SURVIVAL AND GROWTH OF JUVENILE HATCHERY-REARED SPOTTED SEATROUT, *Cynoscion nebulosus*, STOCKED INTO REARING PONDS AT 2 AND 9 DAYS POST-HATCH

A Thesis

by

MANOR GURY

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

Of concern to recreational fisheries is the growth and survival of hatchery-reared sport fish released for stock enhancement purposes. This study was conducted to evaluate the effect of water quality/source on growth and survival of larval spotted seatrout (*Cynoscion nebulosus*) reared in captivity. Trials were conducted both indoors under controlled conditions and in outdoor tanks. For the indoor trials, seatrout eggs were stocked into replicated (n = 4) 83-L tanks at 50,000 eggs/tank for either 2 or 9 days post-hatch (DPH). During this period, tanks received either filtered seawater, raw seawater, or filtered seawater containing marine microalgae. There was no significant difference ($P>0.05$) in survival of either 2 DPH (49.95 ± 13.6%) or 9 DPH (30.73 ± 14.2%) larvae regardless of water source. However, a significant difference was observed between the growth rates of 9 DPH larvae, as the larvae in the clear water treatment were significantly smaller ($P = 0.01$) than the two other treatments. Outdoor tanks (13,000-L) were stocked with 1,500 larvae (2 DPH or 9 DPH) (n = 3) which were reared for 30 days. Growth (TL) and survival rates in the outdoor tanks approximated 1.06 and 1.41 mm/day and 11 and 7.5%, respectively. There was no significant difference ($P =0.15$) in survival which was similar between the two age groups; a highly significant growth rate difference was shown. Results from this preliminary research indicate that current protocols used to rear juvenile spotted seatrout can be improved by increasing indoor rearing period.

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Introduction

Recreational Value

The spotted seatrout (*Cynoscion nebulosus*) (referred to hereafter as seatrout) is one of the most economically important sport fish species in the U.S., found on the eastern Atlantic and Gulf coasts (Perret et al. 1980; Bortone 2003). In the years 2011-2015, with a yearly decline, 8.9 to 11 million anglers spent around $23 billion dollars annually on fishing in the U.S. (Lovell et al. 2013; NOAA 2016). In Texas, about $1.54 billion was spent on fishing trips and equipment (Sabrina et al. 2013). Specifically, this amounted to 2.2 million anglers, residents and nonresidents. Most recreational fishing targets seatrout, accounting for more than 12% of the total catch in the recreational sector. Over the last 10 years, between 15 and 36 million seatrout were caught annually, with an average catch of 29 million fish per year. In 2015, nearly 17 million seatrout were caught (NOAA 2016). In Texas alone, recreational anglers fishing for seatrout spent over $220 million annually (Vega et al. 2011).

Natural Life History and Geographical Distribution

Seatrout is an important species, ecologically, as a predator of invertebrates and small fish species and, conversely, as a prey item for sharks, dolphins and other large estuarine predators (Gold et al. 2003). The seatrout lives its entire life in the estuaries and nearshore waters (Wuenschel et al. 2004) as an integral member of those ecosystem communities. Geographical distribution of this species extends from Massachusetts in the eastern Atlantic through the Gulf of Mexico, along the 400 miles of Texas coast, down the Mexican coast (Pattillo et al. 1997). The waters of the northern Gulf of Mexico from Florida to south Texas serves as habitat for most of the population (Brown-Peterson and Warren 2001). Seatrout, unlike other members of the Sciaenidae, live most of their life in the same estuary system in which they were spawned (Helser

et al. 1993; Blanchet et al. 2001). Thus, each estuary system population can be considered a different genetic population (King et al. 1995). Stock enhancement programs related to this species need to consider this genetic variation. In order to prevent mixing, genetic strains from each estuarine population should be propagated separately. For instance, the Texas Parks & Wildlife Department (TPWD) saltwater hatcheries program uses distinct seatrout broodstock representing specific Texas bay systems for stock enhancement purposes (King et al. 1995).

Survival Rates of Hatchery-Reared Larval Seatrout

Studies show that in nature there is an average daily mortality rate of 38% of seatrout larvae during the first few weeks post-hatch (Peebles and Tolley 1988). In a study by Houde and Taniguchi (1982), seatrout larvae were reared on different first-food sources, at different feed concentrations and reared at different seawater temperatures. Results showed that at 28°C, larval survival rates were between 10.9% and 80.5% when fed at a rate of 25 and 5,000 rotifers/L, respectively. Lemus et al. (2010) examined survival and growth rates of seatrout reared at various live-feed densities. This study demonstrated a mean survival rate of about 58% for 6 DPH seatrout fed a diet of 20 rotifers/mL. The Texas Parks & Wildlife Department (TPWD) saltwater hatcheries program has an average survival rate of seatrout eggs to 2 DPH of around 75% (R. Chavez, TPWD, personal communication). The survival rate of seatrout from the larval stage to the fingerling stage (35 mm TL), in TPWD rearing ponds, is between 20-30% (Vega 2011). Peebles and Tolley (1988) also studied growth as length of seatrout in Florida bays. They found that the combined larval growth rate was approximated 0.4 mm/day. Additionally, Houde and Taniguchi (1982) determined a mean growth rate for all treatments of about 0.33 mm/day for hatchery-reared seatrout larvae reared on 5 rotifers/mL. Houde and Taniguchi (1982) reported that seatrout

larvae have one of the fastest growth rates reported among marine fish larvae growing in the wild. Their study showed that seatrout larvae maximum growth rate was 76.5%/day at 32°C about 0.62 mm/day.

Feeding Behavior

The feeding behavior of larval seatrout is well documented in the scientific literature. During the period of endogenous nutrition, the hatched larvae depend on their yolk sac. As soon as they develop their digestive track and mouth parts, they seek new sources of food and start exogenous feeding (Rønnestad et al. 1999). Larval swimming abilities are limited and they mainly rely on food sources that are located within one to two body lengths (Arnold et al. 1978). Densities of available prey items play a major key to the survival of the newly hatched larvae. In the study by Houde and Taniguchi (1982) densities of live food prey organisms were tested in tanks and demonstrated that 1,000 organisms/L yielded good results. Higher live-food densities in the study did not improve survival rates. However, lower growth and survival rates were observed when low densities of live-food items were examined. Studies (Howell 1979; Scott and Middleton 1979; Jones et al. 1981; Naas et al. 1992) that used microalgae during the first-feeding of marine fish larvae found a wide variety of factors beneficial to larval survival and growth. Those studies showed that water enriched by microalgae ("green" water) increased the growth and survival rate of different species fish larvae. Larval culture improvements were thought to be derived either from the larvae consuming the microalgae (Moffatt 1981) or via nutritionally-rich rotifers in the larval tank (Howell 1979; Scott and Middleton 1979). In addition, Reitan et al. (1993) demonstrated that the rotifers which consumed the microalgae had higher levels of both protein and lipid as compared to rotifers in the clear water tanks. Similar results have been reported in other studies (Øie et al.1994, 1995). Nicolas et al. (1989) suggested that microalgae in

the water may affect the microbial community in the water, which can contribute to the establishment of an early gut flora in the larvae. Seatrout larvae, as with most fish larvae, are visual feeders (Wuenschel and Werner 2004). The addition of microalgae to larval tanks can provide a better visual contrast by light reflections of particles in the water, which allow the larvae to visualize their prey (Naas et al. 1992). All the factors mentioned above may contribute to improving the first-feed of fish larvae (Reitan et al. 1997).

Rationale for Study

The U.S. Gulf coast human population was reported to be 44.2 million in 1995 (EPA 2014); by 2014, the population number had increased to 59.3 million (U.S. Census Bureau 2015) and estimates are that there will be an increase to 61.4 million by 2025, representing a nearly 40% increase. Texas and Florida are the most rapidly growing states (EPA 2014). As the coastal human population grows, fishing pressure on coastal fish populations can be expected to also increase. One fisheries management tool is the use of stock enhancement hatcheries to rear larvae for longer periods indoors (e.g., 9 DPH) to determine whether larval and subsequent juvenile survival rates can be increased. My study was partly designed to further examine the experiment conducted by Naas et al. (1992) in which seatrout larvae were reared in "green" water vs. "clear" water to compare growth and survival rates. By rearing seatrout larvae in green water, further insight into the role of microalgae in larval growth and survival might be gained.

Objectives

The objectives of this study were to evaluate the effects of 1) extended indoor hatchery rearing periods; 2) adding microalgae as food source and color contrast to rearing tanks; and 3) stocking different age groups into outdoor tanks for grow-out on growth and survival of larval seatrout. The goal of this study is to advance current

larviculture methods/protocols used by fish hatchery operations to culture fingerling and juvenile seatrout.

Material and Methods

Study Site and Source of Fish

This study was conducted at the Texas A&M AgriLife Research facility in Flour Bluff, TX. Seatrout eggs were provided by the TPWD Coastal Conservation Association Marine Development Center (MDC), also in Flour Bluff, TX. At the MDC, captive seatrout brood fish were held in hatchery tanks and subjected to standard photoperiod and seawater temperature regimes to induce spawning (McCarty 1990). After spawning, the eggs were collected and placed in a graduated cylinder (liter) in order to separate fertilized from the unfertilized eggs. Numbers of fertilized eggs were calculated volumetrically, with 1 mm of eggs in the cylinder equals 1,700 eggs, and the eggs were transferred into the experimental tanks at the Texas A&M AgriLife facility. In order to minimize effects of external variables (e.g., egg quality and broodstock variables), all the tanks for each trial were randomly stocked from the same egg source, and separated equally into each tank.

Experimental Design

Indoor Experiment

In order to describe seawater treatments, the term "clear" water refers to seawater from the upper Laguna Madre filtered by a diatomaceous filter, chlorinated to 15 ppm (minimum duration of 30 min), UV-sterilized and stored until used. "green" water refers to seawater from the upper Laguna Madre with the addition of *Nannochloropsis* sp. (microalgae) introduced into culture water to achieve a cell concentration of 3.5×10^5 cells/mL. “raw" water refers to seawater obtained from the upper Laguna Madre without any filtration or disinfection, in order to mimic seawater

from the natural environment. This water was stored in ponds until use. Seatrout eggs were stocked into experimental tanks (83 L total volume) at a density of 600 eggs/L or 50,000 eggs/tank (equivalent to the stocking density in the incubation tanks at the TPWD hatchery). Larvae were reared in three separate water sources: clear, green, and raw. Each water source was used to test larvae reared at 2 and 9 DPH. Treatments were replicated four times for each water source × rearing period combination (Table 1). Large raceways (40,000 L) located in nearby greenhouses, functioned as separate water reservoirs for each water source. There was no addition or replacement of larvae during the trial period. After two days, the larvae that were grown for the 9 DPH trials were fed live-food (rotifers) for the next seven days of the indoor tank trials (see *Feeding and Preparation*). A photoperiod of 24 hours light was maintained during the trials. Water quality factors (e.g., temperature, salinity, pH, dissolved oxygen) were monitored daily. Water quality conditions were kept within appropriate ranges for the culture of larval seatrout (Wuenschel and Werner 2004). Measurements were made once a day from each of the experimental tanks.

Outdoor Experiment

Seatrout larvae that were harvested from the indoor experiment were stocked into six outdoor experimental tanks (13,000 L total volume) at a density of 0.1 larvae/L or 1,500 larvae/tank (equivalent to the stocking density in the hatchery ponds). The tanks were filled with raw seawater, five days prior stocking (see *Feeding and Preparation*). All the larvae for this experiment, both 2 and 9 DPH, came from the same indoor "Raw" water tank trial which had the highest growth and survival during the indoor experiment (one tank from the 2 DPH and different tank for 9 DPH, from the same trial). Treatments were replicated three times for each rearing trial period. Dechlorinated fresh water was added, when needed, to the system to compensate for

evaporation. Larvae were grown in experimental tanks to the age of 30 DPH (28 and 21 days, respectively, for the 2 and 9 DPH larvae). There was no addition or replacement of larvae during the trial period. During the study period, temperature, salinity, pH, dissolved oxygen (DO), were monitored once a day. Water quality factors were kept within appropriate ranges for the culture of seatrout (Wuenschel and Werner 2004).

Trials

To minimize effects of variables such as egg quality and physiological/somatic differences among brooders, trials were conducted using eggs from similar broodfish spawns. Each of the six treatments had four replicates. Four replicates per treatment were used in the study to achieve adequate statistical power. A total of three separate trials (Fig. 1) were conducted in the study. Each trial compared growth and survival rates of seatrout larvae reared for periods of 2 and 9 DPH in clear, green, and raw seawater. The experimental control was larvae reared in "clear" water until 2 DPH, which was similar to that used for seatrout by the TPWD hatchery.

Each experimental trial had six different treatments, as shown in Table 1. The larvae were harvested from experimental tanks at the end of each trial period and transported to outdoor tanks for 30 DPH grow-out using established procedures (Colura et al. 1976). This experiment had 4 x 3 x 2 treatment combinations (24 experimental tanks) as shown in Fig. 2.

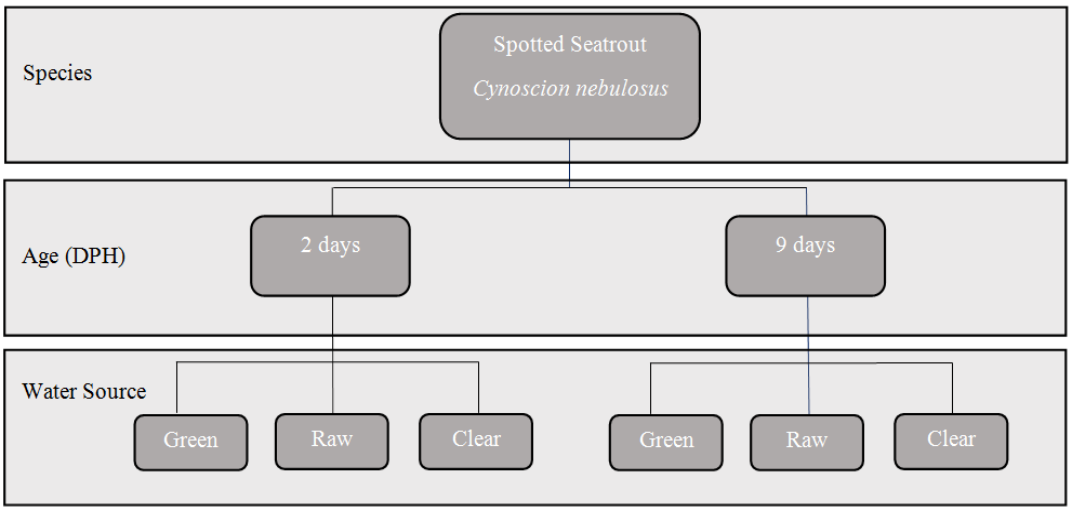
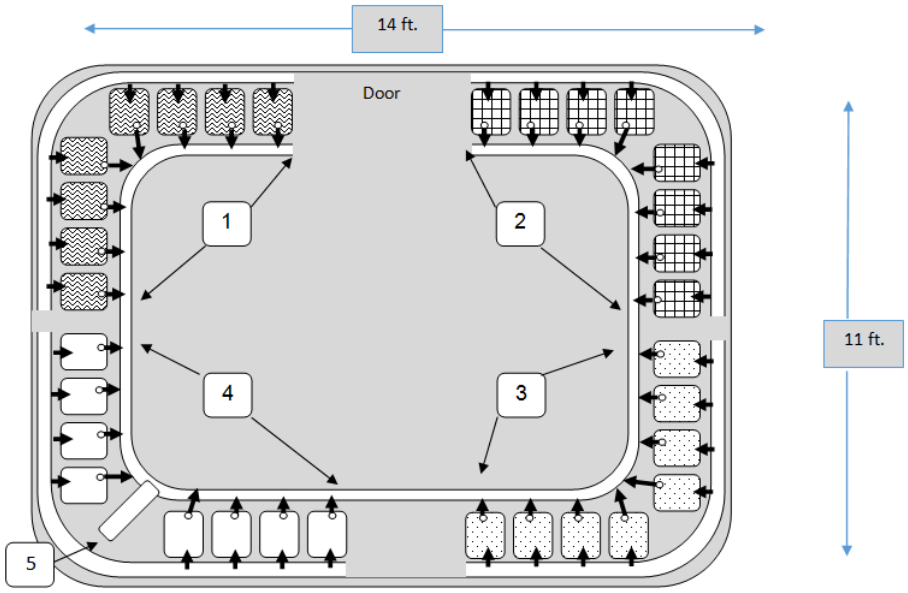


FIGURE 1. *Indoor experiment: experimental design showing the combinations between water sources and rearing period time.*



1. Green water - 8 tanks	2. Raw water - 8 tanks
3. Clear water - 8 tanks	Extra 8 tanks
5. Main Drainage	

FIGURE 2. *Indoor Experiment: experimental system layout.*

Feeding and Preparation

Indoor Experiment

Rotifers, *Brachionus* spp., were used as the main source of live prey for seatrout larvae. As mentioned in the study by Wuenschel and Werner (2004), rotifers can support seatrout larvae from the first-feed stage up to a size of 5 mm length. Rotifers were obtained from Reed Mariculture, Inc. (Campbell, CA) and reared in three conical, 140-L tanks following standard hatchery protocol. Each day, 30-50% of the rotifer population was harvested to feed the larvae and maintain population health. Every three days, all the rotifers were harvested and moved to a new clean tank to prevent bacterial growth. At 2 DPH, seatrout larvae received a rotifer diet at a density of 30-40/mL, about 3 million organisms per tank per day.

Outdoor Experiment

The six outdoor tanks were connected by pumps (bucket with 200-micron mesh net to prevent larvae from escaping, but allowing algae and zooplankton to be dispersed evenly between the tanks. Pump (Hydor model 320 centrifugal pumps with 1,200 liter/hour rate, 1/2" PVC pipe, and 12 L/min flow restrictor) had a water flow of 12 L/min for a 13,000 liter (3435 gallon) tank, and a full tank water turn-over rate every 19 hours (Fig. 3). The tanks were filled five days prior to being stocked, and fertilized with inorganic (urea - 32% N, and phosphoric acid - 54% P) to supplement the algae and zooplankton in the tanks (Colura et al. 1976). Initially, each tank was seeded with 4 million rotifers to establish a standard population of prey items. Twice a week a water sample was taken from each tank to estimate zooplankton densities in the outdoor tanks. Standard hatchery zooplankton sampling protocols were followed. At 10 DPH, the tanks received the addition of dry feed, Rangen Salmon Starter™, to offset the decline of the zooplankton in tanks. Body weights of the larvae were estimated (hatching occurred in treatment tanks) and the larvae were fed at a rate of 10% of their body mass daily. Half of the feed was delivered during the morning, and half in the afternoon.

Harvesting Larvae and Fingerlings

Indoor Experiment

Larvae were harvested by draining tanks into a 50-liter harvest basin. Samples were taken from each tank in order to determine final survival rate and mean total length (n = 20 subsample).

Outdoor Experiment

Harvest from outdoor tanks was accomplished by slowly draining water into the main drainage basin and catching fish in a net. Fish that did not drain out of the tanks were collected by hand. All fish in each treatment tank were counted to estimate the

final survival rate. A total of 30 fingerlings from each tank were collected randomly throughout the harvest, to assess mean specific growth rate.

Larval Enumeration and Growth Indices

Enumeration of seatrout larvae from tanks and prior to stocking into outdoor rearing tanks was conducted using 10 subsamples of 10-mL water each from the indoor harvest box. Each sample was taken after the water was well mixed, in order to have a representative sample of the larvae density in the water. For determination of growth indices, a random sample of 20 larvae from each tank was collected, diagonally across the surface of the harvest box. After collection, the larvae were anesthetized using MS-222, and preserved in chilled water until measured. In the laboratory, the larvae were measured using a dissecting microscope (model Olympus SZX12), Olympus U-CMAD3 camera, and the computer software program QCapture Pro, by QImaging©. The picture image of the larvae was taken at a magnification level of seven. Notochord Length (NL) was measured from the anterior edge of the dorsal jaw to the end of the notochord (Lemus et al. 2010). A growth index was calculated using standard length (SL) measurements.

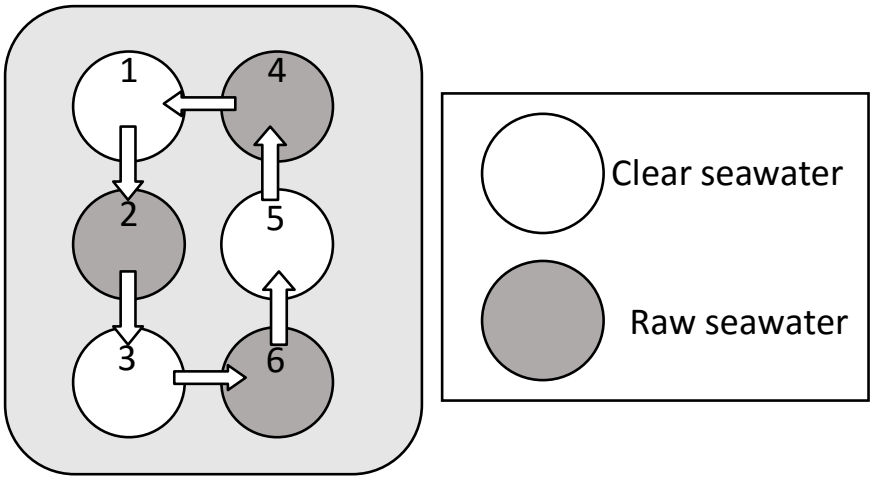


FIGURE 3. *Outdoor Experiment: experimental system layout. The arrows represent the pump moving water from tank to tank (see feeding and preparation - outdoor experiment).*

Fingerling Enumeration and Growth Indices

At harvest (30 DPH), a random sample of 30 fingerlings from each tank was collected for total length and weight measurements to obtain growth trend data. The fingerlings were anesthetized using MS-222 and placed in iced-water. Individual fingerlings were wet-weighed on an analytical scale. After weighing, the fingerlings were placed on ice and taken to the laboratory for length measurements. Length-specific growth rates were determined for fingerling samples by calculating the difference between mean SL for individuals collected on consecutive sampling days from each tank. Growth rates for each sample interval were computed using the equation: GR = (L/T) where GR is the growth rate (mm/day), and L represent the median length (mm TL) of fish samples at harvest (T) (Ricker 1979). This equation was used because eggs were hatched within the same treatment tanks (i.e., no initial weight determination). Coefficient of condition (K) was calculated using the formula: $K = (W*10^5)/L^3$, where W is weight in grams, and L is standard length in mm (Anderson and Neumann 1996). Fish samples that were used to determine number of fingerlings were also used to measure and calculate fingerling growth rates.

Statistical Analysis

The independent variables in the indoor experiment were the different water sources and rearing period of the larvae and, for the outdoor experiment, were the different rearing periods of the indoor experiment prior to stocking in the outdoor tanks. Dependent variables for both experiments were percent survival and growth rate based on length. A one-way fixed ANOVA was performed for each outcome variable. The ANOVAs were conducted as mixed models, for each indoor growth period (2 and 9 DPH) separately: water source (clear vs. green vs. raw water) as fixed factors and trial and tank as random factors. Interactions between water source and growth period were evaluated at significance (α) level of 0.05. In the case of non-significant interactions, *post hoc* tests comparing all levels of the main factors was performed using Shaffer's adjustment of the Tukey HSD procedure (Shaffer 1986; Dickhaus 2012). A significance level of $\alpha = 0.05$ for the *post hoc* comparisons was used. In the case of significant interactions, separate one-way mixed models using either water source or rearing period as fixed factors with trial and tank as random factors were performed for each level of the remaining fixed factor. All models were checked for normality of residuals and homogeneity of variance. All statistical analyses were conducted using R version 3.2.2 and R studio. Individual R packages used in the analysis include nlme version 3.111 (Pinheiro et al. 2013), multcomp version 1.3-1 (Hothorn et al. 2008), and MuMIn version 1.9.13 (Barton 2013), lme4 version 1.1-12 (Banta et al. 2010), car version 2.1-4 (Erickson and Nosanchuk 1977).

Results

Indoor Experiment

Water Quality

Water quality factors (Table 2) were maintained in recommended ranges for growth of seatrout larvae throughout the indoor experimental trials. The mean values

for tank seawater temperature, dissolved oxygen, salinity, and pH were 27.9 ± 1.3 °C (22.5 - 29.5 °C), 5.6 ± 0.2 mg/L (5.1 - 6.6 mg/L), 35.0 ± 0.2 (35 - 36 ppt), and 8.6 ± 0.1 (8.5 - 8.8), respectively.

Survival Rates

The mean survival rate for 2 DPH larvae for all indoor experimental trials (Table 3) was 50±20 % (19 - 89%), with Trial 1 being 45±14 % (19 - 75%), Trial 2 53±20 % (30 - 73%), and Trial 3 62±22% (37 - 90%). Mean survival rate for 9 DPH larvae for all indoor trials was 30±12 % (11 - 65%), with Trial 1 being 29±4 % (19 - 33%), Trial 2 20±9 % (11 - 33%) and Trial 3 51±10 % (40 - 65%).

The numerically lowest larval survival rate in Trial 1 for 2 DPH occurred in the "raw" water tank and in the "green" water tank for the 9 DPH larvae. The numerically highest survival rates for both 2 and 9 DPH were recorded from the "raw" water tanks. For Trial 2, the lowest survival rate for 2 DPH was found in one "green" water tank, whereas for 9 DPH, it was found in one "clear" water tank. The highest survival rate for 2 DPH occurred in a "raw" water tank, whereas for 9 DPH, in a "green" water tank. Trial 3 had the lowest survival rates for both 2 and 9 DPH, both in "clear" water tanks. The highest survival rates for both age groups occurred in "raw" water tanks.

Table 1. *Indoor Experiment: Water quality measurements (range) of the indoor tanks for all three trials.*

Trial #	Water Measurement	Units

Trial 1	Temperature (°C)	22.5 - 29.5
	Salinity (‰)	34 - 36
	Dissolved oxygen (mg/L)	5.0 - 6.6
	pH	8.5 - 8.8
Trial 2	Temperature (°C)	26.9 - 28.3
	Salinity (‰)	36 - 40
	Dissolved oxygen (mg/L)	4.7 - 6.3
	pH	8.3 - 8.6
Trial 3	Temperature (°C)	25.3 - 28.3
	Salinity (‰)	33 - 36
	Dissolved oxygen (mg/L)	5.0 - 6.4
	pH	8.6 - 8.8

ANOVA showed no significant interaction ($P<0.05$) between DPH and water source. Therefore, the analyses were performed for both factors together. For both 2 and 9 DPH age groups the survival rates were not significantly different among the three water sources ($P\geq0.1$).

Table 2. *Indoor experiment: The tanks with the lowest and highest survival rates of seatrout larvae for each of the target ages, by water source, in parentheses are the values.*

Trial #	DPH at harvest	Highest Survival	Lowest Survival
Trial 1	2	Raw (75.11%)	Raw (19.24%)
	9	Raw (32.98%)	Green (10.99%)
Trial 2	2	Raw (89.77%)	Green (54.96%)
	9	Green (33.89%)	Clear (29.31%)
Trial 3	2	Raw (73.28%)	Clear (30.23%)
	9	Raw (65.04%)	Clear (40.30%)

Length Sizes and Growth Rates

Mean larval length at harvest for 2 DPH larvae for all trials (Table 4) was 1.71 ± 0.13 mm (1.48 - 1.88 mm). Trial 1 produced mean larval at lengths of 1.72 ± 0.14 mm (1.48 - 1.81 mm), Trial 2 of 1.67 ± 0.1 mm (1.56 -1.78 mm) and Trial 3 of 1.71 ± 0.15 mm (1.56 -1.88 mm). Mean length at harvest for 9 DPH larvae for all trials was 2.71 ± 0.21 mm (2.14 - 3.24 mm), with means for Trial 1 of 2.83 ± 0.26 (2.48 - 3.24 mm), Trial 2 of 2.35 ± 0.16 (2.14 - 2.58 mm) and Trial 3 of 2.91 ± 0.16 mm (2.78 - 3.13 mm). The highest growth rates were estimated for 2 DPH larvae reared in a "raw" water tank, and 9 DPH larvae grown in a "green" water tank. As for Trial 2, the lowest growth rate for both 2 and 9 DPH larvae occurred in a "clear" water tanks. The highest growth rates occurred in the 2 DPH "raw" water tank and 9 DPH "green" water tank. Trial 3 had the lowest growth rate for both 2 and 9 DPH larvae recorded from the “Clear” water tanks. The highest growth rate for the 2 DPH larvae occurred in the "clear" water tank, and in the "raw" water tank for 9 DPH larvae.

No significant interaction ($P>0.05$) was found between DPH and water sources. Therefore, the analysis was performed for both factors together. As for the growth rate of the larvae, at 2 DPH no significant differences were found among the different water

sources. However, at 9 DPH, both "green" water and "raw" water treatments produced significantly larger sized larvae ($P<0.01$) than the larvae grown in "clear" water treatments (Fig. 4). There was no difference in standard length between "raw" water and "green" water treatments.

Table 3. *Indoor experiment: The Tanks with the higher and low*er *length sizes (SL) of seatrout larvae by water source, in parentheses are the values in mm.*

Trial #	DPH at harvest	High	Low

Trial 1	2	Raw (1.81)	Clear (1.48)
	9	Green (3.24)	Clear (2.48)
Trial 2	2	Raw (1.78)	Clear (1.56)
	9	Green (2.58)	Clear (2.14)
Trial 3	2	Clear (1.88)	Clear (1.56)
	9	Raw (3.13)	Clear (2.78)

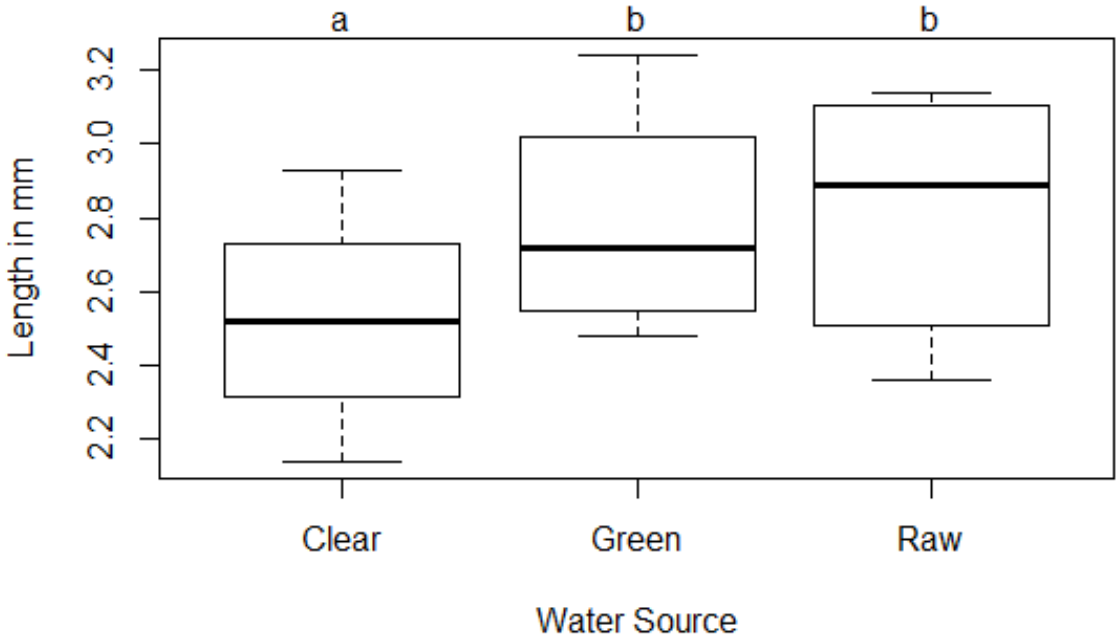


FIGURE 4. *Indoor Experiment: Mean length of 9 Days Post Hatch Spotted Seatrout larvae from different water source. Means with different letters are significantly different (P<0.05).*

Outdoor Experiment

Water Quality

Water quality factors (Table 5) were maintained in the recommended ranges for growth of seatrout larvae throughout the entire outdoor tank trial period. Mean temperature, dissolved oxygen, salinity and pH levels were 30.0 ± 1.2°C (25.3 - 32.2°C), 5.1 ± 0.8 mg/L (1.8 - 8.4 mg/L), 42 ± 1 (40 - 44 ppt), and 9.0 ± 0.1 (8.7 - 9.1), respectively.

Survival Rate

Mean survival rate for fingerlings stocked at 2 DPH was 11 ± 4% (8.6 - 15.6%), whereas those stocked at 9 DPH had a mean survival of 5.3 ± 5.6% (0.9 - 11.7%). Survival rate at the time of harvest was not significantly different between the different age groups *(P>0.15)*.

Length

Mean length of fingerlings stocked at 2 DPH was 31.9 ± 3.9 mm (23 - 41 mm), whereas those at 9 DPH was 42.2 ± 5.1 mm (34 - 59 mm). The length interaction at the time of harvest was significantly different between the different age groups age *(P<0.01)* (Fig. 5).

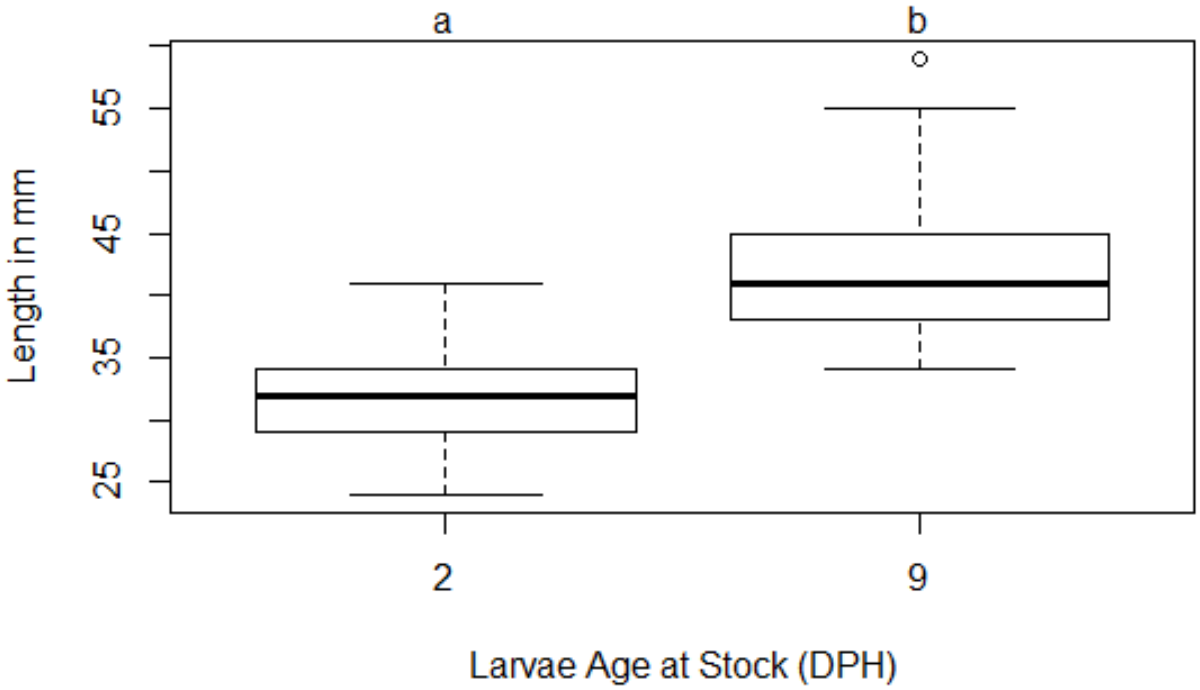


FIGURE 5. *Outdoor Experiment: Mean length of spotted seatrout fingerlings from different age groups. Means with different letters are significantly different (P<0.05).*

Weight

Mean weight of outdoor fingerlings stocked at 2 DPH was 0.26 ± 0.09 g (0.08 - 0.53 g), whereas those at 9 DPH was 0.56 ± 0.22 g (0.27 - 1.32 g). The weight interaction at the time of harvest was significantly different between the different age groups *(P<0.01)* (Fig. 6).

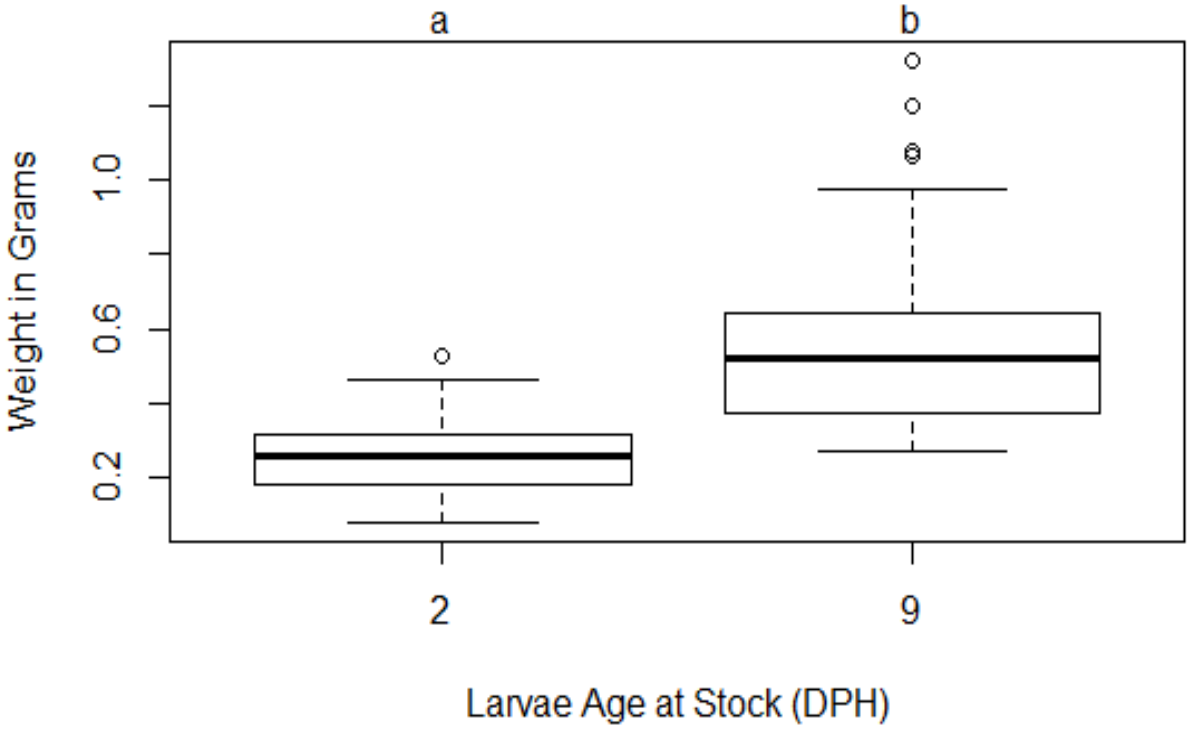


FIGURE 6. *Outdoor Experiment*: *Mean weight of spotted seatrout fingerlings from different age groups. Means with different letters are significantly different (P<0.05).*

Growth Rates

Average growth rate for the fingerlings at the age of 2 DPH was 1.07±0.13 mm/day (0.8 - 1.37 mm/day). As for the fingerlings stocked at the age 9 DPH, the average growth rate was 1.41±0.17 mm/day (1.13 - 1.97 mm/day). Specific Growth Rate interaction at the time of harvest was determined to be significantly different between the different age groups *(P<0.01)* (Fig. 7).

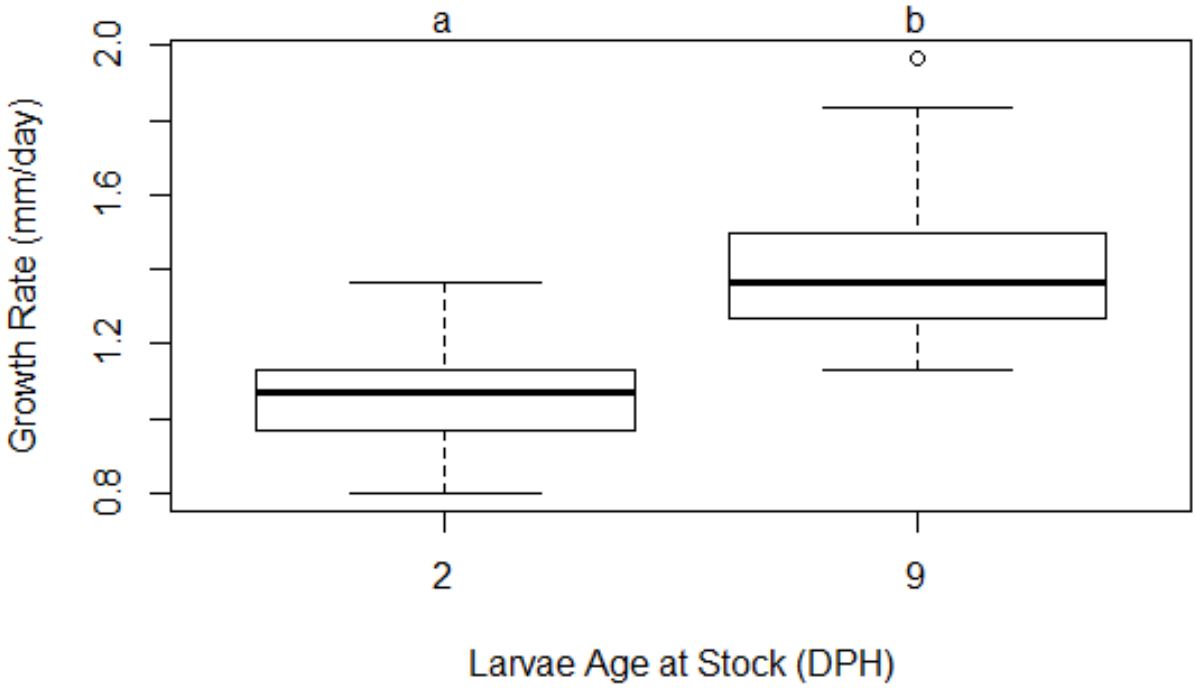


FIGURE 7. *Outdoor Experiment*: *Mean growth rate of spotted seatrout fingerlings from different age groups. Means with different letters are significantly different (P<0.05).*

Fulton's Condition Factor (K)

Average condition factor for outdoor-reared fingerlings at 2 DPH was 0.76 ±0.13 (0.46 - 1.23), whereas those at 9 DPH, was 0.72 ± 0.07 (0.53 - 0.95). K value interaction at the time of harvest was significantly different between the age groups *(P<0.01)* (Fig. 8).

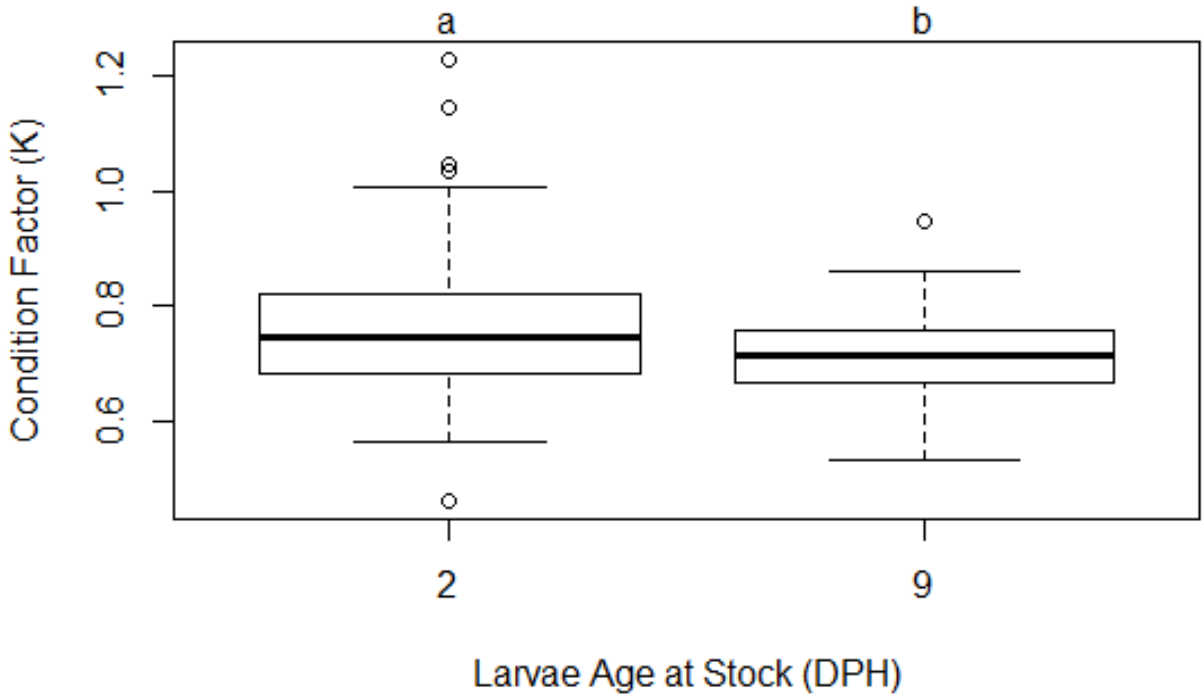


FIGURE 8. *Outdoor Experiment: Mean Fulton's condition factors of Spotted Seatrout fingerlings from different age groups. Means with different letters are significantly different (P<0.05).*

Discussion

Indoor Experiment - Survival Rates

Feeding of early stage fish larvae needs to be frequent as their swimming abilities are limited (Divya et al. 2011), and they can only swim short distances to find food. Larvae that cannot find sufficient amounts of food in the near environment will starve to death. Gopakumar et al. (1999) found that Mauritian anemonefish, *Amphiprion chrysogaster*, larvae reared in indoor tanks and fed with rotifers from first feed to 5 DPH had a 55% survival rate. Rearing fish larvae in a controlled environment beyond their hatching stage as supported by live feed seems practical to support the larvae's feeding needs (Ghan and Sprules 1993; Awaiss and Kestemont 1998). Rotifers were used as a live first feed source to initial feeding because of their small size, high nutritional value, behavior and the fact that they are easy to mass culture (Snell and Carrillo, 1984; Gopakumar et al. 2009). In the present study, seatrout larvae (2 DPH)

fed rotifers during the indoor tank experiment had an average survival of 50%, and 9 DPH larvae averaged a survival rate of 30% The relatively low survival rate in the 9 DPH tanks could have resulted from cohort cannibalism. A study conducted by Manley et al. (2014) found that stocking density affects aggressive and cannibalistic behaviors in larval hatchery-reared seatrout. The study suggested that frequent feedings during the larval rearing stages may reduce aggression and cannibalism, which can result in higher survival and growth rates. In addition, Wuenschel and Werner (2004) determined that due to a fast gut evacuation rate seatrout larvae can be cannibalistic.

Indoor Experiment - Growth

In the indoor tanks system, there was no significant interaction shown among 2 DPH larvae reared in terms of growth regardless of culture water source. One reason for this might be that the nutritional energy of seatrout larvae in the first two days comes from their yolk sac; thus, nutrients derived from water source treatments might not have had an effect. This was not the case with 9 DPH larvae. A study conducted by Divya et al. (2011) evaluated growth and survival of Sebae anemonefish, *Amphiprion sebae*, larvae reared in different water sources supplemented with 10 rotifers/mL. They determined that larvae grown in “green” vs. "clear" water with rotifers were significantly larger. Naas et al. (1992) reported that Atlantic halibut, *Hippoglossus hippoglossus*, larvae had better growth and survival rates when cultured in “green” water as compared to “clear” water. The same trend was observed in the present study in which significantly larger seatrout larvae were harvested from the “raw” and "green" water sources compared to the "clear" water. These results indicate that there are some benefits to growing seatrout larvae using unfiltered or natural water sources with or without plankton rather than filtered water during the first few days of post-hatch development. Naas et al. (1992) found that the larvae in "green" water had a higher

feeding incidence rate during the first days post-hatch compared to "clear" water larvae. This study suggested that several conditions (e.g., particle light refraction visibility) associated with "green" water may have enabled seatrout larvae to search and capture prey organisms.

Outdoor Experiment - Survival Rates

Seatrout fingerlings harvested from the outdoor tanks at 30 DPH had an average survival rate of 11.4% (2 DPH) and 5.3% (9 DPH). As mentioned, cannibalism could be a contributing factor resulting in the relatively low survival rate in the outdoor trials. Another factor could be lack of prey items in the tanks. Wuenschel and Werner (2004) studied the consumption and gut evacuation rates of laboratory-reared seatrout larvae and juveniles. They determined that at 28°C (tank seawater) the gut evacuation rate of a 3.5 to 20.0 mm standard length seatrout was two to four hours. This finding strengthens the hypothesis that lower densities of prey items could cause the lower survival rate. Fincannon (2014) reported that feeding frequency of seatrout should be increased to help reduce cannibalism.

An excess amount of the aquatic insect water boatmen (*Trichocorixa reticulata*) in the outdoor tanks, and their predation on the seatrout larvae was a concern during the trials. Conricote (1996) expressed similar concerns regarding predation of red drum larvae by water boatmen.

Outdoor Experiment - Growth

Outdoor culture ponds are a common method used by aquaculture facilities to grow large numbers of various species of marine and fresh water fishes. One of the most important factors in the efficiency of hatchery operations is to maximize growth rates. Whether it is for commercial sale or stock enhancement purposes, producing a high quality fingerling is a primary goal. This study was designed to determine whether

an indoor treatment, prior to outdoor rearing, would result in larger-sized fingerlings at harvest. However, the results of this study were inconclusive because although seatrout larvae grown to 9 DPH as compared to 2 DPH prior to stocking outdoors were found significantly larger at harvest, their survival rate was lower (5.3%). Jackson et al. (2013) reported that juvenile hatchery-reared seatrout improved their ability to recognize and capture live organisms with increasing experience. This finding supports the current study hypothesis that larvae grown in indoor tanks for a longer period of time and fed live prey can learn to capture live prey. This could have given them an advantage over the 2 DPH larvae in this study. The reason for lower survival is not known.

In case of an overpopulation of water boatmen, some biological oil or other oily substance that is FDA approved for use in an aquaculture setting could be utilized. These types of treatments are used to dispense a thin layer of the substance that will cover the pond surface and asphyxiate the water boatman, and dramatically reduce the water boatman population in the pond.

The coefficient of condition in this study shown a higher value for the 2 DPH tanks compare to the 9 DPH tanks, these results shows that the two treatments had a different diet, partly or fully. The 2 DPH larvae stocked into the outdoor tanks a week prior to the 9 DPH larvae, in this week the 2 DPH larvae were nourished on the natural zooplankton that found in the tank system, while the 9 DPH larvae fed rotifers once a day. Moreover, by the time the 9 DPH larvae were stocked in the outdoor tanks system, the zooplankton population decreased. After the 9 DPH were stocked the feeding of both treatments was the same. This different in the diet might be the reason for the higher value in the coefficient of condition for the 2 DPH.

Conclusions

Results of this study indicated that using unfiltered seawater results in higher yields compared to filtered water. For the outdoor experiment, the 9 DPH larvae had a faster growth rate as compared to the 2 DPH in the outdoor tanks. Application of the finding in this study in an aquaculture-production facility, using raw water for larval culture in most facilities will be economically feasible, if applying raw water for larvae culture, some cost and time can be saved on water treatment. In future studies, water quality monitoring should be performed as frequent as three times a day especially for the outdoor experiment. Also for future studies, a longer period of outdoor pond preparation and fertilization should be implemented before stocking, in order to achieve an initial zooplankton density of at least 250 organisms/L.

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