fertilization success (percent eggs fertilized) is higher in calmer water currents than in rough, turbulent currents (Petersen et al. 1992). The flow rate of the recirculating system may be a problem especially with smaller egg sizes. In sea urchins, larger eggs were found to have significantly higher fertilization rates than smaller eggs (Levitan 1993), primarily because they are easier to find by sperm in a turbulent or fast-moving environment. Recirculating systems may have low fertilization success due to the strong current in the tank which potentially disperses sperm and eggs too quickly. Especially with the

smaller eggs induced in our study, if the flow of the tank was disruptive enough, it could prevent the sperm from fertilizing the eggs in the brief window of the spawning event. To test the effects of water flow on fertilization rates, pompano should be induced to spawn in the tanks with the aeration turned off, and the fertilization rates should be compared to those achieved when fish are spawned with normal tank aeration and pump systems.

Conclusion

The factors that hamper the successful and consistent spawning of pompano in recirculating aquaculture systems are still largely unknown. However, from our study, it is clear that the limiting factors of space and external cues in the recirculating system are not hindering natural group spawning behavior in the pompano. In fact, strong evidence for distinctions between false spawning and true spawning behavior have been found, as well as evidence for multiple spawning in both sexes. While pompano exhibit normal spawning behavior in a recirculating aquaculture system, further study must explore the factors of inconsistent gamete production and fertilization rates found in this species. One method that may improve both these factors is inducing serial spawns within the two natural peaks in the spawning season. In addition, further testing with GnRHa hormones should be done to improve gamete production. Finally, the effects of water movement during spawning should be examined.

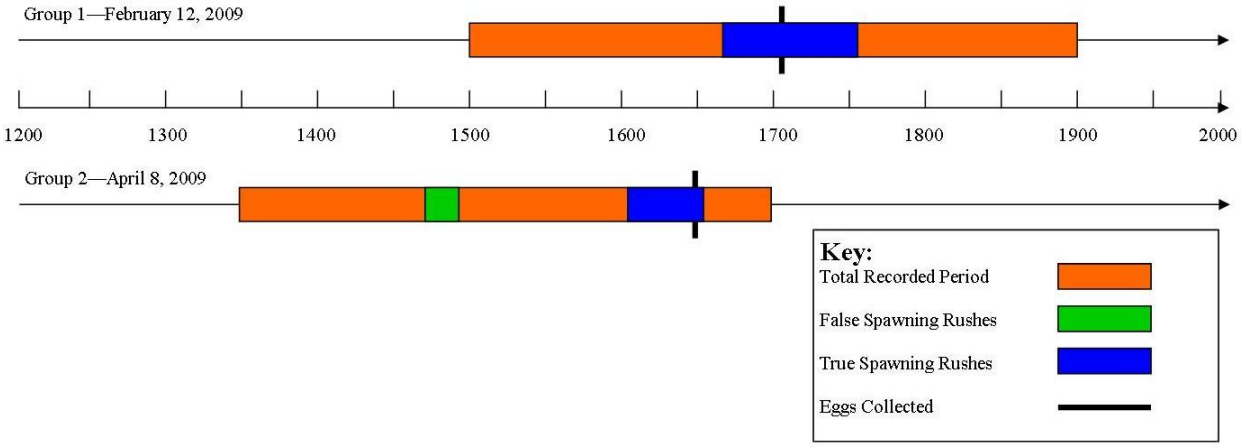

Figure 2-1. Spawning behavior video recording timeline

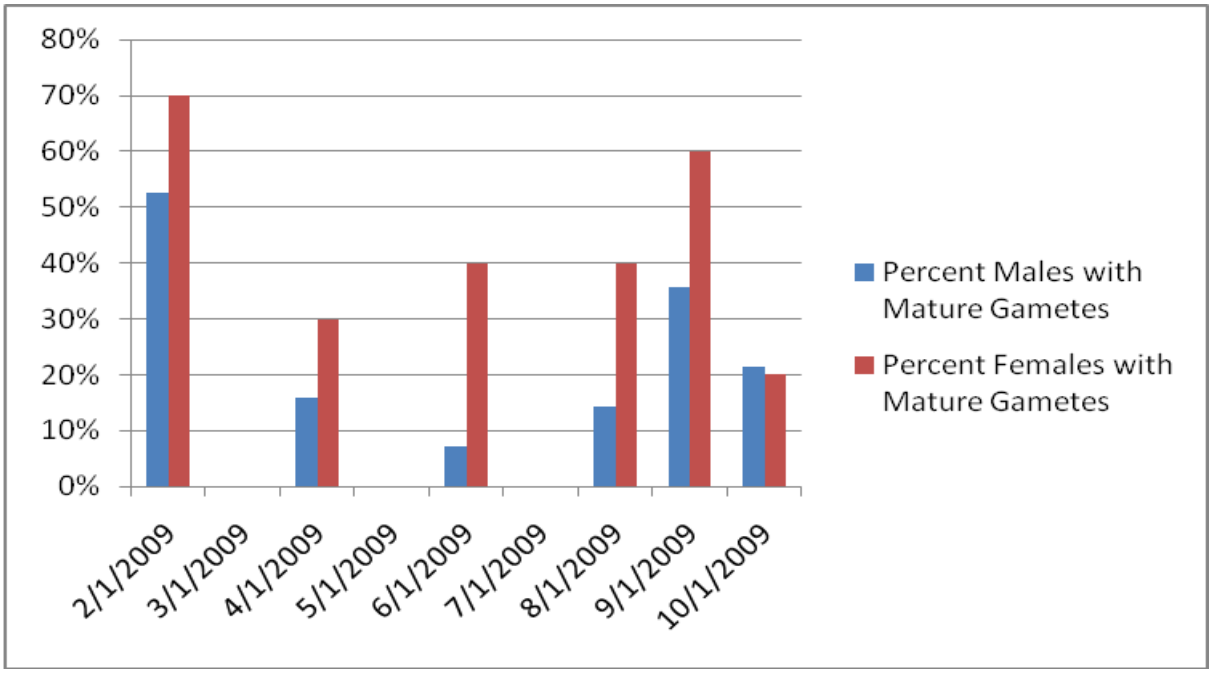

Figure 2-2. Percent individuals with mature gametes

Table 2-1. Proportion males and females with mature oocytes spawning season

	Ripe Males	% Males with Mature Gametes	Ripe Females	% Females with Mature Gametes	Sex Ratio	Injection
2/11/2009	10	52.6%	7	70.0%	19M:10F	HCG F
4/7/2009	3	15.8%	3	30.0%	19M:10F	HCG F
6/12/2009	1	7.1%	4	40.0%	14M:10F	Ovaprim F
8/11/2009	2	14.3%	4	40.0%	14M:10F	Ovaplant F, Ovaprim M
9/14/2009	5	35.7%	6	60.0%	14M:10F	HCG-F
10/13/2009	3	21.4%	2	20.0%	14M:10F	Ovaplant F, Ovaprim M

Table 2-2. Fertilized egg yield of 2009 spawning season

Spawning Date	Total Number of Eggs	Fertilized Eggs Mature Female	Fertilization Rate
2/11/2009	256,000	36,571	43.80%
4/7/2009	224,000	74,666	57.10%
6/12/2009	no eggs	none	no eggs
8/11/2009	130,000	32,500	36.90%
9/14/2009	323,988	53,998	43.10%
10/13/2009	72,800	36,400	28.70%

Table 2-3. Egg diameter and female measurements

Sampling Date	2/11/2009			4/7/2009			6/9/2009		
Female	Weight (g)	Length (cm)	Egg Diameter (µm)	Weight (g)	Length (cm)	Egg Diameter (µm)	Weight (g)	Length (cm)	Egg Diameter (µm)
1	1020	34.5	424-530	1115	35.1	424-530	1240	37.0	239-318
2	745	31.5	318-398	845	32.5	No Sample	995	33.9	398-477
3	2000	45.6	371-477	2195	45.9	504-557	2280	46.1	450-530
4	850	31.6	504-557	925	32.2	318-398	1070	33.8	292-371
5	975	34.2	504-583	1005	34.4	398-504	1035	34.9	504-557
6	670	31.3	398-477	755	32.5	80-133	860	33.0	424-504
7	830	33.2	80-133	920	34.2	No Sample	1070	35.3	80-133
8	900	34.1	424-530	1040	35.4	80-133	1215	37.1	80-133
9	705	31.5	398-504	755	32.1	80-133	770	32.2	185-265
10	685	30.7	53-80	780	31.8	80-133	860	32.8	106-159
	8/11/2009			9/14/2009			10/13/2009		
1	1510	39.5	53-80	1700	40.1	477-557	1755	40.8	159-212
2	955	34.2	477-530	1365	37.2	504-583	1430	38.2	80-133
3	2515	47.0	530-583	2535	47.2	530-610	2520	47.4	133-212
4	1300	36.3	80-133	1445	37.0	318-398	1560	37.0	371-424
5	1160	36.3	504-557	1195	36.5	239-292	1240	36.9	345-398
6	985	34.1	477-530	1000	34.2	504-610	1055	32.0	504-583
7	1270	37.4	80-133	1350	37.8	159-292	1460	38.6	212-292
8	1515	39.7	80-133	1620	40.0	318-371	1745	41.1	239-318
9	860	33.5	53-80	895	33.5	371-451	970	35.0	106-159
10	1075	35.0	106-159	1190	35.7	424-504	1280	36.5	504-583

LIST OF REFERENCES

Alongi, D.M., Chong, V.C., Dixon, P., Sasekumar, A., and F. Tirendi. 2003. The influence of fish cage aquaculture on pelagic carbon flow and water chemistry in tidally dominated mangrove estuaries of peninsular Malaysia. Marine Environmental Research, 55:313-333.

Bell, J.D., Leber, K.M., Blankenship, H.L., Loneragan, N.R., and R. Masuda. 2008. A new era for restocking, stock enhancement, and sea ranching of coastal fisheries resources. Fisheries Science, 16:1-9.

Berglund, A. 1994. The operational sex ratio influences choosiness in a pipefish. Behavioral Ecology, 5: 254-258.

Bourque, B. D., and R. P. Phelps. 2007. Induced spawning and egg quality evaluation of Red Snapper, *Lutjanus campechanus*. World Aquaculture Society, 38:2.

Bromage, N., Randall, C., Duston, J., Thrush, M., and J. Jones. 1993. Environmental control of reproduction in salmonids. In: Muir, J., Roberts, R. (Eds.), Recent Advances in Aquaculture, vol. IV. Blackwell Science, Oxford, pp. 55–66.

Brown, R.C., Woolliams, J.A., and B.J. McAndrew. 2006. Factors influencing effective population size in commercial populations of gilthead seabream, *Sparus aurata*. Aquaculture, 247:219-225.

Campos-Mendoza, A. McAndrew, B.J., Coward, K., and N. Bromage. 2004. Reproductive response of Nile tilapia (*Oreochromis niloticus*) to photoperiodic manipulation; effects on spawning periodicity, fecundity, and egg size. Aquaculture, 231:299-314.

Cejko, B.I., Glogowski, J., Kowalski, R.K., Kucharczyk, D., and K. Targonska. 2008. Description of Pikeperch, *Sander Lucioperca* (L.), semen obtained from males held under different rearing conditions. Polish Fisheries, 16:93-100.

Coleman, F.C., Koenig, C.C., and L.A. Collins. 1996. Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environmental Biology of Fishes, 47:129-141.

Colin, P.L. 2010. Aggregation and spawning of the humphead wrasse *Cheilinus undulates* (Pisces: Labridae): general aspects of spawning behavior. Fish Biology, 76:987-1007.

Donaldson, E.M, and G.A. Hunter. 1983. Induced final maturation, ovulation, and spermiation in cultured fish. In: Hoar, W.S, Randall, D.J, and E.M. Donaldson (Eds), Fish Physiology. Academic Press, New York, USA, pp.351-403.

D'Orbcastel, E. R., Blancheton, J.P., and J. Aubin. 2009. Towards environmentally sustainable aquaculture: Comparison between two trout farming systems using Life Cycle Assessment. Aquaculture Engineering, 40:113-119.

Dou, S.Z, Yamada, Y, Okamura, A, Tanaka, S, Shinoda, A, and K. Tsukamoto. 2007. Observations on the spawning behavior of artificially matured Japanese eels Anguilla japonica in captivity. 2007. Aquaculture, 266:117-129.

FAO (Food and Agriculture Organization). 2006. The State of World Fisheries and Aquaculture 2000. FAO, Rome, Italy.

Finucane, J.H. 1969. Ecology of the Pompano (*Trachinotus* carolinus) and the Permit (*T.* falcatus) in Florida. Trans. Amer. Fish. Soc. 3:478-486.

Forsberg, L. 2008. Genetic aspects of sexual selection and mate choice in Salmonids. Acta Universitatis Upsaliensis. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 436. Sodertorn Doctoral Dissertations 30. 67 pp. Uppsala.

FWRI (Fish and Wildlife Research Institute of the Florida Fish and Wildlife Commission). 2008. Florida's Inshore and Nearshore Species: 2008 Status and Trends Report.

Graham, R. T., and D. W. Castellanos. 2005. Courtship and spawning behaviors of carangid species in Belize. Fishery Bulletin, 103:426-432.

Hamlin, H.J., Moore, B.C., Edwards, T.M., Larkin, I.L.V., Boggs, A., High, W.J., Main, K.L., and L.J. Guillette Jr. 2008. Nitrate-induced elevations in circulating sex steroid concentrations in female Siberian sturgeon (*Acipenser baeri*) in commercial aquaculture. Aquaculture, 281:118-125.

Hoff, F.H., Rowell, C., and T. Pulver. 1972. Artificially induced spawning of the Florida pompano under controlled conditions. World Mariculture Society, 3:53-64.

Hoff, F.H., Pulver, T., and J. Mountain. 1978a. Conditioning Florida pompano, *Trachinotus carolinus*, for continuous spawning. World Mariculture Society, 9:299-309.

Hoff, F.H., Mountain, J., Frakes, T., and K. Halscott. 1978b. Spawning, oocyte development and larval rearing of the Florida pompano, *Trachinotus carolinus*. World Mariculture Society, 9:279-297.

Holmer, M., Perez, M., and C.M. Duarte. 2003. Benthic primary producers – a neglected environmental problem in Mediterranean maricultures? Marine Pollution, 46:1372-1376.

Holt., G.J., Faulk, C.K., and M.H. Schwartz. 2007. A review of the larviculture of cobia *Rachycentron canadum*, a warm water marine fish. Aquaculture, 268:181-187.

Ibarra-Castro, L., and N. J. Duncan. 2007. GnRHa-induced spawning of wild-caught spotted rose snapper *Lutjanus guttatus*. Aquaculture, 272:737-746.

Islam, M.S. 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. Marine Pollution, 50:48-61.

Kloth, T.C. 1980. Observations on the spawning behavior of captive Florida Pompano, *Trachinotus carolinus.* Copeia, 4:884-886.

Kucharczyk, D., Targonska, K., Hliwa, P., Gomulka, P., Kwaitkowski, M., Drejszeff, S., and J. Perkowski. 2008. Reproductive parameters of common carp (*Cyprinus carpio* L) spawners during natural season and out-of-season spawning. Reproductive Biology, 8:285-289.

Kupren, K., et al. 2008. Economic aspects of rearing larval Asp, *Aspius Aspius* (L.), and Ide, *Leuciscus Idus* (L.), in closed recirculating systems. Polish Fisheries, 16:413-429.

Lee, C.S., and A.C. Ostrowski. 2001. Current status of marine finfish larviculture in the United States. Aquacultur*e*, 200:89-109.

Levitan, D.R. 1993. The importance of sperm limitation to the evolution of egg size in marine invertebrates. The American Naturalist, 141:517-536.

Losordo, T.M, Masser, M.P, and R. Rakocy. 1998. Recirculating aquaculture tank production systems: an overview of critical considerations. Southern Regional Aquaculture Center, No. 451.

Main, K.L, Rhody, N., Nystrom, M, and M. Resley. 2007. Species Profile – Florida Pompano*.* Southern Regional Aquaculture Center*,* No. 7206.

Masser, M.P, Rakocy, J, and T.M. Losordo. 1992. Recirculating aquaculture tank production systems: management of recirculating systems. Southern Regional Aquaculture Center, No. 462.

de Mitcheson, Y.S., Cornish, A., Domeier, M., Colins, P.L., Russell, M., and K.C. Lindeman. 2008. A global baseline for spawning aggregations of reef fishes. Conservation Biology, 22:1233-1244.

Muller, R.G., Tisdel, K., and M.D. Murphy. 2002. The 2002 update of the stock assessment of Florida Pompano (*Trachinotus carolinus)*. Florida Fish and Wildlife Conservation Commission, Florida Marine Research Institute, St. Petersbury, FL. p.1-45.

Mylonas, C.C, Papandroulakis, N, Smboukis, A, Papadaki, M, and P. Divanach. 2004. Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. Aquaculture, 237:141-154.

Penney, R.W., Lush, P.L., Wade, A.J., Brown, J.A., and M.P.M. Burton. 2006. Effect of photoperiod manipulation on broodstock spawning, fertilization success, and egg developmental abnormalities in Atlantic Cod, *Gadus morhua*. World Aquaculture Society, 37: 273-281.

Petersen, C.W, Warner, R.R, Cohen, S, Hess, H.C, and A.T. Sewell. 1992. Variable pelagic fertilization success: implications for mate choice and spatial patterns of mating. Ecology, 73:391-401.

Pfeiffer, T., and P. Wills. 2007. Prototype recirculating aquaculture system design for juvenile red drum production as part of the Florida Fish and Wildlife Conservation sommissions Hatchery Network Initiative [abstract]. International Sustainable Marine Fish Culture Conference and Workshop Book of Abstracts, p. 26.

Rainis, S, Mylonas, C.C, Kyriakou, Y, and P. Divanach. 2003. Enhancement of spermiation in European sea bass (*Dicentrarchus labrax*) at the end of the reproductive season using GnRHa implants. Aquaculture, 219:873-890.

Resley, M.J., Nystrom, M.J., and K.L. Main. 2007. Comparison of essential fatty acid profiles in captive and wild Florida pompano *Trachinotus carolinus* eggs. Abstract – Aquaculture 2007, San Antonio, Texas.

Roberts, Jr. D.E., Harpster, B.V., and G.E. Henderson. 1978. Conditioning and induced spawning of the Red Drum (*Sciaenops ocellata*) under varied conditions of photoperiod and temperature. World Mariculture Society*,* 9:311-332.

Rosenberg, A.A., Swasey, J.H., and M. Bowman. 2006. Rebuilding US fisheries: progress and problems. Frontiers Ecol Environ, 4: 303-308.

Shiraishi, T., Ohta, K., Yamaguchi, A., Yoda, M., Chuda, H., and M. Matsuyama. 2005. Reproductive parameters of the chub mackerel *Scomber japonicas* estimated from human chorionic gonadotropin-induced final oocyte maturation and ovulation in captivity. Fisheries Science*,* 71:531-542.

Shiraishi, T., Ketkar, S.D., Kitano, H., Nyuji, M., Yamaguchi, A., and M. Matsuyama. 2008. Time course of final oocyte maturation and ovulation in chub mackerel *Scomber japonicas* induced by HCG and GnRHa. Fisheries Science*,* 74:764-769.

Solomon, J.J. and D.M. Tremain. 2009. Recruitment timing and spatial patterns of estuarine use by young-of-the-year Florida pompano, *Trachinotus carolinus*, in northeastern Florida. Marine Science, 85:133-148.

Van der Meeren, T. and V. Ivannikov. 2006. Seasonal shift in spawning of Atlantic cod (*Gadus morhua* L.) by photoperiod manipulation: egg quality in relation to temperature and intensive larval rearing. Aquaculture Research, 37: 898-913.

Watson, R. and D. Pauly. 2001. Systematic distortions in world fisheries catch trends. Nature, 414: 534-536.

Wedekind, C., Rudolfsen, G., Jacob, A., Urbach, D., and R. Muller. 2007. The genetic consequences of hatchery-induced sperm competition in a salmonid. Biological Conservation, 137:180-188.

Weirich, C., and K.L. Riley. 2007. Volitional spawning of Florida Pompano, *Trachinotus carolinus*, induced via administration of gonadotropin releasing hormone analogue. Applied Aquaculture, 19:47-60.

Weirich, C., Riley, K., and M. Davis. 2005. Florida pompano: Induced reproduction via pelleted GnRHa and preliminary observations regarding larval production. Global Aquaculture Advocate, 8:75-77.

Zohar, Y., and C. C. Mylonas. 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. Aquaculture, 197:99-136.

BIOGRAPHICAL SKETCH

Elizabeth Ann Dippon was born in Gainesville, FL in 1986. When she was three, she and her parents moved to Oregon for her father to pursue a career with the Bureau of Land Management. Elizabeth was raised in Portland and has loved animals and music from a very young age. She has enjoyed playing the piano since age 6 and learned the marimba in high school. Elizabeth enjoyed much success with music during high school, earning three 2nd Place Awards at the State Music Festival for Marimba. She graduated as Valedictorian from Westview High School in 2004. She earned a B.A. in music performance as well as a B.S. in zoology from the University of Florida in 2008. Elizabeth pursued an M.S. in zoology in 2010 from the University of Florida.

Elizabeth was married to Jesse J. Reynolds in May 2008. Elizabeth and her husband Jesse are very active in health and fitness. They own a youth performance center and personal training studio, Accel Sports, Inc. in Jacksonville, FL as well as a nutritional business, Advocare. Elizabeth loves helping her husband coach a nationally-ranked Olympic Weightlifting team and helping people achieve their physical and financial goals. Elizabeth is committed to helping others in health and personal development and attributes all her accomplishment to her Lord and Savior Jesus Christ. She is also especially thankful for all the support from her family, her husband, her friends, and Henry.