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The Effects of Stocking Density and Feeding Frequency on Aggressive and Cannibalistic Behaviors in Larval Hatchery-Reared Spotted Seatrout, *Cynosion nebulosus*

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The University of Southern Mississippi

THE EFFECTS OF STOCKING DENSITY AND FEEDING FREQUENCY ON AGGRESSIVE AND CANNIBALISTIC BEHAVIORS IN LARVAL HATCHERY-REARED SPOTTED SEATROUT, *CYNOSCION NEBULOSUS*

by

Christopher Burton Manley

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ABSTRACT

THE EFFECTS OF STOCKING DENSITY AND FEEDING FREQUENCY ON AGGRESSIVE AND CANNIBALISTIC BEHAVIORS IN LARVAL HATCHERY-REARED SPOTTED SEATROUT, *CYNOSCION NEBULOSUS*

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The Spotted Seatrout (*Cynoscion nebulosus*) is a euryhaline fish that inhabits coastal regions from Massachusetts to Texas. Due to its predaceous nature and proximity to coastal shorelines it is the most important marine finfish in the Gulf of Mexico. Because of this importance, Spotted Seatrout has been identified as a possible candidate for stock enhancement. Stock enhancement is the practice of releasing hatchery-reared juveniles to increase local recruitment beyond existing levels. However, raising enough Spotted Seatrout in an intensive aquaculture system has proven difficult due to aggression and cannibalism during the larval stages.

The manipulation of stocking density as well as feeding frequency might be used to reduce aggression and cannibalism. The goal of this study was to determine the effects of stocking density and feeding frequency on aggressive and cannibalistic behaviors in larval hatchery-reared Spotted Seatrout at both small- and production- scales. For the first two small-scale experiments, fish larvae were filmed and behaviors were quantified. Three different aggressive behaviors were quantified: nip (aggressor strikes prey causing prey to dart), chase (aggressor moves more than one body length toward prey), and capture (predator captures and holds prey but unable to consume). From these experiments, the author found that in all stocking densities (15, 30, and 60 fish L^{-1}) there was a significant increase in aggression and cannibalism with time since feeding; and the author identified a density threshold of 30 fish L^{-1}, beyond which the intensity of aggressive behaviors did not increase. These results implied that aggression in early stage hatchery-reared Spotted Seatrout might be alleviated by increasing feeding frequency, and furthermore, the Spotted Seatrout possibly could be cultured at densities higher than the current protocol allows.

From the second experiment, the author concluded that the 2 h feeding frequency treatment elicited fewer aggressive and cannibalistic acts than the 1 h, 4 h, and 8 h treatments. Lower levels of aggression and cannibalism associated with the 2 h feeding frequency treatment could be attributed to a balance between the optimal amount of live feed and the gut evacuation rate of larval Spotted Seatrout. In the third large-scale production experiment, I found a significantly lower per capita mortality and correspondingly higher specific growth rate for the 30 fish L^{-1} treatment than for the lower density 15 fish L^{-1} treatment. This difference resulted in a 45% higher production yield within the high density treatment.

Due to logistical constraints, only two replicates were available for the production experiment. Thus, the statistical power of this experiment was low; however, directional trends consistently suggested biological importance. Feeding every 2 h suggested an apparent decrease in per capita mortality, leading to a 15% increase in production. The observed higher specific growth in the high density treatment likely reflected a decrease in aggression. Also, size heterogeneity did not differ between density treatments, reinforcing the case that using a higher stocking density and a 2 h feeding frequency schedule should be ideal for the production of larval Spotted Seatrout. Overall, the results from the production-scale run were consistent with the results of the two earlier small-scale experiments, suggesting that a stocking density of 30 fish L^{-1} and a feeding frequency of 2 h should be effective for reducing aggression and cannibalism in the culture of Spotted Seatrout.

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CHAPTER I

GENERAL INTRODUCTION

Need for Aquaculture

The human population is predicted to rise to 9.2 billion people by 2050, and this increase will create a substantially increased demand for food and recreation (FAO, 2010). Fish provides a major subsistence food source for humans as well as a source of recreation. Currently, per capita consumption of seafood is approximately 17 kg per year, creating $98.4 billion in revenue (FAO, 2010). The recreational fishery in the Gulf of Mexico region alone produces an economic impact of almost $10 billion annually (FAO, 2010). A continuously growing world population will lead to a subsequent growth in demand for fish. In addition, accumulating evidence for the nutritional benefits of fish is increasing consumption rates in areas of the world not historically known for fish consumption. When combined with the multi-billion dollar recreational fishing industry, enormous pressure will be placed on fisheries resources in the future. Continued dependence on wild fisheries is not feasible because wild fishery production has remained stable since the 1980s and is not expected to increase substantially (FAO, 2010). Currently, almost 90% of known fisheries resources are over-exploited, fully-exploited, depleted, or recovering (FAO, 2010). Thus, comprehensive strategies for preserving and rebuilding fish stocks as well as meeting the increased demand for fisheries products must be pursued.

Aquaculture and Stock Enhancement

Aquaculture is the practice of raising aquatic organisms such as fish, crustaceans, mollusks, and algae in a man-made system. Aquaculture now provides approximately 28% of the global supply of fish and is filling the gap between the supply of wild fisheries products and global demand, currently making it the fastest growing animal-producing industry in the world, with an average growth of 8.3% per year (FAO, 2010). In 2008, aquaculture produced 52.5 million tonnes of product with a value of $98.4 billion (FAO, 2010). This rapid growth has supported an increase in the per capita consumption of raised aquatic organisms from 0.7 kg per capita per year in the 1970s to 7.8 kg per capita per year in 2008 and helped relieve the pressure placed on capture fisheries (FAO, 2010).

Aquaculture also can help enhance natural fishery stocks by stock enhancement. Stock enhancement is the process of releasing cultured fish into the wild, often with the goal of increasing recruitment (Lorenzen et al., 2010). Stock enhancement has been attempted many times in history, but few efforts have successfully benefitted natural populations or increased fishery yields. Efforts have failed due to a combination of the lack of hatchery technology to produce fish large enough to survive, the inability to identify hatchery fish, and the lack of understanding of the effect stocking has on the ecosystem (Blankenship and Leber, 1995). Some of the species that are currently being studied for stock enhancement include Atlantic Cod (*Gadus morhua*), Red Drum (*Sciaenops ocellatus*), Striped Mullet (*Mugil cephalus*), White Seabass (*Atractoscion nobilis*), and Spotted Seatrout (*Cynoscion nebulosus*) (Lorenzen et al., 2010). These initiatives are beginning to show some success, mainly due to fact that most follow the concept of responsible stock enhancement as originally defined by Blankenship and Leber (1995) and modified by Lorenzen et al. (2010). The concept comprises a set of general guidelines used to ensure the success of the program such as producing genetically fit juveniles for release, defining a quantitative measure of success, considering appropriate ecological, biological, and life history patterns, employing appropriate tagging technology to identify released fish, and optimizing the size of released fish and the release site (Lorenzen et al., 2010).

Problems and Solutions in Marine Aquaculture

Commercial aquaculture is a relatively new field and is well-developed for only a few species, primarily shrimp and freshwater finfish. With respect to marine finfish, there is still much work to be accomplished to fulfill the global demand. The supply of marine finfish is primarily wild caught. Aquaculture contributes only 3.4% of the total marine finfish supply (FAO, 2010).

The low production of marine finfish is due in part to the lack of commercial-scale technologies for larval culture (Lee and Ostrowski, 2001). There are many limiting factors that contribute to the lack of larval culture, but the most common is a poor understanding of larval nutrition and intracohort aggression (Lee and Ostrowski, 2001). The majority of marine finfish will initiate exogenous feeding by consuming zooplankton. In hatcheries, first foods typically consist of rotifers (*Brachionus plicatilis*, or *B. rotundiformis*) or *Artemia* because they are easy to culture in an indoor aquaculture system (Lee and Ostrowski, 2001). However, rotifers and *Artemia* naturally do not have the nutritional profile necessary for the growth and ontogeny of finfish larvae. To solve this problem, live feeds are fed special diets to enrich them with the highly unsaturated fatty acids (HUFA) required by the larvae (Lee and Ostrowski, 2001). Unfortunately, the specific nutritional requirements for most species of marine fish larvae are not yet known; therefore, standard hatchery feeding practices may or may not successfully produce larvae. Research is required to determine adequate nutritional profiles for live feeds that would facilitate an expansion of larviculture capability for marine fish.

Cannibalism

Intracorhort aggression and cannibalism is a primary cause for high mortality rates during larval rearing (Lee and Ostrowski, 2001). Cannibalism accounts for 15-90% of the total mortalities that occur during the larval rearing phase of finfish due to the aggressive behavior of fish larvae caused by their high growth and food consumption rate (Hecht and Pienaar, 1993; Baras and Jobling, 2002). Cannibalism is the act of attacking and consuming a member of one's own species (Hecht and Pienaar, 1993). Intracohort cannibalism is the act of attacking one's own siblings within the same year class (Baras and Jobling, 2002). Cannibalistic acts in fish populations originally were thought to occur only in a laboratory or aquaculture setting because of the high rearing densities and increased competition (Hecht and Pienaar, 1993). However, it is now known that cannibalism occurs in the wild within naturally occurring fish populations (Kohlmeier and Ebenhoh, 1995). Cannibalism occurs mainly within highly fecund species that show little to no parental care, especially if the productivity of the area is low (Baras and Jobling, 2002). This strategy allows stronger, faster growing individuals in a population to survive periods of low food availability and removes less fit individuals from the population.

Cannibalism falls into two types based on the gape size limitations of the species. In a system wherein all the larvae are of similar size, Type 1 cannibalism is prevalent. Type 1 cannibalism is defined by the inability of the larvae to fully consume conspecific prey due to a mismatch between the gape size and prey size (Baras and Jobling, 2002).

Often, Type 1 cannibals will asphyxiate from the prey, thereby causing the death of both individuals. Type 2 cannibalism is defined by the ability of the predator to fully consume conspecific prey (Baras and Jobling, 2002). Type 2 cannibalism occurs when the gape size of the predator is not limited by the predator/prey size ratio.

In most cases, a feedback loop exists between Type 1 and Type 2 cannibalism (Baras and Jobling, 2002). Type 1 can lead to Type 2 because Type 1 may involve the consumption of a portion of the conspecific. Since the nutritional value of consuming the prey is greater than that provided by the background food source, the cannibalistic predator gains more energy than non-cannibalistic conspecifics (Baras and Jobling, 2002), resulting in an increased growth of the cannibal and an increase in size heterogeneity which further exacerbates Type 2 cannibalism.

Causes of cannibalism in aquaculture fall primarily into two categories: genetic (innate) and environmental. Genetic factors that cause cannibalism range from inherent differences in growth rates to a genetic predisposition for cannibalistic behavior (Hecht and Pienaar, 1993; Baras and Jobling, 2002). Studies have shown that individuals expressing a cannibalistic phenotype exhibit a greater probability of cannibalistic behaviors (Baras and Jobling, 2002). Ironically, some of the same genetic traits that make aquaculture feasible can also lead to cannibalism (Baras and Jobling, 2002).

Cannibalism may also be induced by environmental factors that affect the behavior of the fish (Hecht and Pienaar, 1993; Baras and Jobling, 2002). The environmental factors that affect rates of cannibalism in aquaculture alter either the ability of the fish to find and capture prey or alter the size heterogeneity within the system. Size heterogeneity comprises variability in the sizes of fish within the system, and plays a major role in cannibalistic behavior. Baras and d'Almeida, (2001) studied the effects of size heterogeneity on cannibalism in Sharptooth Catfish (*Clarias gariepinus*). They concluded that cannibalism was higher in groups with initial size heterogeneity than in groups with no size heterogeneity. Other examples of environmental factors that potentially affect cannibalism include food availability, stocking density, light, turbidity, and temperature (Hecht and Pienaar, 1993; Baras and Jobling, 2002). Alteration of these environmental factors also will influence the organisms' feeding behavior by altering visual cues used to recognize and capture prey (Hecht and Pienaar, 1993; Baras and Jobling, 2002). Consequently, cannibalistic behaviors also will be altered.

Stocking density is a major concern in the operation of aquaculture programs. Maximizing the output of individuals per unit volume will lower the facility's footprint and maximize production. The ideal stocking density should result in the maximum survival of individuals per unit volume without compromising the health of the organisms (Hecht and Pienaar, 1993; Baras and Joblin, 2002). However, maximum stocking density is limited by the biology of the organisms (Hecht and Pienaar, 1993; Baras and Joblin, 2002). High densities can intensify stress which may lead to an increase in disease.

The effects of stocking density on cannibalism also vary among species. If a species is territorial or establishes hierarchies, an increase in density could decrease the occurrence of cannibalism (Hecht and Pienaar, 1993; Baras and Joblin, 2002). By increasing the number of organisms per unit volume, the organism's ability to hold the territory or fend off competitors might diminish, causing aggression to diminish as well (Hecht and Pienaar, 1993; Baras and Joblin, 2002). Cannibalism also can be reduced at higher densities if the organisms being cultured are visual feeders. By increasing the density in the tanks, a schooling effect could make it difficult for the cannibal to single out and attack a conspecific (Baras and Joblin, 2002). Previous studies suggest that this schooling effect occurs in Yellowtail (*Seriola quinqueradiata*) (Sakakura and Tsukamoto, 1998) and Perch (*Perca fluviatilis)* (Baras et al., 2003).

In other species, an increase in density may lead to an increase in the rate of cannibalism because the chance of encounter between the conspecific prey and the cannibal is greater at higher densities (Hecht and Pienaar, 1993; Baras and Joblin, 2002). A positive correlation between density and cannibalism is evident in Fat Snook (*Centropomus parallelus*) (Correa and Cerqueira, 2007) and Nile Tilapia (*Orechromis niloticus*) (Fessehaye et al., 2006)). Other studies have shown an increase in aggression along with no difference in the frequency of cannibalism (Haylor, 1991) relative to stocking density. African Sharptooth Catfish (*Clarias gariepinus*) showed a significant decrease in aggression with an increase in density but no difference in cannibalism with respect to density (Haylor, 1991). The author of the study determined that the trend was possibly due to aggression also being linked to territoriality (Haylor, 1991). Atlantic Cod (*Gadus morhua*) showed no difference in cannibalism or aggression with respect to density (Uglem et al., 2009).

Frequent feeding should reduce cannibalism for a species that is driven by hunger more than by territoriality. Frequent feeding could satiate the larvae and reduce the motivation for consuming a conspecific (Baras and Jobling, 2002). Also, multiple feedings per day can reduce cannibalism by increasing the frequency of encounters with feed, instead of with conspecifics (Baras and Jobling, 2002). Increasing the feeding frequency has been shown to reduce cannibalism in Japanese Flounder (*Paralichthys* *olivaceus*) (Dou et al., 2000) and Australian Snapper (*Pagrus auratus*) (Tucker et al., 2006). Alternatively, an increase in feeding frequency may increase the number of cannibalistic events by stimulating the foraging behavior of the predator (Biswas et al., 2006; Kaiser et al., 1995). This stimulation trend was evident for Mrigal (*Cirrhinus mrigala*), Rohu (*Labeo rohita*) (Biswas et al., 2006), and African Catfish (*Claria gariepinus*) (Kaiser et al., 1995).

Spotted Seatrout (*Cynoscion nebulosus)*

The Spotted Seatrout (*Cynoscion nebulosus*) is a euryhaline fish resident of marshes and estuaries ranging from Massachusetts to Texas (Blanchet et al., 2001). Tagging and genetic data have indicated that Spotted Seatrout rarely migrate out of their natal estuary where they remain throughout their lifetime. Spotted Seatrout are capable of spawning multiple times in the Gulf of Mexico from March to October, a protracted spawning season usually associated with warmer temperatures and longer photoperiods (Blanchet et al., 2001). Spawning aggregations for Spotted Seatrout occur in areas of deep water with relatively strong currents located between barrier islands and channels in open water areas (Saucier and Baltz, 1993). Eggs of Spotted Seatrout are either buoyant or demersal depending on salinity (Perret et al., 1980). In highly saline waters, pelagic eggs can be carried by local currents. Once hatched, larvae tend to be associated with demersal vegetation and shell rubble where adequate protection from predators and plentiful food are present (Blanchet et al., 2001). As larvae age, they eventually metamorphosize into the juvenile phase and begin to associate more with marsh edge habitats, sea grass beds or other areas with bottom vegetation (Baltz et al., 1993; Blanchet et al., 2001). Adult Spotted Seatrout remain in the same general areas as juveniles;

however, adults school and move further from shore to shell beds, harder reef structures, grass beds, or other coastal habitats.

Spotted Seatrout migrate seasonally in the Gulf of Mexico (Baker and Matlock, 1993). In the Mississippi Sound during the summer months, adult Spotted Seatrout will travel farther offshore and inhabit areas around the Mississippi barrier islands (Horn Island, Cat Island, Ship Island). During the fall and winter, they migrate back inshore into estuaries. As water temperatures cool, they travel up rivers and inhabit deep holes that act as a thermal refuge. During the spring, Spotted Seatrout will migrate again offshore to the coastal barrier islands.

Prey selection by Spotted Seatrout depends on the life stage of the species. In general, Spotted Seatrout are opportunistic carnivores but tend to feed on certain types of estuarine prey types that change ontogenetically, from copepods as larvae, to mysids as postlarvae and early juveniles, to grass shrimp and small penaeids as later stage juveniles, to adult penaeid shrimp and other fishes as adults (Moody, 1950). Larval and juvenile Spotted Seatrout target invertebrates small enough to be easily taken without much energy input. The majority of these invertebrates are copepods, mysid shrimp, grass shrimp, and juvenile shrimp. Spotted Seatrout larger than 40-150 mm will begin to feed on fish as well as invertebrates (Moody, 1950). The primary food source for adult Spotted Seatrout is fish but may also include large invertebrates such as penaeid shrimp (Tabb, 1961).

Owing to its abundance, edibility, aggressive behavior, and ease of access, the Spotted Seatrout is the most popular recreational fish in the Gulf of Mexico. However, because of its popularity, there are concerns that current fishing pressure will compromise the sustainability of the regional stock. Indeed, Fulford and Hendon (2010) found that the Mississippi Spotted Seatrout stock is highly dependent on annual recruitment, and that fishing mortality is close to a level which may exceed that of maximum sustainable yield.

The University of Southern Mississippi's Thad Cochran Marine Aquaculture Center

The University of Southern Mississippi's Thad Cochran Marine Aquaculture Center currently cultures a variety of marine finfish, including the Spotted Seatrout (*Cynoscion nebulosus*), for stock enhancement research. The program is a joint effort among USM GCRL, the Mississippi Department of Marine Resources, and the Coast Conservation Association. The goal of the program is to evaluate the feasibility of stock enhancement as a tool that can be used in combination with traditional fisheries management techniques to insure sustainability of important marine resources.

Historically, the Spotted Seatrout has been cultured with marginal success in both extensive and intensive systems (Arnold et al., 1978; Colura et al., 1992; Tucker, 1988). Spawning is induced primarily by photothermal manipulation; however, induction by hormonal injection also has been used (Arnold et al., 1978; Colura et al., 1992; Tucker 1988). In extensive systems, culture ponds are fertilized 28 days or longer to promote optimum growth and densities of planktonic algae and zooplankton which serve as the first prey source for stocked larvae. Two-day-post-hatch Spotted Seatrout larvae are stocked at densities of 45,000 to 2,075,000/ha (Colura et al., 1992). The ponds require little maintenance after stocking, except for some addition of fertilizer if zooplankton densities decline (Colura et al., 1992). An average recovery of 28.7% of the number of fish stocked at 20 to 30 days is considered normal (Colura et al., 1992). Similar to most pond aquaculture methods where the pond is open to the environment, fluctuations occur in water quality parameters which affect the growth and survival of both the Spotted Seatrout and the planktonic prey. These fluctuations make it difficult to maintain optimum conditions for growth and survival of Spotted Seatrout, creating highly variable production for each pond.

Intensive systems are enclosed systems in which organisms are cultured at high densities, thus allowing for an increase in production and greater control of environmental parameters. Historically, in intensive systems using Spotted Seatrout, larvae are reared a relatively low densities (<5 fish L^{-1}) to reduce mortality and to ensure fast growth. Spotted Seatrout in intensive systems were first fed with rotifers enriched with algae to maintain optimum nutritional levels (Arnold et al., 1978; Tucker, 1988). Rotifers were maintained at a concentration of 10-20 rotifers/ml for 10 days (Arnold et al., 1978; Tucker, 1988). Algae was regularly added to the larval rearing tanks to maintain proper enrichment of the rotifers. As the larvae grew, larger *Artemia* were introduced at a density of 3-5 *Artemia*/ml (Arnold et al., 1978; Tucker, 1988). During the period of *Artemia* introduction, rotifer concentrations were lowered as *Artemia* concentrations were increased gradually to wean fish larvae off of rotifers. These methods generally yield 60-65% survival at 10 day post-hatch and 30% survival after day 10. (Arnold et al., 1978). Notably, the main causes of the mortality within such systems are sub-optimal nutrition during weaning as well as cannibalism at 10 days post-hatch (Arnold et al, 1978). Tucker (1988) reported cannibalism rates as high as 100 attempts per day. Arnold et al. (1978) reported an overall decline from 500 larvae to 8 larvae within 30 days. Clearly, for Spotted Seatrout to be effectively reared within intensive systems, procedures to reduce cannibalism must be explored.

At the USM Gulf Coast Research Laboratory (USM GCRL), efforts have been made to increase survival of Spotted Seatrout during the larval rearing stage by exploring proper weaning protocols, using enriched rotifers and *Artemia* and exploring the optimum feed densities for each day post-hatch. Original protocols followed Arnold et al (1978) and produced survival rates of 25% after 24 days. Currently, USM GCRL stocks newly hatched larvae into 1500 L tanks at a stocking density of 15 larvae L^{-1}, and employs a strict feeding protocol to maintain live feed prey densities at optimum levels based on residual counts of prey prior to each feeding. Prior to each feeding, water samples are collected, and the number of live feed organisms per milliliter are counted giving a residual live feed count. This residual count is then subtracted from the live feed concentration for that day to give the amount of live feed to be added. The feeding protocol initially starts at a 1 rotifer ml^{-1} concentration and gradually increases to an 8 rotifer/ ml concentration ending at day 10 post-hatch. *Artemia* concentration starts at 0.5 Artemia/ml on day 10, and thereafter increases to 8 Artemia/ml by day 21 post-hatch. Rotifers and *Artemia* are enriched with a commercial enrichment medium to increase their nutritional profile. In addition, to counteract cannibalism, GCRL protocol entails 3 feedings per day, every 8 h (5am, 1pm, and 9pm), to ensure the larvae are in continual contact with live feeds. This modified protocol has increased the survival rate from 25% to 60% (personal observation).

Objectives

The main goal of this study is to determine the effects of stocking density and feeding frequency on the expression of aggression and cannibalistic behaviors of early hatchery-reared Spotted Seatrout (*Cynoscion nebulosus*) to maximize survivorship during the larval rearing process. The research will be accomplished by testing two subsidiary hypotheses: (1) the frequency of occurrence in aggression and cannibalism will increase with the density of Spotted Seatrout; and (2) the frequency of occurrence in aggression and cannibalism will decrease with the frequency of feeding. At the conclusion of these two experiments, a production run was tested to determine if the small scale results would translate to a production scale run.

CHAPTER II

STOCKING DENSITY EFFECTS ON AGGRESSIVE AND CANNIBALISTIC BEHAVIORS IN LARVAL HATCHERY-REARED SPOTTED SEATROUT, *CYNOSCION NEBULOSUS*

Abstract

Cannibalism and aggression are major sources of mortality in the larviculture of the Spotted Seatrout, *Cynoscion nebulosus*. High stocking density can either increase cannibalism by increasing the likelihood of encounters between aggressors and prey or decrease cannibalism by interfering with normal territorial or aggressive behaviors. The goal of this study was to assess the effect of stocking density on cannibalism and aggressive behavior in hatchery-reared Spotted Seatrout.

Seven-day-old larvae were stocked randomly into three replicates of three different densities (15 (233 total fish), 30 (465 total fish), and 60 (930 total fish) L^{-1}) in 15.5-L aquaria. Feeding was conducted every 8 h based on residual rotifer or *Artemia* counts. Growth was determined 6 days post-stocking at the conclusion of the experiment. To quantify cannibalism and aggressive behaviors, three cameras filmed three tanks for 9 h each day. Recorded behaviors were quantified for three selected 30-minute segments per tank per day: 1 h, 4, and 7 post feeding. Aggressive acts were scored as: nip (aggressor strikes prey causing prey to dart), chase (aggressor moves more than one body length toward prey), and capture (predator captures and holds prey but unable to consume).

In all stocking densities there was a significant increase in aggression and cannibalism with time since feeding. Growth was significantly higher in the lower

density treatment. An observed density threshold existed at a stocking density of 30 fish L^{-1}, beyond which the intensity of aggressive behaviors did not increase. Based on the results of this study, aggression in early stage hatchery-reared Spotted Seatrout might be alleviated with increased feeding frequency. Further, Spotted Seatrout could possibly be cultured at densities higher than the current protocol allows.

Introduction

Aquaculture provides a way to supplement the growing demand for overexploited wild fisheries stocks; however, marine finfish production is currently limited by the lack of commercial-scale hatcheries with the capacity to provide plentiful finfish juveniles economically (Lee and Ostrowski, 2001). Many factors contribute to this bottleneck including cost of production, successful spawning of broodstock, and, especially, high mortality during the larval rearing stage. The occurrence of high mortality can be attributed to suboptimal nutrition and rearing conditions as well as aggression and cannibalism.

Aggression and cannibalism contribute significantly to mortality in aquaculture settings, even where conditions appear to be ideal (Baras and Jobling, 2002; Hecht and Pienar, 1993). The prevalence of aggression and cannibalism in an aquaculture setting can be attributed to intrinsic genetic effects that result in the heterogeneous growth of larvae in addition to extrinsic factors that mediate the aggressive behavior of larvae (Baras and Jobling, 2002; Hecht and Pienar, 1993). Because extrinsic factors can be manipulated, current aquaculture research has focused on managing environmental conditions such as stocking density to control aggression and cannibalism.

The influence of stocking density on aggression and cannibalism varies among species depending on the social structure (Hecht and Pienaar, 1993). An increase in density for a territorial or hierarchical species may lead to a reduction of aggression (Baras and Jobling, 2002; Hecht and Pienar, 1993), as high densities may swamp competitive defenses, thereby making aggression ineffective due to the trade-off in time required for feeding. Previous studies suggest that this is the case for several aquaculture species including Japanese Amberjack (*Seriola quinqueradiata*) (Sakakura and Tsukamoto, 2007), Greater Amberjack (*Seriola dumerili*) (Miki et al., 2011), and Yellow Perch (*Perca fluviatilis*) (Baras et al., 2003). On the other hand, in some species higher density may translate into more intense aggression and frequent cannibalism. This phenomenon is evident under aquaculture conditions for Fat Snook *(Centropomus parallelus*) (Correa and Cerqueira, 2007) and Nile Tilapia (*Orechromis niloticus*) (Fessehaye et al., 2006). Possible explanations for a positive relationship between density and aggression include enhanced prey encounter frequency for aggressors and increased rates of conspecific interactions at higher densities (Baras and Jobling, 2002; Hecht and Pienar, 1993).

In Mississippi (USA), the Spotted Seatrout, *Cynoscion nebulosus,* the most popular recreational finfish in the Gulf of Mexico, is being considered for stock enhancement as part of a comprehensive management approach. The Spotted Seatrout is a euryhaline fish that mainly inhabits marshes and estuaries along the Atlantic and Gulf of Mexico coasts from Massachusetts to Texas (Blanchet et al., 2001). Spotted Seatrout have been reared successfully in both extensive and semi-intensive systems (Arnold et al., 1978; Colura et al., 1992; Tucker, 1988). However, semi-intensive and intensive systems have proven to be problematic in part due to the high prevalence of cannibalism once larvae have reached 10 days post hatch (Arnold et al., 1978; Tucker, 1988). Large-scale cultivation of Spotted Seatrout in intensive systems, therefore, requires a better understanding of the behavioral ecology of the species as well as a practical density for optimizing rearing success.

To understand the aggressive nature of Spotted Seatrout the author 1) use video analysis to elucidate aggressive behaviors shown by larval stages of hatchery-reared Spotted Seatrout to better understand the behavioral ecology of Spotted Seatrout and 2) examine the effects of stocking density on the expression of aggressive and cannibalistic behaviors and growth and survival to illuminate ways to increase survival of Spotted Seatrout in intensive culture conditions.

Materials and Methods

Animal husbandry

Seven-day-old Spotted Seatrout larvae (mean total length 2.9 mm) were obtained on 21 August 2012 from a single cohort cultured at The University of Southern Mississippi's Thad Cochran Marine Aquaculture Center. Eggs were obtained from tank spawns of captive adults maintained under photothermal control similar to the methods described in Arnold et al. (1978) and incubated at a density of 1 ml^{-1} at 30 ppt and 30 °C for 24 h. Newly hatched larvae were stocked at 15 L^{-1} into 1500 L tanks and fed beginning on day 1 post hatch. The larvae were held in the 1500 L tanks until they were moved to the experimental system at 7 days post hatch. Prior to the experiment, 20 larvae were measured (total length, TL) alive using the ProgRes Capture Pro microscope imaging program (JENOPTIK Optical Systems LLC, Jupiter FL, USA) and randomly allocated among three replicates of three different densities (15 (233 total fish), 30 (465 total fish), and 60 (930 total fish) fish L^{-1}). The nine 15.5 L clear, square, aquaria

(22.86cm X 25.4cm X 26.67cm) were connected to a recirculating system with a common water supply with a flow rate of 3.3 ml sec^{-1}. Water recirculated through mechanical, carbon, and ultraviolet filters, and water temperature was maintained at 30.7 ± 0.34°C and salinity at 30 ± 0.29 ppt. The experimental system was continuously illuminated by overhead fluorescent lighting at an intensity of 378 lux. White dividers were placed between each tank to ensure that behaviors of fish were not affected by adjacent tanks. Because the tanks were illuminated overhead, the dividers did not have an effect on light intensity between tanks. Temperature, salinity, pH, dissolved oxygen, ammonia, nitrite, nitrate, and alkalinity were monitored daily using Hach© (Hach Co., Loveland CO, USA) test strips and a YSI© 566 MPS probe (YSI Inc., Yellow Springs OH, USA). Dissolved oxygen was maintained above 8 mg L^{-1}, and pH was maintained at 7.66 ± 0.23 throughout the entire experiment. Ammonia ranged from 0.25 to 0.35 ppm, nitrite from 0 to 0.15 ppm, and nitrate from 25 to 50 ppm. Maintenance included daily siphoning and mortality counts. Equivalently aged Spotted Seatrout from the same cohort were used to replace those that died to maintain the correct densities throughout the experimental period. Daily percent mortality was calculated as the number of mortalities in each tank was divided by the original number of larvae in each tank. Daily survival was calculated by subtraction using the daily percent mortality. Overall percent survival was calculated as the average of daily percent survival over 5 experimental days.

Larvae were fed enriched s-type rotifers (*Brachionus* sp.) on days 1-2 and enriched *Artemia* on days 2-5. Rotifers and *Artemia* were cofed on day 2. Feeding was conducted every 8 h. The amount of food added at each feeding interval was based on residual prey densities obtained by collecting three 1 ml water samples from each tank prior to feeding, which is the standard protocol used to determine residual prey densities at The University of Southern Mississippi's Thad Cochran Marine Aquaculture Center. The total number of live food items (rotifer or *Artemia*) per milliliter was calculated from each sample using a dissecting microscope to estimate the residual number of live food items per milliliter. The residual number was then subtracted from the target concentration, between 0-8 individuals ml^{-1}, depending on days post hatch (DPH) to determine the adjusted number of live food items per milliliter that needed to be added to each tank. The target concentrations for each day were based on the standard Spotted Seatrout feeding protocol used at the Thad Cochran Marine Aquaculture Center and were as follows: Day 0-1, 8 rotifers ml^{-1}; Day 2, 6 rotifers ml^{-1} and 1 *Artemia* ml^{-1}; Day 3, 2 *Artemia* ml^{-1}; Day 4, 3 *Artemia* ml^{-1}; and Day 5, 4 *Artemia* ml^{-1}. After five days, 20 fish larvae were randomly selected from each tank and measured (TL) alive using the ProgRes Capture Pro microscope imaging program (JENOPTIK Optical Systems LLC, Jupiter FL, USA).

Experimental design

To quantify aggressive behaviors and mortality, each of the three video cameras (Sony Handycam DCR-SR45) were used to film the nine tanks for nine h each day. Filming commenced one day after stocking (8 DPH). Footage was analyzed for 3 selected 30 minute segments per tank per day using the Noldus Observer XT program (Noldus Information Technology Inc. Leesburg VA, USA). The selected observation periods were 1 h after feeding, 4 h after feeding, and 7 h after feeding. Aggressive acts were scored as: nip (aggressor strikes prey causing prey to dart), chase (aggressor moves more than one body length toward prey), and capture (predator captures and holds prey but unable to consume, i.e., Type 1 cannibalism). Total acts were scaled to the number of individuals per treatment to standardize data to the number of acts per individual for each tank at each observation period.

To guard against observer bias, a different investigator scored three randomly selected observations in a blind comparison. At the completion of the blind comparison, the mean for the blind observations was tested against that for the original observations using a t-test. No difference in results was obtained between the two observers. In addition, no differences were found when one observer counted the same video multiple times.

Data analysis

Two-way repeated measures (RM) ANOVA (GLM procedure in SPSS version 15.0.1) tested the response variables: (1) number of nips, (2) number of chases, and (3) number of captures. The two within-subjects factors (Day and Time) accounted for variability and dependence among the five consecutive days and the three consecutive feeding times per day. The between-subjects factor (Density) represented Low, Medium, and High density levels as described above. Due to the failure of one of the video cameras, missing data for observations of one replicate observation for each of the three density treatments on 1 day were imputed as means of the other two replicates to enable the RM ANOVAs.

As the directionality of expected differences in behavioral frequencies across density levels was unknown *a priori*, the significance of between-subjects effects was interpreted as a two-tailed hypothesis. The RM ANOVA model included an intercept (overall mean) and two two-way interaction terms (Density×Day, Density×Time). To scale behavioral responses in terms of per capita rates relative to density treatments, frequencies were normalized relative to the number of fish within each of the three density levels. To stabilize variances of those normalized response variables that could greatly exceed one, data were transformed as log ratios [e.g., log(number nips/number fish)]. Accordingly, the number of nips and the number of chases were transformed as log ratios. However, as normalized numbers of captures ranged between zero and one, this response variable was arcsine square root transformed (Zar, 1984). Levene's tests confirmed that error variances of the responses were equal ($P > 0.05$) across the 15 Day by Time levels of the two-way RM ANOVA within-subjects factors for the log ratio responses. However, the arcsine-square root number of captures response was significantly heterogeneous for six of the eleven within-subject levels that could be tested (i.e., the 11 non-zero levels). Mauchley's tests did not reveal any violations of sphericity for the three behavioral responses; however, Box's tests of equality of covariance matrices could not be completed for these responses. The multivariate form of RM ANOVA was used to conservatively interpret significance; and as the most robust multivariate metric (Swearer et al., 2003), Pillai's Trace conveyed the significance of the model terms. Finally, potential trends across the within-subjects factors of Day and Time were diagnosed for the best fit by linear, quadratic, cubic, or higher order (up to fifth order) polynomial contrasts.

One-way ANOVA tested the significance among treatment differences in total length and percent survival. To ensure normality, the length data were log transformed, and the mortality data arcsine transformed. One-way repeated measure (RM) ANOVA (GLM procedure in SPSS version 15.0.1) tested the response variable: percent survival.

The within subject factor (Day) accounted for variability and dependence among the five consecutive days. The between subjects factor (Density) represented Low, Medium, and High. Since values of percent survival fell between 0 and 1, they were transformed as arcsine-square root values.

Results

For all stocking densities there was a significant increase in the number of occurrences of each aggressive behavior with increasing time after feeding as well as with increasing days post hatch (Table 1). Interaction effects between day and density and between time and density were not significant for any scored behavior.

Table 1

Within subjects and between subjects effects for the Two-way Repeated Measures ANOVA on behavior variables for all densities. Values in bold indicate a significant difference between multiple days.

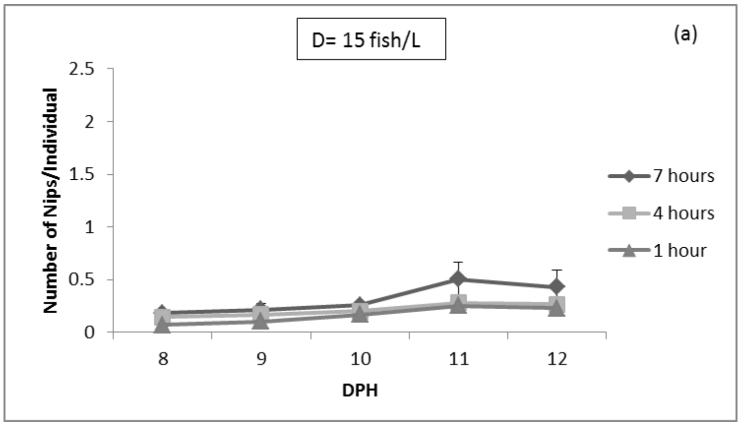
Variable	Effect	F	df	P	Power[a]
Nip	Day	84.194	4	**0.002**	1.00
	Density	13.291	2	**0.006**	0.945
	Day x Density	1.375	8	0.331	0.307
	Time	87.665	2	**<0.001**	1.000
	Time x Density	0.993	4	0.448	0.226
Chase	Day	110.905	4	**0.001**	1.000
	Density	2.288	2	0.183	0.301
	Day x Density	2.321	8	0.128	0.506
	Time	44.573	2	**0.001**	1.000
	Time x Density	0.737	4	0.585	0.175

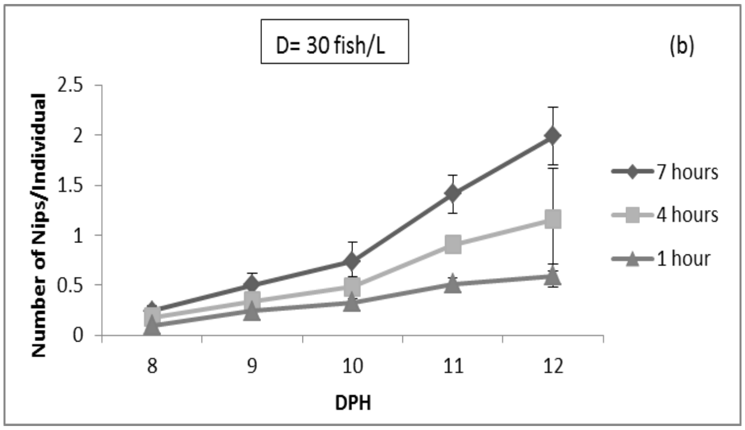
Table 1 (continued).

Capture	Day	34.707	4	**0.008**	0.988
	Density	21.470	2	**0.002**	0.995
	Day x Density	1.523	8	0.283	0.339
	Time	89.270	2	**<0.001**	1.000
	Time x Density	2.670	4	0.084	0.561

[a] Power represents the probability of rejecting the null hypothesis when it is false.

The number of nips per individual was significantly greater in the two higher density treatments (30 and 60 fish L^{-1}) than in the low density treatment (15 fish L^{-1}). The 30 and 60 fish L^{-1} treatments were not significantly different in the frequency of nipping behavior (Figure 1). For all density treatments, the number of nips per individual correlated positively with increased time after feeding. There also was a positive correlation between the number of nips per individual and the number of days post hatch (Figure 1).





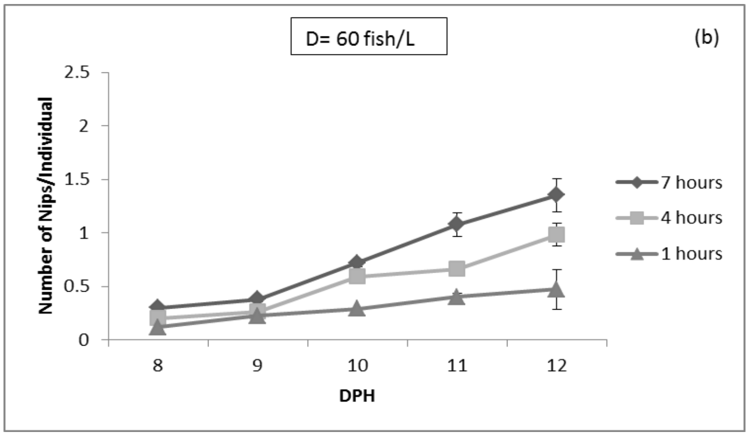


Figure 1. The number of nips per individual at each stocking density (15 fish L^{-1}, 30 fish L^{-1}, and 60 fish L^{-1}) with increasing days post hatch (DPH) for larval *Cynoscion nebulosus* at three time intervals after feeding: 1 h, 4 h, and 7 h. Values represent mean ± SE. Treatments with different letters are significantly different at $P<0.05$ (Two-way repeated measures ANOVA). D represents stocking density.

For the number of chases per individual, there were no detectable differences among density treatments. However, there was a significant positive correlation between the number of chases per individual and time after feeding as well as for the number of days post hatch (Figure 2).

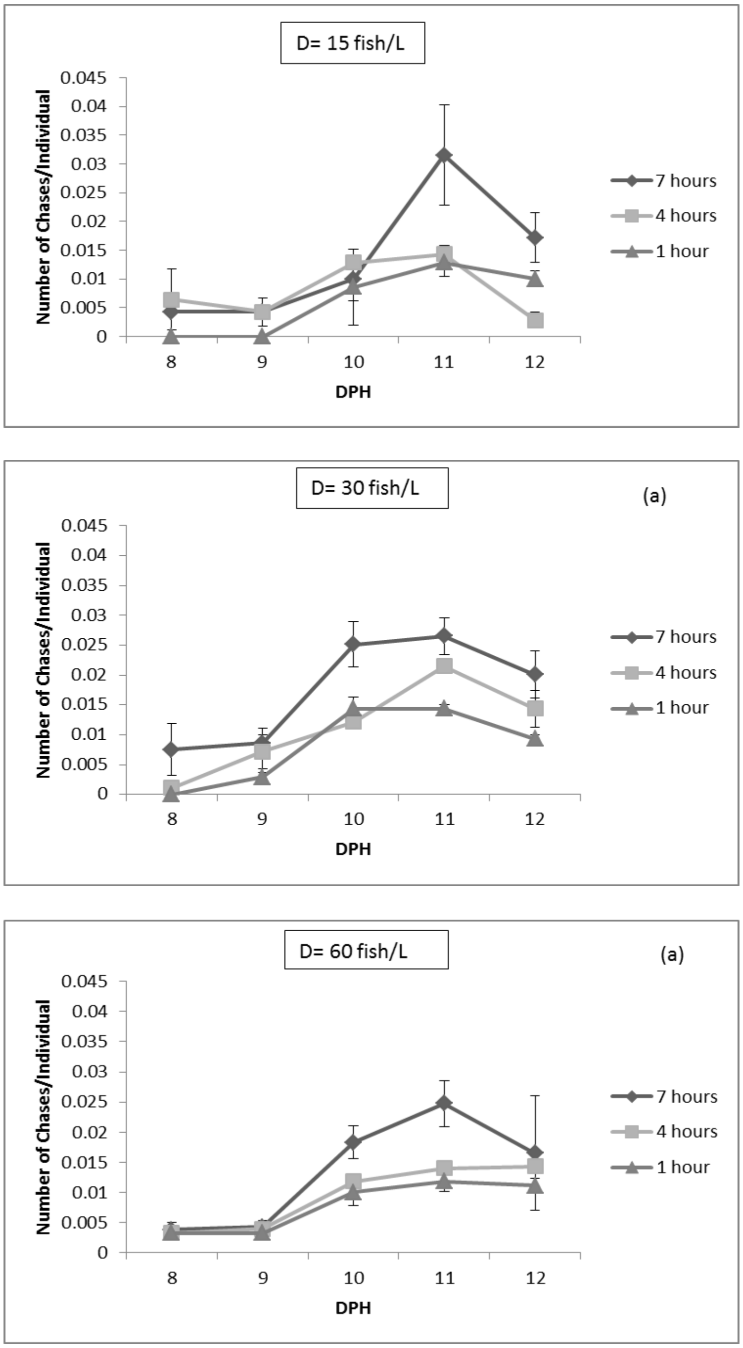


Figure 2. The number of chases per individual at each stocking density (15 fish L^{-1} , 30 fish L^{-1}, and 60 fish L^{-1}) with increasing days post hatch (DPH) for larval *Cynoscion nebulosus* at three time intervals after feeding: 1 h, 4 h, and 7 h. Values represent mean ± SE. Treatments with different letters are significantly different at $P<0.05$ (Two-way repeated measures ANOVA). D represents stocking density.

Capture behavior paralleled the nipping behavioral pattern: the number of captures per individual was significantly greater for the two higher density treatments (30 and 60 fish L^{-1}) than for the low density treatment (15 fish L^{-1}). Similarly, significant positive correlations were found between captures per individual and times after feeding as well as days post hatch (Figure 3).

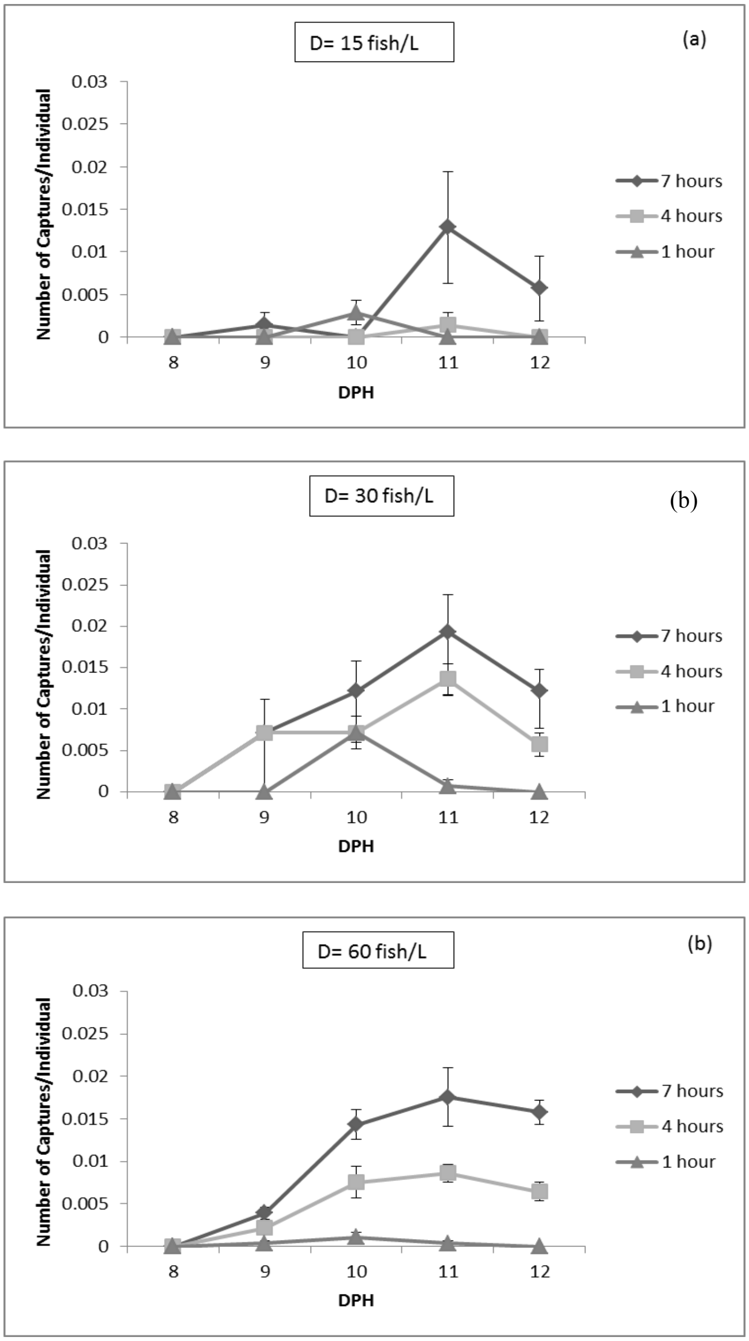


Figure 3. The number of captures per individual at each stocking density (15 fish L^{-1}, 30 fish L^{-1}, and 60 fish L^{-1}) with increasing days post hatch (DPH) for larval *Cynoscion nebulosus* at three time intervals after feeding: 1 h, 4 h, and 7 h. Values represent mean ±

SE. Treatments with different letters are significantly different at $P<0.05$ (Two-way repeated measures ANOVA). D represents stocking density.

Percent survival did not differ significantly among the density treatments or with increasing days post hatch. Overall the 15, 30, and 60 fish L^{-1} had survival rates of 88.4%, 84.6%, and 92.0%, respectively. Furthermore, the difference in percent survival among densities was not detectable when data were averaged across the five-day period. The lower density treatment had significantly higher growth than the two higher density treatments. The total lengths of the 15, 30, and 60 fish L^{-1} had total lengths of 12.2 mm, 10.8 mm, and 11.0 mm, respectively. Mean size did not differ significantly between the two higher density treatments.

Discussion

In all three density treatments there was a significant increase in aggression and cannibalism with time elapsed following feeding. A similar trend has been shown for other cultured marine finfish including Greater Amberjack (*Seriola dumerili*) (Miki et al., 2011) and Japanese Flounder (*Paralichthys olivaceus*) (Dou et al., 2000). Such an increase in aggression may be attributable to a commensurate decrease in the availability of planktonic feed during the later stages of the feeding interval. Intraspecific aggression may function as an adaptive interference mechanism by elevating the feeding rate of the aggressor (Rincón and Grossman, 2001), especially in the face of changing predator densities (Alanärä and Brännäs, 1996) and food availability (Rakocinski et al., 1983). Increased growth depensation would exacerbate this syndrome through positive feedback effects, eventually leading to accelerated rates of mortality.

Displaced innate aggression may inadvertently lead to cannibalism, or conspecifics may provide an alternative food source for aggressive species during periods of low food availability within intensive aquaculture settings (Dionne, 1985). Dou et al. (2000) suggested that hunger brought about by low food availability is the main cause of cannibalism in Japanese Flounder (*Paralichthys olivaceus)*. To ensure proper growth and development and minimize aggression, larval finfish are typically fed just before or just after they evacuate their guts (Baras and Jobling, 2002). Larval Spotted Seatrout will undergo gut evacuation within 2-4 h after feeding at 24°C (Wuenschel and Werner, 2004). Thus, the time required for gut passage may explain why a marked increase in both aggression and cannibalism was observed from 4 to 7 h after feeding in the present study. In light of this, the main causative factor for aggression and cannibalism in Spotted Seatrout appeared to be induced by hunger.

Aggression and cannibalism also increased along with the number of days post hatch at all three densities in this study. For most finfish species, such a temporal trend typically results from the development of teeth and increasing gape size (Baras and Jobling, 2002). In addition, Spotted Seatrout might have learned how to recognize conspecifics as alternative prey. Indeed, Jackson et al. (2013) found that the ability of early juvenile hatchery-reared Spotted Seatrout to recognize and capture novel prey improves with increasing experience. In addition, learning through the observation of other conspecifics might influence development of aggressive and novel feeding behavior. Thus, the observed commensurate increase in aggression and cannibalism with days post hatch may reflect some combination of factors including the development of teeth and increased gape size as well as learned behavior including possible effects of social learning.

The frequencies of both nipping and capture behaviors per individual were significantly higher for the high and medium density treatments than for the low density treatment. However, the two higher density treatments did not differ significantly from each other for nipping or capture. Correa and Cerqueira (2007) showed that juvenile Fat Snook (*Centropomus parallelus*) became more aggressive and cannibalistic at high densities. A positive correlation between density and aggression, or cannibalism, might emerge simply as an outcome of the frequency of contact between individuals (Baras and Joblin, 2002; Correa and Cequeira, 2007; Hecht and Pienaar, 1993). Conversely, Miki et al. (2011) found the opposite trend in hatchery-reared Greater Amberjack (*Seriola dumerili*). The authors suggested that this inverse relationship at high densities might result from enhanced schooling behavior acting to confuse potential cannibals, leading to the difficulty of singling out and attacking any one individual. There appeared to be a threshold effect in the density versus frequency of aggression, or cannibalism, in the present study on larval hatchery-reared Spotted Seatrout. A density threshold appeared to exist at ~ 30 fish L^{-1}, below which a positive relationship between density and aggression, or cannibalism, occurred, and above which the per capita rate of aggression, or cannibalism, remained stable.

As signified by changes in mean length, growth was significantly higher in the low density treatment than in the two higher density treatments; however, the medium and high density treatments did not differ significantly. Slower growth rates with increasing density have been documented in aquaculture settings for other finfish species including African Catfish (*Clarias gariepinus*) (Hossian et al., 1998) and Meagre (*Argyrosomus regius*) (Roo et al., 2010). Multiple factors known to contribute to slower growth at high densities within aquaculture settings include increased competition for food resources, effects of decreased water quality, and effects of stress due to decreased tank space (Roo et al., 2010). Energy costs associated with aggression may represent one proximate cause of growth reduction.

Parallel trends for larval Spotted Seatrout involving a density threshold for growth, aggression and cannibalism suggest linkages among these factors. The lack of a significant difference in growth between the medium and high density treatments further revealed that effects of density dependence were not uniform across all density levels. Density dependent effects on growth may have been mediated by the per capita level of stress as conveyed by the intensity of aggressive and cannibalistic behaviors. Accordingly, medium and high density treatments exhibited similar per capita frequencies of aggressive and cannibalistic acts. This increase in aggression would cause higher stress levels in these treatments. As signified by elevated levels of stress hormones, stress is known to reduce growth for both Eurasian Perch (*Perca fluviatilis*) and Rainbow Trout (*Oncorhynchus mykiss*) within aquaculture conditions (Jentoft et al., 2005). Despite differences in aggression, relative survival did not differ among density treatments in the present study. Thus, the extent to which stress may have been elevated with increasing density did not translate into a corresponding difference in survival.

This study suggests that the main influence on aggression and cannibalism in hatchery-reared Spotted Seatrout larvae is hunger and food availability. If an adequate amount of live feed is not available in the rearing system, larvae will become aggressive and consume each other as an alternative food source or as a byproduct of aggression. Although significant, density likely plays a lesser role as a factor influencing aggression and cannibalism. Within the range considered, there seems to be a threshold at a density of 30 fish L^{-1}, after which aggression and cannibalism levels off. This study suggests Spotted Seatrout raised at densities higher than 30 fish L^{-1} may not exhibit an increase in per capita rates of aggression or cannibalism. A reason behind this threshold could be due to the cannibal being overwhelmed by the number of prey items. The high number of pray items will prohibit the potential cannibal from singling out an individual prey item. The density threshold found in this study further supports the interpretation that aggression in Spotted Seatrout is mainly driven by hunger. Alternatively, if aggression acts as leverage on feeding gain, the trade-off between the cost of increased aggression and feeding gain may reach its limit at some threshold density. The ability of an aquaculture facility to increase the density of culture could increase production and reduce the cost per fish. However, if the increase in density decreases the growth of the larvae such that additional days are required for larval rearing, the cost savings could be eliminated. Nevertheless, the calculation is valuable when considering how to optimize production.

Future study is needed to determine if an increase in the daily frequency of feeding will reduce aggression and cannibalism in hatchery-reared larval Spotted Seatrout. Increasing the frequency of feeding so that a more consistent supply of live feed is available within the rearing system might remove the inclination of larvae to consume each other as an alternative food source. Such a study of feeding frequency also would help test alternative hypotheses about factors driving aggression and cannibalism within intensive culture scenarios. Other future studies might include increasing the amount of food given at each feeding. An increase in the amount of food may lead to an increase in satiation and prohibit the need to seek out alternative food sources. Also, a large-scale experiment based on the results of this study would be beneficial for testing the feasibility of raising Spotted Seatrout at high densities in a production system.

Conclusion

The data from this study suggest that at densities exceeding 30 fish L^{-1} aggression, including cannibalism, will not increase. The data from this study suggests that it is possible to culture Spotted Seatrout at higher densities than current protocols would allow. By concluding that aggression and cannibalism is governed more by the need for a constant supply of live feeds instead of stocking densities may allow an alternative method to counteract cannibalism in the larval rearing process. More frequent feedings during the larval rearing stages may diminish aggression and cannibalism and allow for higher survivorship and higher growth rates which will increase production of a facility.

CHAPTER III

THE RELATIONSHIP BETWEEN FEEDING FREQUENCY AND AGGRESSIVE BEHAVIORS IN LARVAL HATCHERY-REARED SPOTTED SEATROUT, *CYNOSCION NEBULOSUS*

Abstract

Cannibalism is a major source of mortality in the intensive culture of the Spotted Seatrout, *Cynoscion nebulosus*. Increased feeding frequency, particularly in the live feed stage, may promote fewer attacks on conspecifics and increased growth due to less energy spent on aggression, or more efficient digestion of smaller ration parcels. The goal of this study was to examine the effects of feeding frequency on cannibalism and aggressive behaviors in larval hatchery-reared Spotted Seatrout. Ten-day-old Spotted Seatrout larvae reared using the standard protocol of enriched s-type rotifers and enriched *Artemia* were stocked at 30 fish L^{-1} into three replicates of each of four feeding frequency treatments (every 8 h, 4 h, 2 h, and 1 h) in which the same total ration was divided accordingly. Larvae were fed enriched *Artemia* for eight days. Four video cameras monitored blocks of three tanks each from 2 h before feeding until 1 h after feeding. Two, pre-selected 30 min segments (30 min before and 30 min after feeding) relative to each feeding event per tank per day were scored separately for the number of nips, chases, and captures (Type 1 cannibalism). The 2 h frequency treatment elicited the fewest aggressive and cannibalistic acts. Lower aggression and cannibalism associated with the 2 h feeding frequency treatment could be attributed to a balance between the amount of live food per feeding event (degree of satiation) and the gut evacuation time of larval Spotted Seatrout.

Introduction

Marine finfish comprise a major portion of consumer seafood demand globally, yet only 3.4% of marine finfish is obtained from aquaculture (FAO, 2010). This gap in finfish production can be attributed primarily to a shortage of commercially available marine finfish larvae. High mortality during the larval rearing process due to suboptimal nutrition, cannibalism, and aggression prevents the economically efficient culture of certain species and contributes greatly to this bottleneck (Lee and Ostrowski, 2001).

Intracohort aggression often has been linked to the mortality of aquaculture organisms. Cannibalism in aquaculture settings is expressed as either of two types. In Type 1 cannibalism, the aggressor is unable to fully consume its prey; in Type 2 cannibalism; the predator is fully able to consume its prey (Baras and Jobling, 2002; Hecht and Pienaar, 1993). An organism also may be aggressive or cannibalistic toward a conspecific when defending its own space (Baras and Jobling, 2002, Hecht and Pienaar, 1993). This tendency may be overcome by increasing the stocking density to limit the aggressor’s effectiveness. Satiation also may play a role in mediating aggression and cannibalism. During periods of low food availability, aggression and cannibalism may tend to increase when conspecifics present the only alternative prey type (Baras and Jobling, 2002, Hecht and Pienaar, 1993). Elevated aggression due to low food availability can be counteracted by increasing the frequency and volume of feedings. For Spotted Seatrout (*Cynoscion nebulosus*), the author found that satiation plays a more critical role in intracohort aggression than stocking density (Manley et al., 2014).

Feeding frequency can be controlled easily in an aquaculture setting and may provide an effective means of reducing cannibalism and aggression. Increasing the amount of food or the number of feedings per day should increase the frequency of encounters between larvae and food and lengthen the period of satiation, thereby leading to less cannibalism and aggression (Baras and Jobling, 2002, Hecht and Pienaar, 1993). However, the effect of feeding frequency varies among species. In species for which cannibalism and aggression are mediated by satiation, more frequent feeding may lead to a reduction in aggression and cannibalism. A negative relationship between more frequent feedings and aggression appears to be true for the Australian Snapper (*Pagrus auratus*) (Tucker et al., 2006). Alternatively, more frequent feeding could exacerbate aggression if it is induced by conspecific foraging behavior. For example, higher feeding frequency induced increased aggression 1 h before feeding events in African Catfish (*Clarias gariepinus*) larvae (Kaiser et al., 1995). For some species, including Atlantic Cod, *Gadus morhua*, (Folkvord and Ottera, 1993) and Yellowtail Flounder, *Limanda ferruginea*, (Dwyer et al., 2002), increased feeding frequency has no effect on aggression.

The Spotted Seatrout (*Cynoscion nebulosus*) is a euryhaline fish that inhabits estuaries of the western Atlantic Ocean from Massachusetts to Mexico (Blanchet et al., 2001). Due to its predatory nature and inshore accessibility, the Spotted Seatrout is the most popular recreational fish in the Gulf of Mexico. This popularity has engendered concern about the sustainability of the Spotted Seatrout population and spurred interest in using stock enhancement for management of this species in Mississippi (USA).

The Spotted Seatrout has been reared successfully in extensive aquaculture systems (Colura et al., 1992). However, mortalities associated primarily with aggression and cannibalism at 10-31 days post-hatch have limited production of Spotted Seatrout in intensive culture systems (Arnold et al. 1978, Tucker 1988). Thus, large-scale, economic production of Spotted Seatrout requires techniques for lowering mortality due to aggression and cannibalism during the larval rearing process.

Materials and Methods

Animal husbandry and experimental set-up

Ten-day-old Spotted Seatrout (mean total length 5 mm) were obtained on 23 April 2013 from a single cohort cultured at The University of Southern Mississippi's (USM) Thad Cochran Marine Aquaculture Center (CMAC). Eggs were obtained from a tank spawn of captive adults maintained under photothermal control similar to the methods described in Arnold et al. (1978) and incubated at a density of 1 egg ml^{-1} at 25 ppt and 28°C for 24 h. One-day-old larvae were stocked into eight 1500 L tanks at a density of 15 fish L^{-1}. The tanks were kept on a recirculating system involving mechanical, carbon, and ultraviolet filtration. Prior to experiments at 7 days post hatch, larvae were harvested from the 1500-L tanks and randomly stocked at a density of 30 fish L^{-1} (465 total fish) into three replicates of each of four feeding frequency treatments (every 8 h, 4 h, 2 h, and 1 h).

A total of twelve 15.5 L clear aquariums (22.86 cm X 25.4 cm X 26.67 cm) were used for the experiment. The twelve 15.5 L aquariums were connected to a recirculating filtration system with a common water supply and a flow rate of 3.3 ml sec^{-1}. White dividers were placed between each tank, and a black curtain was placed behind the tanks to ensure that adjacent tanks or maintenance did not affect fish behaviors. The experimental system was continuously illuminated by overhead fluorescent lighting at an intensity of 378 lux. Water circulated through mechanical, carbon, and ultraviolet filters; water temperature was maintained at 28.03 ± 0.36°C and salinity at 28.35 ± 0.71ppt.

Temperature, salinity, pH, dissolved oxygen, ammonia, nitrite, nitrate and alkalinity were monitored daily using Hach© test strips (Hach Co., Loveland CO, USA) and a YSI© 566 MPS probe (YSI Inc., Yellow Springs OH, USA). Dissolved oxygen was maintained at 5.59 ± 0.39 ppm, and pH was maintained at 8.46 ± 0.16 throughout the experiment. Ammonia ranged from 0-0.25 ppm, nitrite from 0.15-1 ppm, and nitrate was steady at 50 ppm. Maintenance included daily siphoning and mortality counts. At 18 days post hatch, the total length (TL) of 20 randomly chosen live larvae from each tank were measured to the nearest 0.01 mm using the ProRes Capture-Pro microscope imaging program (JENOPTIK Optical Systems LLC, Jupiter FL, USA).

Larvae were fed enriched *Artemia* for eight days. The same total daily ration was used for each treatment; the amount of food added for each feeding event per treatment was determined by subdividing the total number of *Artemia* in the daily ration evenly among the number of feeding events for that treatment. The daily ration was based on the standard protocol utilized at CMAC. Food for the 1 h, 2 h, and 4 h frequency treatments was dispersed using an automated feeder, whereas food for the 8 h treatment was dispersed manually according to standard practice at the time when the automated feeder was refilled.

Automated feeder

The automated feeder was constructed from a 22.7-L plastic bucket, with nine 0.32 cm holes drilled into the bottom. An appropriately sized fitting was attached to each of the holes in the bucket and a 91-182 cm length of 0.32 cm diameter vinyl tubing (Pentair Aquatic Eco-Systems Inc., Apopka FL, USA) was added to connect each of the nine holes to nine solenoid valves (Xylem Inc., Rye Brook NY, USA). Tubing from the solenoid valves was then placed into tanks subjected to the 1 h, 2 h, or 4 h feeding frequency treatments via delay on/off relays (Dayton Co., Dayton OH, USA) to control the volume of *Artemia* dispensed into each tank at each feeding. The relays were connected to on/off timers (Pentair Aquatic Eco-Systems Inc., Apopka FL, USA) set for the designated feeding intervals. The reservoir of the feeder was constantly aerated using an air stone to ensure that the *Artemia* were evenly distributed in the bucket. A 2-L bottle of ice was maintained in the feeder reservoir to ensure that the *Artemia* remained cool enough to retain their enrichment.

Experimental design

To quantify aggressive behaviors, each of the four video cameras (Sony Handycam DCR-SR45) monitored blocks of three tanks from 2 h before feeding until 1 h after feeding. Recordings were examined using the Noldus Observer XT program (Noldus Information Technology Inc. Leesburg VA, USA) at 2 pre-selected 30 min segments relative to each feeding event per tank per day: (1) 30 min before and (2) 30 min after feeding. The frequency of each type of aggressive act was tabulated. Aggressive acts were scored as: nip (aggressor strikes prey causing prey to dart), chase (aggressor intently moves more than one body length toward prey), and capture (predator captures and holds prey, but is unable to consume it, i.e., Type 1 cannibalism). To ensure that bias was not a factor in the analysis, three randomly chosen observations also were scored by a blind observer independently of the original observer. An ANOVA determined that there were no differences between observers or repeated measurements.

Data analysis

Behavioral responses (number of nips, number of chases, and number of captures) were examined using a Repeated Measures ANOVA within the linear mixed-effects models (MIXED; LMM) procedure in SPSS ver. 18.0.0. The LMM procedure was chosen because of its ability to address correlated data (e.g., repeated measures), heterogeneous data via alternative covariance structures, unbalanced experimental designs, and time-dependent covariates (i.e., density) (SPSS, Inc. 2005). Thus, data did not require transformation or imputation of missing observations. Factors for the LMM RM ANOVA model used in this study included the Frequency treatment (main), Day (repeated), Time (repeated), Density (as covariate), and the three two-way interactions between the Frequency treatment and the two repeated factors as well as between the two repeated factors. Individual tanks were regarded as subjects within the LMMs. The restricted maximum likelihood (REML) approach aided determination of model significance. Based upon the best balance between parsimony (number of parameters vs. BIC (i.e., Schwarz's Bayesian Criterion)) and best fit of the variance structure (highest proportion of significant covariance elements), the Diagonal covariance structure was used for the LMMs. The repeated measures aspect of the model entailed a two-way combinatorial design between Day and Time (i.e., before vs. after feeding). Differences between factor levels were examined using the EMMEANS procedure based on adjusted LSD tests. Additional follow-up, post-hoc tests of interaction means made via the EMMEANS procedure compared frequency treatments separately at both levels of Time at the adjusted mean density for each behavioral response.

A One-way repeated measures ANOVA tested for differences in mortality among frequency treatments. Mortality data were log transformed (i.e., log n+1) to normalize and homogenize variance in the data. The within-subject factor accounted for variability and dependence across the 8 consecutive days; a between-subject factor accounted for differences among the feeding frequency treatments. A One-way repeated measures ANOVA tested for differences in total length among treatments. The same within and between subject factors were represented in the mortality data analysis.

Results

For all three behaviors, the three main factors including Frequency, Day, and Time were significant (F = 2.55 - 92.40; P = 0.035 - <0.001). The Density covariate was significant for the number of nips (F = 12.07; P = 0.002), almost significant for the number of captures (F = 3.85; P = 0.060), and non-significant for the number of chases (F = 0.16; P = 0.688). In addition, the Frequency × Day interaction was not significant for any response (F = 0.68 - 1.94; P = 0.062 - 0.815). However, both the Frequency × Time and Day × Time interactions were significant for the number of nips (F = 13.81 and 2.73; P = <0.001 and 0.029) and the number of chases (F = 12.08 and 3.13; P = <0.001 and 0.014), but neither of these interactions were significant for the number of captures (F = 0.92 and 1.92; P = 0.435 and 0.103).

As conveyed by significant Frequency × Time interactions in addition to the main Time factor, the three aggressive behaviors differed relative to before vs. after feeding. Thus, follow-up tests of interaction means considered differences in aggressive responses among frequency treatments at each level of time. The number of nips was significantly lower after feeding than before feeding for the 2 h, 4 h, and 8 h treatments (P = <0.001), but not for the 1 h frequency treatment (P = <0.780), for which the number of nips did not differ between before and after feeding (Figure 1). The 2 h treatment exhibited the fewest nips before feeding (P = <0.001), whereas, the number of nips was high and similar before feeding for the other three frequency treatments. After feeding, the number of nips was significantly higher for the 1 h treatment compared to both the 2 h and 4 h treatments (P = <0.001), and the number of nips after feeding for the 4 h treatment was significantly higher than for the 8 h treatment (P = <0.001), for which the number of nips was lowest after feeding.

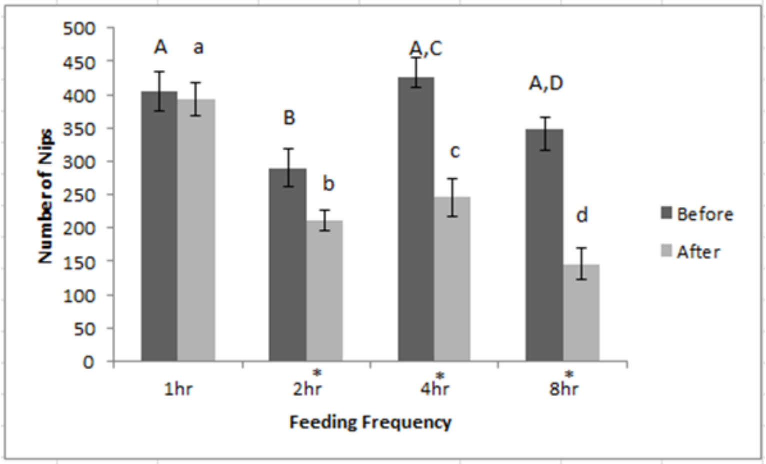


Figure 4. Mean number of nips before and after feeding at each feeding frequency treatment (1 h, 2 h, 4 h, and 8 h) for larval *Cynoscion nebulosus*. Values represent mean ± SE. Capital letters denote significant differences between treatments in the before-feeding group. Lowercase letters denote significant differences between treatments in the after-feeding group. Asterisks denote significant differences between before and after groups for each treatment.

The number of chases decreased significantly after feeding vs. before feeding for the 4 h and 8 h treatments (P = <0.001), although not for the 1 h and 2 h treatments (Figure 2) (P = 0.326-0.583). The 2 h treatment again exhibited the lowest number of chases before feeding (P = 0.002-0.034), whereas, the 1 h, 4 h, and 8 h treatments all exhibited similar numbers of chases before feeding (P = 0.228-0.662). After feeding, the number of chases was lowest for the 8 h treatment (P = <0.001-0.049); the number of chases was lower for the 2 h and 4 h treatments than for the 1 h treatment (P = <0.001-0.001) (Figure 2).

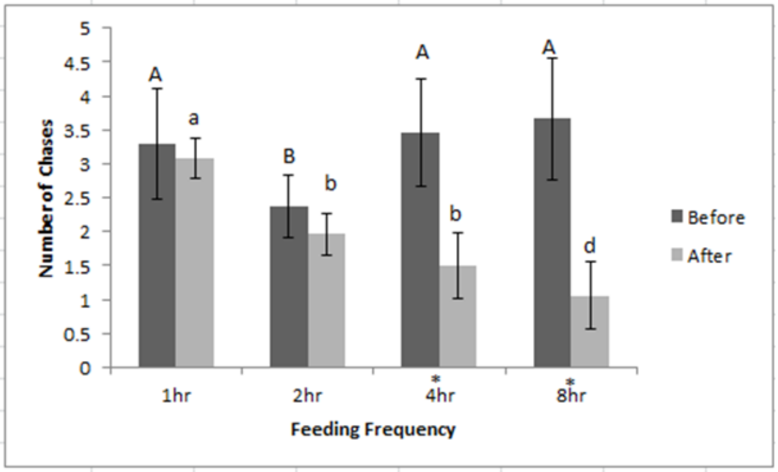


Figure 5. Mean number of chases before and after a feeding at each feeding frequency treatment (1 h, 2 h, 4 h, and 8 h) for larval *Cynoscion nebulosus*. Values represent mean ± SE. Capital letters denote significant differences between treatments in the before-feeding group. Lowercase letters denote significant differences between treatments in the after-feeding group. Asterisks denote significant differences between before and after groups for each treatment.

The number of captures decreased significantly after feeding vs. before feeding for the 4 h treatment (P = 0.015), although not for the 1 h, 2 h, and 8 h treatments (Figure 3) (P = 0.112-0.817). Before feeding, the number of captures was significantly fewer for both the 2 h and 8 h treatments than for the 1 h and 4 h treatments (P = <0.001-0.013), for which captures were significantly higher before feeding (Figure 3). After feeding, captures were significantly lower for the 2 h and 8 h treatments compared to the 1 h treatment (P = 0.002-0.013).

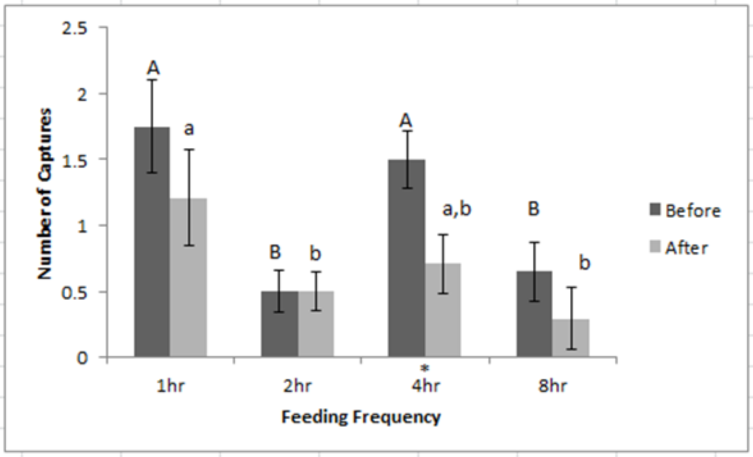


Figure 6. Mean number of captures before and after feeding at each feeding frequency treatment (1 h, 2 h, 4 h, and 8 h) for larval *Cynoscion nebulosus*. Values represent mean ± SE. Capital letters denote significant differences between treatments in the before-feeding group. Lowercase letters denote significant differences between treatments in the after-feeding group. Asterisks denote significant differences between before and after groups for each treatment.

There was no difference in the number of daily mortalities among frequency treatments (P = 0.478-0.979). However, fish from the 2 h and the 8 h treatments were significantly larger in total length (TL) at the conclusion of the experiment (P = <0.001-0.001); fish from the 1 h treatment were significantly smaller in TL (P = <0.001-0.001).

Discussion

Overall, the 2 h feeding frequency treatment elicited the fewest aggressive and cannibalistic acts before each feeding. This trend could reflect two related physiological factors: satiation and gut evacuation (Baras and Joblin, 2002). Satiation may play a major role in mediating aggression and cannibalism. When not satiated, larvae seek alternative prey sources which are conspecifics. Such a scenario was shown in African Catfish (*Clarias gariepinus*), where groups of larvae that were fed 2-4% body weight daily displayed higher rates of cannibalism than groups fed 6-10% body weight (Al-Hafedh and Ali, 2004). In another African catfish study, food availability had the greatest influence on cannibalism in an aquaculture system (Hecht and Appelbaum, 1988). The author concluded that for some aggressive species, an increase in the satiation level will deter aggressive or cannibalistic tendencies if aggression is due to satiation level. This decrease in aggression while satiation concurs with optimal foraging theory which contends that it is beneficial to decrease activity when satiated to conserve energy (Pyke, 1984).

Our results indicate that the 2 h treatment provided sufficient food to keep the larvae relatively satiated, thereby reducing their motivation to be aggressive. As the daily ration was subdivided across the feeding frequency regimes, the smaller ration fraction delivered in the 1 h frequency treatment might not have satiated the larvae enough to hinder aggression. The effects of satiation also may help explain the marked differences between pre- and post-feeding aggression for the 4 h and 8 h treatments. Introduction of relatively large ration fractions at each feeding appears to have led to marked decreased aggression after feeding events.

Gut evacuation time also should mediate satiation. If larvae fully evacuate their intestinal tracts between feedings, they should be very motivated to seek food. Gut evacuation times in Spotted Seatrout of a comparable age and held at a similar temperature to those in this study range from 2-4 h (Wuenschel and Werner, 2004). This effect could explain why fish from the 2 h treatment exhibited the fewest aggressive acts. The intestinal tracts of larvae in the 2 h feeding frequency regime may not be evacuated completely between feedings, thus some degree of satiation would be maintained. In contrast, larvae in the 1 h frequency treatment were not likely fed enough to be effectively satiated. A strong positive relationship between cannibalism and starvation level seen for Japanese Flounder (*Paralichthys olivaceus*) (Dou et al., 2000) and Yellowtail Amberjack (*Seriola quinquevadiata*) (Sakakura and Tsukamoto, 2007) suggests that feeding soon after gut evacuation is critical for minimizing aggression. Indeed, in the present study, a high frequency of aggressive acts in the 4 h treatment and more nips and chases in the 8 h treatment support the idea that larvae were fully evacuating their guts within 4 h. Also, fuller guts following feeding appeared to elicit a marked decrease in aggression after feeding when the frequency of feeding was lower, as has been documented for yellowtail kingfish (*Seriola lalandi*) (Moran, 2007).

The growth response in the present experiment appeared complex. Fish in the 2 h and the 8 h treatments were largest, whereas fish from the 1 h treatment were smallest at the conclusion of the experiment. An opposite trend was found for hatchery-reared Florida Pompano (*Trachinotus carolinus*), which showed a direct correspondence between feeding frequency and growth when fed with the same ration (Weirich et al., 2006). For cultured Whitemouth Croaker (*Micropogonias furnieri*), growth was lowest at the highest feeding frequency (Aristizabal-Abud, 1990). In the present study, both the 1 h and the 4 h treatments exhibited a high frequency of aggressive acts, which possibly translated into increased energy expenditures and/or stress levels. Jentoft et al. (2005) documented that higher stress negatively impacts growth in teleost fish. Higher growth in the 2 h and the 8 h treatments also might indicate lower stress associated with a decrease in cannibalism. However, the fact that there were no significant differences in mortality among treatments suggests a lack of any differences in cannibalism-related mortality among treatments, despite definite differences in aggression. Thus, some components of aggression might be directed at goals other than cannibalism, such as vying for access to food (Manley et al. 2014).

There also were no significant differences in size heterogeneity, which suggests the lack of any growth depensation among the frequency treatments. Size heterogeneity has been positively correlated with aggression and cannibalism (Baras and d'Almeida, 2001; Baras et al., 2000; Kestemont et al., 2003; Moran, 2007; Skov et al., 2003). Feeding to satiation at a sufficient frequency to ensure constant satiation possibly promoted constant growth rates for all individuals in the tank. However, the observed lack of size heterogeneity in this study reinforces the interpretation that satiation and gut evacuation play large roles in mediating aggression for Spotted Seatrout.

Conclusion

This study indicates that aggression in hatchery-reared larval Spotted Seatrout is physiologically mediated by satiation and gut evacuation. Providing enough feed to sustain the satiation should minimize intraspecific larval aggression of this species within intensive aquaculture settings. Also, feeding larvae soon after gut evacuation should reduce aggression by restricting the time without access to food. By optimizing the balance between feeding frequency and satiation, Spotted Seatrout larvae within the 2 h treatment exhibited lower aggression and better growth in the present study. However, future studies are needed to determine if feeding every 2 h would be feasible and successful in a full-scale production scenario.

CHAPTER IV

MANIPULATING FEEDING FREQUENCY AND STOCKING DENSITY TO ENHANCE YIELD IN LARGE-SCALE PRODUCTION OF HATCHERY-REARED SPOTTED SEATROUT, *CYNOSCION NEBULOSUS*

Abstract

Cannibalism and aggression are major causes of mortality in larval Spotted Seatrout during the larval rearing process and present a major bottleneck in the intensive production of this species. Previous experimental-scale studies revealed that the frequency of aggressive behaviors increases with stocking density up to a density of 30 fish L^{-1}, above which aggression or cannibalism levels off. Other small-scale studies revealed that a feeding frequency of every 2 h minimized aggression and cannibalism compared to feeding frequencies of every 1, 4, or 8 h. The goal of this study was to couple findings from the previous experiments within the context of a large-scale production run to examine whether or not reduced cannibalism and aggression can elicit better yields. Two density and feeding regime treatment levels were fully crossed. One-day-old Spotted Seatrout were randomly stocked in duplicate at two different densities (15 fish L^{-1} and 30 fish L^{-1}) into eight 1500-L tanks. Water was recirculated in tandem through biological, bead, and UV filters. Siphoning and mortality counts were completed daily. From Day 0 through Day 10, feeding followed the standard CMAC protocol. Beginning on Day 11 and continuing until Day 24, the total ration (standard protocol x 1.5 to ensure satiation) from each density level were assigned to a regime involving three feedings, each spaced 8 h apart (standard protocol). For the other four tanks, the total ration was subdivided into 12 feedings, each spaced 2 h apart. The 30 fish L^{-1} density

level exhibited a lower mortality rate than the 15 fish L^{-1} level. Moreover, the 8 h feeding regime exhibited a slightly higher rate of mortality per individual than the 2 h feeding regime. This trend suggests that higher densities and feeding every 2 h may be used to increase production as well as survivorship in the larval production of Spotted Seatrout.

Introduction

Cannibalism and aggression represent major sources of mortality in the intensive culture of some marine finfish larvae. In some cases, mortality brought about by cannibalism and aggression can be so severe it precludes the culture of a particular species (Baras and Jobling, 2002, Hecht and Pienaar, 1993). The majority of studies focusing on alleviating cannibalism have been small laboratory-scale experiments which are more controllable, less labor intensive and less expensive than large-scale production systems. However, it is questionable whether the results from a small-scale study will translate equitably to a production-scale run. It is therefore critical that a production-scale experiment be conducted prior to applying small-scale experimental results to a production protocol.

In some instances, results from small-scale experiments do not apply the same in production-scale studies. For example, Summer Flounder (*Paralichthys dentatus*) larvae stocked under varying densities within a small-scale 60-L system showed no difference in growth or survival, whereas larvae grew significantly larger at a lower density in a large-scale commercial system (1,000 L) under the same experimental conditions (King and Howell, 2000). The author suggested that decreased growth in the high density treatment could be linked to either higher stress or a lower rate of food consumption at high densities (King and Howell, 2000). However, they did not explain why the commercial-scale results differed from the small-scale results. Conversely, studies on the effects of stocking density using African Catfish (*Clarias gariepinus*) showed an inverse relationship between stocking density and growth in both small- and large-scale systems (Hengsawat et al., 1997, Hossain et al 1998).

The Spotted Seatrout, *Cynoscion nebulosus* is an aggressive euryhaline predaceous fish that inhabits salt marshes and estuaries from Massachusetts to Texas (Blanchet et al., 2001). It occurs relatively close to shore and is the most important recreational finfish in the Gulf of Mexico. Due to its importance, stock enhancement of Spotted Seatrout is being considered as part of a comprehensive management approach in Mississippi.

Historically, Spotted Seatrout have been successfully cultured within extensive systems (Colura et al., 1992). However, intensive culture has proven difficult for this species. Previous attempts to intensively culture Spotted Seatrout have been hindered by the detrimental effects of aggression and cannibalism (Arnold et al., 1978, Tucker, 1988).

In two former experiments, the author examined the effects of feeding frequency and stocking density on aggressive behaviors of larval hatchery-reared Spotted Seatrout in small-scale systems at The University of Southern Mississippi’s Thad Cochran Marine Aquaculture Center (CMAC). In terms of stocking density, although increasing stocking density from 15 fish L^{-1} to 30 fish L^{-1} elicited a commensurate increase in the frequency of aggressive acts, aggression did not continue to increase at densities higher than 30 fish L^{-1} (Manley et al., 2014). Also, mortality did not increase with increasing stocking densities. The density threshold suggests that Spotted Seatrout larvae could be reared at densities higher than what was previously thought. In terms of feeding frequency, the balance between satiation and gut evacuation rate appeared to be a key factor controlling aggression and cannibalism, and a feeding frequency of every 2 h was ideal, compared to feeding frequencies of every 1, 4, or 8 h (Manley et al., in review).

In this chapter the author applied the findings from previous small-scale studies of the effects of stocking density and feeding frequency on the survival and growth of Spotted Seatrout larvae to the level of a full-scale production run. I wanted to see whether or not beneficial effects on aggression were consistent between small and large-scale scenarios; and further, to determine if alleviation of cannibalism and aggression can be a vehicle for enhancing the intensive production of Spotted Seatrout.

Methods

Animal husbandry

Newly hatched Spotted Seatrout were obtained on 13 June 2013 from a single cohort cultured at the CMAC. Eggs were obtained from tank spawns of captive adults maintained under photothermal control, similar to the methods described in Arnold et al. (1978), and incubated at a density of 1 mL^{-1} at 30 ppt and 30 °C for 24 h. Two density and feeding regime treatment levels were fully crossed and duplicated (i.e., eight tanks). One-day-old Spotted Seatrout were stocked randomly at two different densities (15 fish L^{-1} (22,500 total larvae) and 30 fish L^{-1} (45,000 total larvae) into 1500-L tanks. Logistics precluded more than two replicates for each treatment combination. Initially, feeding followed the standard CMAC protocol from Day 0 through Day 10, wherein larvae were fed rotifers every 8 h. Beginning on Day 11, the total ration of 1.5 × standard protocol ration (i.e., 1.8×10^7 – 5.4×10^7 *Artemia*/tank) was allocated for two replicates of each density level, following a regime involving three feeding events separated by 8 h intervals (standard protocol). For the other four tanks, the total ration was subdivided into 12 feeding events, each separated by 2 h intervals. The 2 h frequency feedings were dispensed using an automated feeder. The 8 h frequency feedings were dispensed manually at a time of day which coincided with refilling of the automated feeder.

Experimental tanks were connected to an aquaculture system in which water was recirculated through mechanical, carbon, and ultraviolet filters. Water temperature was maintained at 28.29 ± 0.35°C and salinity at 26.40 ± 0.18 ppt. Water quality was monitored twice daily at 0600 and 2000 CST. Temperature, salinity, pH, dissolved oxygen, ammonia, nitrite, and alkalinity were monitored using Hach© (Hach Co., Loveland CO, USA) test strips and a YSI© 566 MPS probe (YSI Inc., Yellow Springs OH, USA). Dissolved oxygen was maintained at 5.28 ± 0.71 ppm, and pH was maintained at 8.17 ± 0.35 throughout the experiment. Ammonia ranged from 0-0.5 ppm and nitrite from 0-3.0 ppm. Siphoning and mortality counts were conducted daily at 0500 and 1800 CST. Every two days, six randomly selected larvae from each tank were measured (TL) alive using the ProRes Capture Pro microscope imaging program (JENOPTIK Optical Systems LLC, Jupiter FL, USA). At 25 DPH all tanks were harvested and larvae were enumerated. In addition, one hundred randomly selected live larvae from each tank were measured (TL) using the ProRes Capture Pro microscope imaging program (JENOPTIK Optical Systems LLC, Jupiter FL, USA).

Automated feeder

A total of three automated feeders per tank were used for the 2 h feeding frequency replicates. The automated feeders were constructed from four 22.7-L plastic buckets with three 0.32 cm holes drilled into the bottom. An appropriately sized fitting was attached to each of the holes in the bucket and a 91-182 cm length of 0.32 cm diameter vinyl tubing (Pentair Aquatic Eco-Systems Inc., Apopka FL, USA) connected each of the three holes to solenoid valves (Xylem Inc., Rye Brook NY, USA). Tubing from the solenoid valves was placed into tanks via delay on/off relays (Dayton Co., Dayton OH, USA) to control the volume of *Artemia* dispensed into each tank at each feeding. The relays were connected to on/off timers (Pentair Aquatic Eco-Systems Inc., Apopka FL, USA) set for the designated feeding intervals. The feeders were constantly aerated using an air stone to ensure that the *Artemia* were evenly distributed within the buckets. Two liter containers of ice were maintained in the feeders to ensure that the *Artemia* remained cool enough to retain their enrichment.

Data analysis

Mortality, Specific Growth, and the Coefficient of Variation in Length (CV Length) were examined as Repeated Measures ANOVA's within the Linear Mixed-Effects Models (MIXED; LMM) procedure in SPSS ver. 18.0.0. The LMM procedure was selected because of its ability to address correlated data (e.g., repeated measures), heterogeneous data via alternative covariance structures, unbalanced experimental designs, and time-dependent covariates (i.e., changes in density) (SPSS, Inc. 2005). Mortality was calculated based on daily total mortality counts normalized by the number of larvae presumed alive (i.e., per capita number of dead larvae) over the 10 day period (i.e., repeated factor levels = 10). Numbers of larvae presumed alive were derived by subtraction of recovered dead larvae. Specific Growth was calculated as a log ratio of the mean lengths between the 2 day time interval (i.e., $\ln(\text{length}_{\text{time2}}/\text{length}_{\text{time1}})/2$ day) to estimate daily growth rates over five time intervals (i.e., repeated factor levels = 5).

Coefficient of Variation (CV) in Length (CV Length) was based on the lengths of 6 fish measured for each tank at each of 6 daily time points, separated by 2 day intervals (i.e., repeated factor levels = 6), as CV = (100 × (SD (length)/MEAN(length)) (Zar 1984). Individual tanks were regarded as subjects within the LMMs. Factors for the LMM RM ANOVA models used in this study included Density (2 levels), Feeding (2 levels) and Day (repeated, fixed). The Diagonal covariance structure, which is the default structure for repeated effects, was used for the LMMs. The restricted maximum likelihood (REML) method helped determine overall model significance, and significance of the model terms was determined by F-tests.

A one way ANOVA was conducted on the harvested number of fish to determine significant differences between treatments with concern to production.

Results

Per capita mortality was significantly lower for the 30 fish L^{-1} density treatment than for the 15 fish L^{-1} density treatment ($F = 61.13$, $P = <0.001$) (Figure 7). Although the 2 h feeding frequency treatment appeared to have lower per capita mortality than the 8 h frequency treatment, the difference was not significant ($F = 0.08$, $P = 0.791$). The Density × Feeding interaction also was not significant ($F = 1.16$, $P = 0.321$). Mortality did vary significantly across dates ($F = 31.68$, $P < 0.001$).

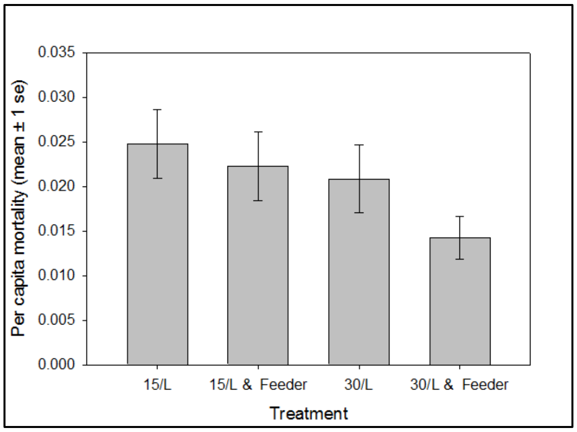


Figure 7. Per capita mortality for each treatment (15 fish/L, 15 fish/L & feeder, 30 fish/L, and 30 fish/L & feeder) for larval *Cynoscion nebulosus*. Treatments labeled with "& feeder" represent the 2 h feeding schedule. Values represent mean ± 1 SE.

Specific growth was significantly higher for the 30 fish L^{-1} density treatment than for the 15 fish L^{-1} density treatment ($F = 4.84$, $P = 0.036$) (Figure 8). However, Specific Growth was not different between feeding frequency treatments ($F = 1.41$, $P = 0.244$). Again, the Density × Feeding interaction was not significant ($F = 0.02$, $P = 0.900$), but Specific Growth did vary significantly across dates ($F = 107.99$, $P < 0.001$).

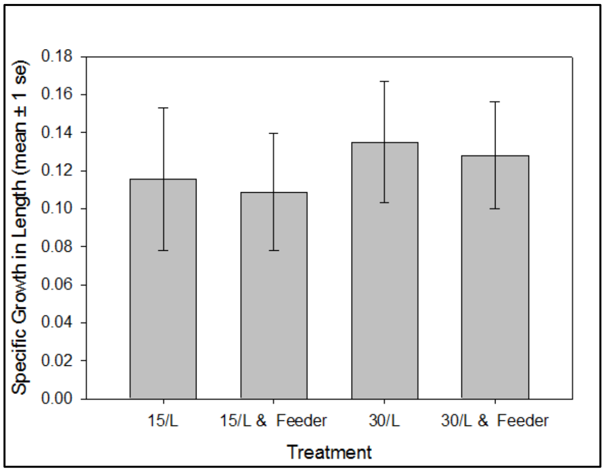


Figure 8. Specific growth for each treatment (15 fish/L, 15 fish/L & feeder, 30 fish/L, and 30 fish/L & feeder) for larval *Cynoscion nebulosus*. Treatments labeled with "& feeder" represent the 2 h feeding schedule. Values represent mean ± 1 SE.

Size heterogeneity was undetectable relative to all LMM terms: differences in CV Length were not significant relative to Density (F = 0.05, P = 0.821), Feeding (F = 0.98, P = 0.328), the Density × Feeding interaction (F = 0.01, P = 0.925), and Day (F = 1.35, P = 0.318). However, CV Length was visually lower for the 2 h feeding frequency treatment, suggesting that more frequent feeding may lead to lower size heterogeneity (Figure 9).

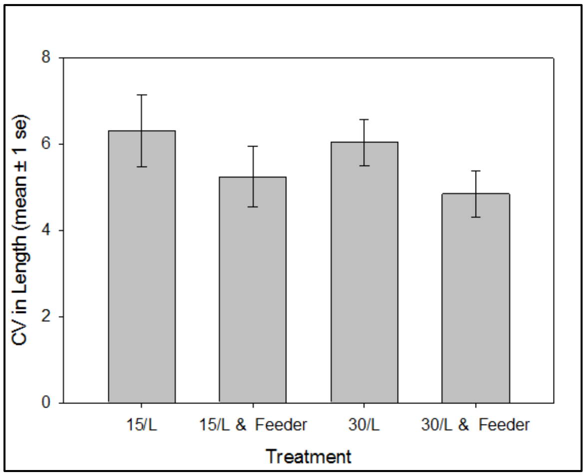


Figure 9. Coefficient of variation for each treatment (15 fish/L, 15 fish /L & feeder, 30 fish/L, and 30 fish/L & feeder) for larval *Cynoscion nebulosus*. Treatments labeled with “& feeder” represent the 2 h feeding schedule. Values represent mean ± 1 SE.

There were no significant differences among the four treatments in the numbers of larvae produced (F = 0.46, P = 0.725) (Figure 10). Nevertheless, the 30 fish L^{-1} density treatment did exhibit 42% higher production than the 15L density treatment, and the 2 h feeding frequency treatment exhibited 15% higher production than the 8 h frequency treatment.

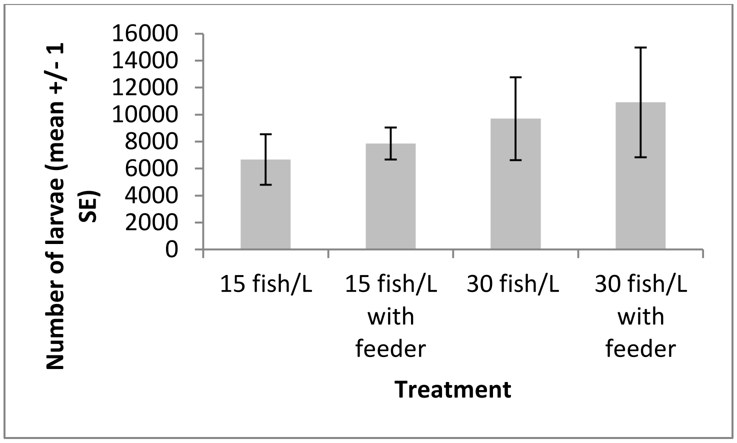


Figure 10. The number of *Cynoscion nebulosus* larvae harvested for each treatment (15 fish/L, 15 fish/L & feeder, 30 fish/L, and 30 fish/L & feeder). Treatments labeled with "& feeder" represent the 2 h feeding schedule. Values represent mean ± 1 SE.

Discussion

Per capita mortality was significantly lower and Specific growth significantly higher in the 30 fish L^{-1} than in the 15 fish L^{-1} treatment. The 30 fish L^{-1} treatment also yielded 42% greater production in terms of absolute numbers of larvae at the end of the experiment. These trends might reflect lower levels of aggression and cannibalism in the high density treatment (Manley et al., 2014). A stocking density threshold was observed in small-scale studies of larval Spotted Seatrout, wherein stocking density and aggression were positively correlated until densities reached 30 fish L^{-1}, above which aggression and cannibalism did not increase (Manley et al., 2014). In this study, it appears that fish at densities of 30 fish L^{-1} might have exhibited lower aggression leading to a lower per capita mortality and higher Specific growth. A possible explanation for this trend is that high stocking densities moderate the practicality of territoriality and social dominance, thus leading to less aggression and cannibalism (Macintosh and De Silva, 1984). Another possible reason for less aggression and cannibalism at higher stocking densities is that a "schooling effect" might prohibit the cannibal or aggressor from seeking out and targeting an individual (Miki et al., 2011). A similar trend in which larval finfish showed higher survival at high stocking densities due to less aggression was shown for Atlantic Cod (*Gadus morhua*) and Greater Amberjack (*Seriola dumerili*) (Baskerville-Bridges and Kling, 2000; Miki et al., 2011). Higher specific growth in the 30 fish L^{-1} density treatment also might indicate less stressful conditions, as suggested by lower per capita mortality as a potential reflection of lower aggression in the high density treatment. Stress has been shown to reduce growth for both Eurasian Perch (*Perca fluviatilis*) and in Rainbow Trout (*Oncorhynchus mykiss*) (Jentoft et al., 2005).

Unfortunately, logistical constraints precluded using more than two replicates per treatment combination for this large-scale production run, thereby limiting the statistical power of some comparisons. However, even the non-significant differences may point to biological significance. For example, although not significant, per capita mortality appeared lower for the 2 h feeding frequency treatment than for the 8 h frequency treatment. The lower per capita mortality for the 2 h treatment was consistent with the observed 15% higher production. Again, this trend may have been caused by less aggression and cannibalism in the high feeding frequency treatment. The lower per capita mortality for the 2 h treatment also is consistent with the results from small-scale experiments which showed that feeding every 2 h minimized aggression by keeping the larvae sufficiently satiated (Manley et al., in review). By maintaining larvae sufficiently satiated, aggression and cannibalism are diminished because the larvae are not seeking out an alternative (conspecific) food source or vying for access to a temporally limited food supply.

The coefficient of variation of length (CV) is an indicator of size heterogeneity as an outcome of growth depensation within the culture units. Size heterogeneity is a known determinant of cannibalism and aggression in an aquaculture setting (Baras and Jobling 2002, Hecht and Pienaar, 1993). There is a positive correlation between size heterogeneity and cannibalism because size heterogeneity facilitates the consumption of smaller fish. In species like the Sharptooth Catfish (*Clarias gariepinus)*, size heterogeneity was shown to be the main factor controlling cannibalism (Baras and d'Almeida, 2001). In the present study, there were no significant differences in the CV of length relative to density or feeding frequency. Thus, the benefits of stocking at higher densities were not diminished by greater size heterogeneity relative to lower densities. Also, although not significant, an apparent lower CV of length for the 2 h feeding frequency treatment suggests that feeding more often may lead to less size heterogeneity. A possible explanation for this apparent decrease in CV might be that increased feeding frequency enables all the larvae to feed more equitably.

Aside from the biological benefits of stocking 30 fish L^{-1} and feeding every 2 h, there also are economic benefits. The feeding protocol at CMAC is based on tank volume instead of the number of larvae, allowing for the same amount of feed to be given for any stocking density. By feeding based on tank volume allows the cost of production between a 15 fish L^{-1} and a 30 fish L^{-1} stocking density run to be the same. Also, the cost of feeding every 8 h and every 2 h is the same because the total amount of food administered is the same, and with the help of automated feeders, the amount of labor is the same. Having the same operating cost coupled with an increase in production, stocking at 30 fish L^{-1} and feeding every two h is economically ideal. For example, during 2012 the larval rearing production runs at TMAC, utilizing a stocking density of 15 fish L^{-1}, produced a total of 236,703 larvae. The cost of Spotted Seatrout juveniles is unknown, however Cobia juveniles (*Rachycentron canadum*), which are cultured under similar conditions, are sold at \$1.00-\$2.50 per juvenile (Kaiser and Holt 2005). By applying the cost of cobia juveniles at \$2.50/juvenile to Spotted Seatrout in 2012, CMAC would have produced \$591,758 worth of Spotted Seatrout. The data from this study suggest that by stocking at 30 fish L^{-1} production would increase by 42%. If there was a 42% increase in production in 2012, TMAC would have produced 108,883 more juveniles with a monetary increase of \$272,208. By feeding every 2 h, production would increase 15%, leading to 35,505 more larvae with a monetary increase of \$88,764. The combined total production when stocking at 30 fish L^{-1} and feeding every 2 h would on average be 381,041 larvae valued at \$952,602.50.

Conclusion

The results of the present study resonate with the findings from previous small-scale experiments, and these findings together support the conclusion that stocking at high densities and feeding more often may enhance the yield of larval Spotted Seatrout. Interestingly, the present study supports the idea that benefits of reduced aggression and cannibalism observed in the previous small-scale experiments can translate to a production-scale system. From these studies, it is evident that a minimum stocking density of 30 fish L^{-1} and a feeding frequency of every 2 h is an appropriate protocol for commercially raising larval Spotted Seatrout. Assuming a lower per capita yield is acceptable, increasing the stocking density to 30 fish L^{-1} led to a 42% increase in production, and increasing the feeding frequency to every 2 h led to a 15% increase in production. Using the same quantity of feed at higher densities and implementing higher feeding frequencies using an automated feeder maintains the same cost of production compared to the standard protocol. By using high stocking densities and feeding ever 2 h in turn promotes an increase in yield without incurring an increase in the cost of production, thereby increasing the potential profit margin for the facility utilizing a protocol involving a higher stocking density and more frequent feeding.

CHAPTER V

GENERAL CONCLUSION

Marine finfish make up a major part of the worldwide consumer demand for fishery products. Only a small percentage of marine finfish is supplied through aquaculture, putting an enormous pressure on wild fisheries (FAO, 2010). The main cause for the lack of aquaculture production is the inability to mass produce juveniles for use in commercial grow out facilities (Lee and Ostrowski, 2001). A primary determining factor for this juvenile bottleneck is low survivorship in the larval rearing stages of production caused by suboptimal nutrition and aggression among conspecifics. The high prevalence of aggression is especially true for Spotted Seatrout in intensive systems. To sustainably fulfill consumer demand and alleviate the pressure on the capture fisheries, more marine finfish must be produced through aquaculture. To assist in accomplishing this goal, methods to reduce aggression-related mortalities in larval Spotted Seatrout were investigated in this research project.

In Chapter II, the author showed that increases in stocking density lead to an increase in aggression and cannibalism in larval Spotted Seatrout when densities change from 15 fish L^{-1} to 30 fish L^{-1}. However, at densities higher than 30 fish L^{-1} aggression and cannibalism leveled off. A similar positive relationship between stocking density and aggression was found for Fat snook (*Centropomus parallelus*) (Correa and Cerqueira, 2007). The positive relationship between stocking density and aggression may reflect increased encounters with conspecifics at higher stocking densities in turn leading to increased aggression. Conversely, in greater amberjack (*Seriola dumerili*) there was an inverse relationship between stocking density and aggression (Miki et al., 2011). This might have been due to a swarming effect in which high densities prevent a likely cannibal or aggressor from singling out a target, thus decreasing the likelihood of an aggressive act. Both outcomes may be true for Spotted Seatrout, resulting in a nonlinear relationship between density and aggression. At low densities there was a positive relationship between stocking density and aggression; however, at densities of 30 fish L^{-1} or higher a cannibal may not be able to single out a target. These findings are crucial because they indicate that Spotted Seatrout can be cultured at densities higher than previously thought.

In all three density treatments there was a significant increase in aggression with time since feeding. This trend may be attributed to a decrease in the amount of planktonic live feeds available to the larvae. During periods of low food availability, larvae may more readily switch to an alternative food source, which in an aquaculture setting includes conspecifics. Similarly, it was concluded that food availability was the main determining factor in aggression in Japanese flounder (*Paralichthys olivaceus*) (Dou et al., 2000). These findings suggest that aggression in larval Spotted Seatrout is mediated by hunger, and more frequent feedings may lead to longer satiation times that would translate into reduced aggression.

In Chapter III the author showed that increasing feeding frequencies alone does not reduce aggression or cannibalism. However, a balance exists between where larvae must be fed enough to be fully satiated and how they can be fed often enough to ensure constant satiation. As found in Chapter II, satiation plays a crucial role in controlling aggression and cannibalism in larval Spotted Seatrout. An increase in satiation level will deter cannibalism and aggression because the need for satiated larvae to target an alternative food source (i.e., conspecific) is reduced. Also, the feeding should be frequent enough to complement gut evacuation times. Feeding before the gut is fully evacuated will foster a minimum continuous level of satiation and repress the motivation to seek an alternative food source. Wuenschel and Werner (2004) concluded that Spotted Seatrout raised in similar conditions to this study underwent gut evacuation 2-4 h after feeding. Accordingly, in the present study the 2 h feeding frequency treatment appeared ideal because it promoted satiation and coincided with gut evacuation. These results are crucial because they demonstrate that both satiation and gut evacuation are critical components when trying to minimize aggression in larval Spotted Seatrout.

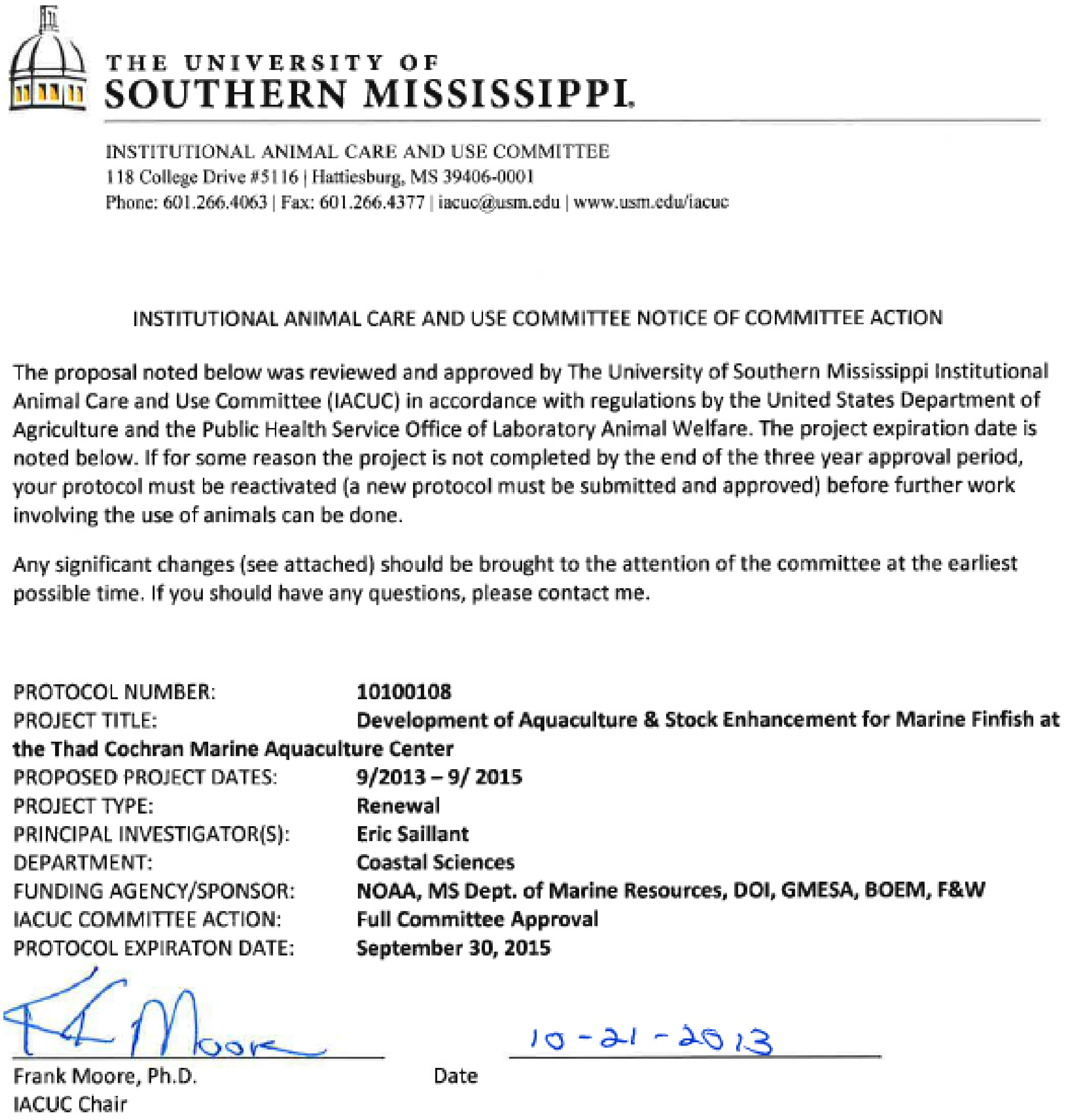
The first two experiments for this project were performed in small-scale aquaria, leaving no way to determine if these results would translate to a larger the production-scale setting. Thus, Chapter IV tested the results from the previous two studies against the protocol used at USM GCRL CMAC to determine whether increasing stocking density and feeding frequency improved overall larval production. Interestingly, there was a significantly lower per capita mortality and higher specific growth rate in the high density treatment. Based on the results from Chapter II, the reason for this outcome can be hypothesized to be less aggression in the higher density tanks, although aggression levels were not measured for this experiment. Although not significant, the 2 h feeding frequency treatment was consistent with a reduction in per capita mortality which also may have contributed to increased larval production. Even though low statistical power hampered the author’s ability to detect statistically significant differences, the directional trends still appeared to be biologically significant and commercially relevant. Any increase in production of larvae is ideal in a commercial hatchery where the cost of production of marine larvae is high.

In terms of economics, assuming the market price cost per Spotted Seatrout juvenile is \$2.50, increasing the stocking density to 30 fish L^{-1} would lead to an increase in the production of larvae valued at \$272,208.45, and feeding every 2 h, would lead to an \$88,763.63 increase in larval production.

When raising Spotted Seatrout larvae in intensive systems, it is imperative to feed often enough to foster satiation and to feed in concert with gut evacuation rates to reduce aggression and cannibalism. Also, raising larvae at densities of 30 fish L^{-1} or higher appears to decrease aggression and increase the production of larvae. The work conducted for this thesis has afforded valuable insights into the causes of cannibalism in the larviculture of Spotted Seatrout and offered potential modifications of current methodology for reducing aggression and cannibalism in Spotted Seatrout and other marine fishes.

APPENDIX A

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE



THE UNIVERSITY OF
SOUTHERN MISSISSIPPI.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
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INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER:	**10100108**
PROJECT TITLE:	**Development of Aquaculture & Stock Enhancement for Marine Finfish at the Thad Cochran Marine Aquaculture Center**
PROPOSED PROJECT DATES:	**9/2013 – 9/ 2015**
PROJECT TYPE:	**Renewal**
PRINCIPAL INVESTIGATOR(S):	**Eric Saillant**
DEPARTMENT:	**Coastal Sciences**
FUNDING AGENCY/SPONSOR:	**NOAA, MS Dept. of Marine Resources, DOI, GMESA, BOEM, F&W**
IACUC COMMITTEE ACTION:	**Full Committee Approval**
PROTOCOL EXPIRATON DATE:	**September 30, 2015**

FR Moore

Frank Moore, Ph.D.
IACUC Chair

Date 10-21-2013

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