EXTENDED INCUBATION FEEDING PROTOCOL AND HYPERSALINE ACCLIMATION OF LARVAL SPOTTED SEATROUT (*CYNOSCION NEBULOSUS)*

A Thesis

by

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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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December 2019

ABSTRACT

Spotted seatrout (*Cynoscion nebulosus*) is a popular sport fish and highly targeted by anglers on the Texas coast. To offset pressures by anglers and natural events, Texas Parks and Wildlife Department implemented a stock enhancement program in which hatchery-reared fish are released into the wild to augment natural stocks. One of these hatcheries is located on a hypersaline lagoon resulting in fish often being challenged by hypersaline conditions in outdoor rearing ponds. The objectives of this study were to determine: 1) a feeding protocol for use during extended incubation that produces the highest survival and growth of larvae and 2) whether gradual hypersaline acclimation during extended incubation leads to increased growth and survival of larvae when subjected to hypersaline conditions.

Two feeding trials (12 days each) were conducted to evaluate rotifer enrichments used in the diet of larval spotted seatrout. Treatments in both feeding trials (100 larvae/60 L tank, 4 replicates/treatment) included Algamac-3050®, Easy Dry Selco®, Ori-one®, and RotiGrow Plus®. After the feeding trials, a salinity experiment (t=17 days, 100 larvae/60 L tank, 4 replicates/treatment) was conducted in which larvae were subjected to hypersaline (50 ppt) conditions at 3, 6, 9, and 12 days post hatch (dph). Initial salinity was 35 ppt. Starting at day 3, salinity was raised by 1 ppt per day until larvae were subjected to 50 ppt at their assigned treatment day.

In both feeding trials, survival was significantly ($P<0.05$) affected by treatment. In the first trial, Algamac-3050® had significantly higher survival (75.2±6.3%) than the other three treatments, whereas in the second trial, Algamac-3050® (83.2±2.8%) and Easy Dry Selco® (79.8±6.0%) had significantly higher survival than the other two treatments. In both feeding trials, larvae in the Algamac-3050® treatment exhibited significantly higher growth (i.e., length, weight,

specific growth rate, and percent weight gain) than any of the other treatments. Based on these results, Algamac-3050® was selected for use in the salinity trial.

In the salinity trial, survival was affected by treatment, with larvae exposed to hypersaline (50 ppt) conditions at 3 dph having lower survival (8.2±5.8%) than the other three groups. Survival improved significantly when fish were acclimated until 6 (52.5±7.0%) or 9 (65.0±7.7%) dph, although there was no improvement in survival between 9 and 12 (65.3±7.1%) dph. Growth parameters followed the same trend as survival, with the 3 dph group experiencing the least growth. Acclimation until 6 or 9 dph improved growth, but further acclimation to 12 dph did not yield any further improvement.

The results of this study suggest that larval rearing of spotted seatrout in hypersaline conditions may be improved by increasing the incubation time beyond three days, and by gradually acclimating larvae to a higher salinity during this extended incubation.

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Introduction

The spotted seatrout (*Cynoscion nebulosus*) is a member of the Sciaenidae family, which includes croakers and drums. Spotted seatrout are distributed from Massachusetts to the Bay of Campeche on the Yucatan Peninsula in Mexico and are most abundant in the northern Gulf of Mexico (Pattillo et al. 1997). They are found mainly within estuaries, particularly among areas with seagrass habitats. In Texas estuaries, adults typically spawn from April until October. Spawning usually takes place in areas of deeper water adjacent to shallow areas, especially those with seagrass beds (Neahr et al. 2010).

Recreational saltwater fishing is an economically important industry in the Gulf of Mexico. In 2015, this industry accounted for 15,400 jobs and $1.9 billion in revenues in Texas alone (NMFS 2017a). Spotted seatrout are one of the most popular fish targeted by anglers in the Gulf of Mexico. In 2016, Gulf coast recreational anglers caught 23 million spotted seatrout, which made it the most commonly caught species, as well as the largest harvest by weight (NMFS 2017b). Although it remains a common and popular fish, spotted seatrout landings in the Gulf have decreased by 51% from 2006 to 2015 (NMFS 2017a). Although landings have been increasing since dropping to a low in 2014, the 2016 catch of 23 million was still below the 10-year average of nearly 28 million fish (NMFS 2017b). Texas fisheries managers have utilized a variety of tools to maintain a sustainable spotted seatrout recreational fishery.

Texas Parks and Wildlife Department (TPWD) uses several fisheries management methods for the long-term management of important recreational species. Some of the practices that have been used for the management of spotted seatrout in Texas include long-term coastwide monitoring, a ban on gill nets in bays and estuaries, daily bag and possession limits, slotted size limits, and closure of the commercial fishery (Anderson and Karel 2009). In addition

to these traditional methods, TPWD implemented a saltwater stock enhancement program for spotted seatrout in 1993, in which hatchery-reared juvenile fish are released into the wild to supplement natural recruitment (Vega et al. 2011). The program currently stocks an average of 4.7 million fingerlings into Texas bays annually; production goals are to increase that number to eight million fingerlings annually (McDonald et al. 2013). To date, over 125 million fingerlings have been released into Texas bays (TPWD 2019). Further increases in production are necessary to mitigate population declines caused by factors such as coastal habitat degradation, the state's growing human population and recreational fishing pressure (Vega et al. 2011), as well as natural events such as red tide and freeze events (King et al. 1995). As pressure on spotted seatrout stocks continues to increase, the success of stock enhancement is important to both recreational anglers and coastal communities that rely on recreational fishing as a source of revenue (Vega et al. 2011).

A notable characteristic of this species is their affinity for a home estuary, which they rarely leave (Bortone 2003). Early concerns regarding genetic variation led to the development of a management strategy that recognized three separate populations representative of the upper, middle, and lower Texas coast (King et al. 1995). Additional research has indicated that the genetic structure of the spotted seatrout population in Texas may be different from bay to bay (genetic isolation by distance), leading to a policy in which TPWD returns hatchery-reared fingerlings to the estuary where broodstock were collected (Anderson and Karel 2009). One of the estuaries in which spotted seatrout are stocked by TPWD is the Laguna Madre, a shallow bay that stretches from Corpus Christi to the Rio Grande. The northern portion of the bay is known as the Upper Laguna Madre, while the southern portion is known as the Lower Laguna Madre. The upper and lower portions are divided by an area of mud flats that is only periodically inundated

(Spiller and Blankinship n.d.). The completion of the Gulf Intracoastal Waterway (GIWW) in 1949 allowed for a permanent connection between the upper and lower Laguna Madre (Laguna Madre 1998). There are few connections between the Laguna Madre and the Gulf of Mexico, and freshwater inflows from rainfall and streams are limited. This coupled with high evaporation rates and shallow depths causes the Laguna Madre to be typically saltier than the Gulf, making it a negative estuary, also known as a hypersaline lagoon (Tunnell 2001). The Upper Laguna Madre is consistently saltier than the Lower Laguna Madre and before the dredging of the GIWW would sometimes reach salinities in excess of 100 ppt (Grand 2014). Although the Upper Laguna Madre no longer experiences such extreme high salinity, annual salinity in recent years has been as high as 48.7 ppt, with hypersalinity (salinity above 44.1 ppt) events lasting months or even years (Olsen 2014). Despite high salinity conditions, the Laguna Madre contains about 80% of the remaining coastal seagrass habitat in Texas, making it a crucial nursery ground for many species (Spiller and Blankinship n.d.). Although spotted seatrout are an euryhaline species, extremes of high or low salinity in the estuaries may negatively impact spawning, hatching, and early life stages (Kucera et al. 2002a).

Bottlenecks to Production

Despite the success of TPWD's stock enhancement program, there are several bottlenecks to production. There are only three saltwater hatchery facilities on the Texas coast: Sea Center Texas (SCT) in Lake Jackson (upper coast), Perry R. Bass Marine Fisheries Research Station (PRB) in Palacios (middle coast), and the Coastal Conservation Association Marine Development Center (MDC) in Corpus Christi (lower coast) (Puritz et al. 2014). All three facilities raise fingerlings in outdoor ponds, but only SCT and MDC house broodstock for egg production. Although conditions can be controlled to optimize spawning and egg hatching,

larvae are transferred out of this controlled environment and stocked into outdoor rearing ponds within days (3-4) of hatching (Puritz et al. 2014). One of the major obstacles is that once the larvae are released into rearing ponds, water quality parameters such as temperature, salinity, and pH are subject to ambient environmental fluctuations which may not be optimal for larval grow-out and survival (McDonald et al. 2013).

In addition to environmental challenges, another bottleneck to marine fish production is providing appropriate and nutritionally suitable feeds, especially during early larval stages (Lemus et al. 2010). Many marine species are reared using a combination of rotifers, *Artemia* nauplii, and copepods (Palmtag et al. 2006; Seychelles et al. 2009; Lemus et al. 2010; Lindley et al. 2011). Current TPWD protocols do not require exogenous feeding of spotted seatrout larvae during incubation, since they are stocked into outdoor ponds rich in zooplankton by the time exogenous feeding begins. However, if incubation were to be extended, exogenous feeds would need to be provided during the indoor larval rearing process. Colura et al. (1992) determined that larval spotted seatrout feed on zooplankton such as rotifers, polychaete larvae, copepod nauplii, and copepods when reared in earthen culture ponds. When larval spotted seatrout are reared in environmentally controlled culture tanks, it is vital to provide a source of nutrition to approximate that which they would be consuming in the wild or in earthen ponds. Hendon and Rakocinski (2016) found that early growth is a critical factor in determining survival rates of hatchery-reared spotted seatrout. To promote optimal growth and survival, larvae must be provided with feeds containing appropriate nutrient profiles, especially with respect to essential fatty acids such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) (Seychelles et al. 2009; Ma and Qin 2014; Hache et al. 2017). These essential nutrients are often provided to early larval stage fish by enriching live feeds, such as rotifers,

before feeding them to the larvae. Enrichment nutrients have traditionally been provided to rotifers in the form of a variety of marine microalgae, but in recent years many production facilities have switched to using commercial enrichment preparations because of the high cost, time, and space requirements to produce live microalgae (Ma and Qin 2014) and the variability in nutrient profiles of live microalgae (Palmtag et al. 2006; Seychelles et al. 2009). Studies have shown that regardless of the enrichment type or formulation used, the response of fish larvae (in terms of growth and survival) to different enrichments is species specific (Hache and Plante 2011; Ma and Qin 2014). Although enriched rotifers are used for early larval feeding, they are insufficient for meeting nutrient requirements in later developmental stages. Ludwig et al. (2008) and Lemus et al. (2010) suggested that the earlier larvae are weaned onto *Artemia* nauplii or a dry feed, the better they will perform in terms of growth and survival.

Study Rationale

As there are only three saltwater hatcheries producing spotted seatrout fingerlings for stock enhancement on the Texas coast, it is important that production be optimized. The MDC facility is located on the Upper Laguna Madre from which it obtains seawater for outdoor pond production. As previously described, this body of water often experiences high salinities, particularly in the summer months when spotted seatrout production is at its peak. In the summer months, the salinity of the incoming water at this facility is often above 40 ppt and frequently reaches 50 ppt and above (TPWD, unpublished data). This can result in low survival rates when larvae are stocked into the outdoor plastic lined-ponds at this facility. Improved larval rearing protocols for hypersaline conditions could increase production when pond salinities are beyond optimal environmental conditions for spotted seatrout fingerling production.

Objectives

There have been few studies examining larval acclimation to hypersaline conditions (Genz and Grosell 2011; Laverty and Skadhauge 2012; Lema et al. 2018). Most larval research efforts have focused on rearing spotted seatrout in optimal or brackish conditions (Banks et al. 1991; Colura et al. 1992; Kucera et al. 2002a; Holt and Holt 2003; Wuenschel et al. 2004; Lemus et al. 2010; King 2013; Manley et al. 2014; Manley et al. 2015; Gury 2017). Given the high seawater salinities often encountered at the MDC, larval rearing techniques that improve growth and survival under these conditions need to be investigated. The purpose of the present study was to improve hatchery techniques used to culture spotted seatrout larvae that are reared in hypersaline ponds, with the goal of producing more fingerlings for stock enhancement efforts. The objectives of this study were to (1) compare enrichment preparations fed to rotifers that are fed to larval spotted seatrout during extended tank incubation periods, and (2) determine whether gradual hypersaline acclimation during extended tank incubation increases growth and survival rates of larvae when stocked into hypersaline rearing conditions.

Methods and Materials

Study Site and Source of Spotted Seatrout Larvae

Experiments were performed between November 2018 and June 2019 at the Perry R. Bass Marine Fisheries Research Station (PRB) near Palacios, Texas. Spotted seatrout larvae were produced by broodfish collected from the Laguna Madre and housed at the Marine Development Center (MDC) in Corpus Christi, Texas. Spotted seatrout eggs were obtained from MDC broodfish that were held in 13,250-liter tanks and conditioned to spawn using established temperature and photoperiod manipulations similar to those described by Arnold et al. (1978). Eggs were collected using an egg collector box lined with 300-µm mesh supplied with water

from a surface skimmer located in the spawning tank (Kucera et al. 2002b). Eggs were then transported to the PRB the morning of collection, where they were stocked into a 60-L tank for incubation until hatch-out. The following morning, newly hatched (0 days post hatch) larvae were stocked into experimental tanks.

Experimental Design

In both the feeding studies and the salinity study, newly hatched spotted seatrout larvae were stocked into 66.2 L cylindrical experimental tanks (60 L working volume) at a density of 100 fish per tank. Blue Fortiflex® large capacity plastic buckets made of high-density polyethylene resin from Tractor Supply Company (SKU: 2229804) were used as experimental tanks. The layout consisted of 16 tanks (4 treatments x 4 replicates per treatment) distributed in randomized blocks (Fig. 1, Table 1). Experimental tanks were placed within empty fiberglass tanks (2'h x 6'dia). This was done because the larger tanks could not be removed as they were part of a system that was connected to the plumbing and water supply of the building and had to remain intact.

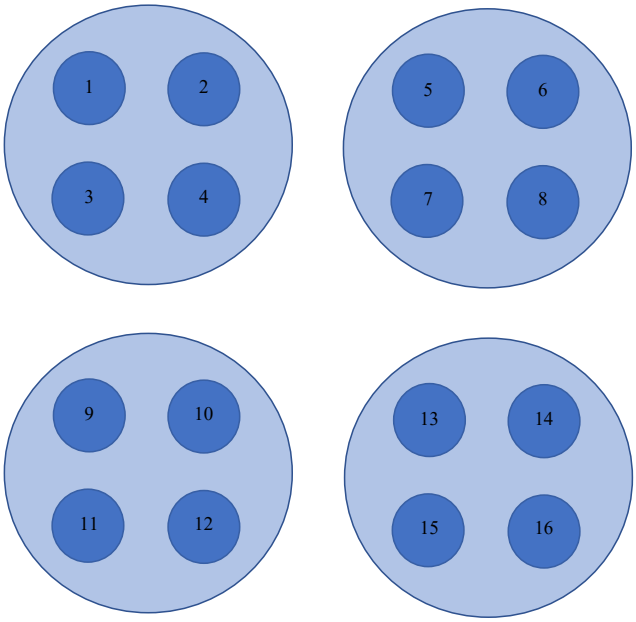

FIGURE 1. Experimental layout. Each group of four tanks contained one of each treatment type.

TABLE 1. Tank treatment assignments for feeding and salinity trials. In the salinity study groups A, B, C, and D were subjected to hypersaline (50 ppt) conditions at 3, 6, 9, and 12 dph, respectively.

Cool Water Feeding Study		Warm Water Feeding Study		Salinity Study	
1	Easy Dry Selco	1	Rotigrow Plus	1	A
2	Ori-one	2	Algamac 3050	2	D
3	Rotigrow Plus	3	Easy Dry Selco	3	C
4	Algamac 3050	4	Ori-one	4	B
5	Algamac 3050	5	Algamac 3050	5	C
6	Easy Dry Selco	6	Rotigrow Plus	6	D
7	Rotigrow Plus	7	Ori-one	7	A
8	Ori-one	8	Easy Dry Selco	8	B
9	Easy Dry Selco	9	Easy Dry Selco	9	A
10	Algamac 3050	10	Ori-one	10	D
11	Ori-one	11	Rotigrow Plus	11	B
12	Rotigrow Plus	12	Algamac 3050	12	C
13	Rotigrow Plus	13	Ori-one	13	C
14	Easy Dry Selco	14	Easy Dry Selco	14	B
15	Ori-one	15	Algamac 3050	15	D
16	Algamac 3050	16	Rotigrow Plus	16	A

Water quality was maintained by 50% daily water exchanges. Ammonia, nitrite, and nitrate levels were monitored daily using Hach test strips (Hach Co., Loveland, CO, USA). Dissolved oxygen (DO), temperature, salinity, and pH were checked daily using a YSI meter (ProPlus-4-15G104158, YSI Inc., Yellow Springs, OH, USA). Dissolved oxygen was maintained by aeration provided via air stones connected to an AMETEK Rotron blower system (Woodstock, NY, USA). Temperature was maintained via a climate-controlled room and Hydor® 100-watt submersible aquarium heaters (Sacramento, CA, USA). Two feeding studies were conducted. The first study conducted was noted as cool (21.4±0.7°C, November 3-15, 2018) and the second was noted as warm (26.2±0.5°C, May 9-21, 2019). Salinity was adjusted as needed by the addition of either softened (<1.5 grains per gallon; Hach total hardness test strips; Hach Co., Loveland, CO, USA) fresh well water (henceforth referred to as treated fresh water) or sea salt (Instant Ocean Sea Salt; Spectrum Brands Inc., Madison, WI, USA). In the feeding studies, salinity was maintained between 34.0-36.0 ppt throughout the experiment. In the salinity study conducted from June 5, 2019 to June 22, 2019, salinity was adjusted based on the assigned experimental treatment. Larvae were subjected to a 12:12 light/dark photoperiod. In both the feeding and salinity study, larvae were fed enriched rotifers, *Artemia* nauplii, and a dry feed (Rangen Salmon Starter #0; Rangen, Inc., Angleton, TX, USA) depending on developmental age. Feeding protocols were developed based on Wuenschel et al. (2004), Manley et al. (2014), and the TPWD pond feeding SOP (Tyler Schacht TPWD hatchery manager, personal communication, August 25, 2018).

Rotifer Culture Systems

Rotifers (*Brachionus plicatilis*, L-type) (Reed Mariculture Inc., Campbell, California, USA) were used for feeding the early stage larvae in both the feeding and salinity experiments.

For both feeding trials and the salinity trial, rotifers were cultured at a salinity of 16-20 ppt and a temperature of 25-27°C. Salinity was maintained by addition of treated fresh water or Instant Ocean sea salt as needed. Temperature was maintained with the use of Hydor® 100-watt submersible heaters.

The rotifer culture system for the cool and warm feeding trials consisted of four 5-gallon (18.93 L) buckets, each set up as a separate static system. Each bucket was filled with 16 L of water and supplied with aeration via air stones connected to an AMETEK Rotron blower system. The four independent rotifer cultures were fed either: (1) Ori-one® powder (manufactured by Skretting, Westbrook, Maine, USA), (2) RotiGrow Plus® liquid (manufactured by Reed Mariculture Inc., Campbell, California, USA), (3 and 4) Nanno 3600® liquid (manufactured by Reed Mariculture Inc., Campbell, California, USA). Rotifers fed Nanno 3600® were harvested and placed in two separate 5-gallon (18.93 L) buckets for enrichment 8 hours prior to feeding to fish larvae. These two batches of rotifers were enriched with either Easy Dry Selco® powder (manufactured by INVE Aquaculture, Salt Lake City, Utah, USA) or AlgaMac-3050® powder (manufactured by Aquafauna BioMarine Inc., Hawthorne, California, USA).

The rotifer culture system for the salinity trial consisted of a single 115 L cylindrical culture tank (working volume 95 L). Aeration and particle suspension were provided via an open-ended airline (1/4-inch inner diameter) connected to an AMETEK Rotron blower system. Continuous supplemental oxygenation was provided by an air stone connected to an oxygen tank, as per TPWD rotifer culture protocol at the MDC. Rotifers were cultured using Nanno 3600® and enriched in separate 5-gallon (18.93 L) buckets using AlgaMac-3050®. Rotifers were enriched for 8 hours prior to feeding to larvae.

Objective 1: Feeding Study

During the first three days after hatching, larvae rely on their yolk-sac as a source of intrinsic nutrition (Banks et al. 1991; Kucera et al. 2002a; Holt and Holt 2003). At three days post hatch (dph), extrinsic feeding generally begins. TPWD's standard operating procedure is that at 3 dph larvae are transported from incubation tanks and stocked into outdoor rearing ponds to begin feeding on zooplankton, thus no source of feed is provided during this brief incubation period. The salinity study required incubation beyond the yolk-sac absorption period; therefore, it was necessary to determine the best feeding protocol that would maximize growth and survival once larvae began extrinsic feeding on rotifers in the incubation tanks.

To develop the best feeding protocol for use during extended incubation of larval spotted seatrout, four different commercial rotifer enrichment products were evaluated. Rotifers were enriched using the following four commercial enrichment products: Ori-one® powder (manufactured by Skretting, Westbrook, Maine, USA), Easy Dry Selco® powder (manufactured by INVE Aquaculture, Salt Lake City, Utah, USA), AlgaMac-3050® powder (manufactured by Aquafauna BioMarine Inc., Hawthorne, California, USA), and RotiGrow Plus® liquid (manufactured by Reed Mariculture Inc., Campbell, California, USA). Enrichment was provided following the manufacturer's instructions. Ori-one® and RotiGrow Plus® served both as rotifer culture diets and enrichments. Easy Dry Selco® and AlgaMac-3050® were enrichments only; therefore, rotifers receiving these two enrichments were cultured on Nanno 3600® and enriched prior to feeding.

Newly hatched (0 dph) larvae (n=100 per tank) were stocked into experimental tanks (n=16; 4 treatments x 4 replicates). Each tank had a volume of 66.2 L and was filled to a volume

of 60 L. Feeding of enriched rotifers to larvae began at 2 dph and continued to the end of the study at 12 dph. Rotifer densities in experimental tanks were maintained at or above 6 rotifers/mL. Rotifers were added to the experimental tanks once daily between 1600-1630 hours.

Artemia nauplii (INVE Aquaculture, Salt Lake City, Utah, USA) were fed to larvae beginning at 5 dph and continued until 12 dph. *Artemia* cysts were hydrated in treated fresh water for 15-20 minutes and then added to a 10 L cone filled with 25-26°C saltwater (18-20 ppt) for hatching. After 24 hours the *Artemia* nauplii were transferred to another 10 L cone for enrichment using Easy DHA Selco® (INVE Aquaculture, Salt Lake City, Utah, USA). After 24 hours of enrichment at 25-26°C and 18-20 ppt, nauplii were fed to fish larvae. *Artemia* densities in experimental tanks were maintained at or above 2 nauplii/mL. *Artemia* were added to the experimental tanks once daily between 1630-1700 hours.

Dry feed (Rangen Salmon Starter #0; Rangen, Inc., Angleton, TX, USA) was fed to larvae beginning at 7 dph and continued until 12 dph. Dry feed was given at a rate of 0.5g twice daily at 0800 and 1600 hours. All treatments received the same feeding regime regarding *Artemia* and dry feed. Both the cool and warm feeding studies were terminated when larvae were 12 dph (i.e., 12 days total time).

At the conclusion of both studies, all fish were euthanized via rapid chilling in an ice bath (AVMA Panel on Euthanasia, 2013) counted to determine survival, weighed, and measured (standard length) to calculate growth and body condition. Thirty (n=30) individuals from each tank were randomly selected for growth measurements. If there were fewer than 30 survivors in a tank, all fish collected from that tank were used. Results from the two feeding trials were used to determine a single rotifer enrichment treatment for use during the salinity study.

Objective 2: Salinity Study

To determine whether a gradual hypersaline acclimation period during an extended incubation increased growth and survival of larvae when stocked into hypersaline conditions, larvae were subjected to various lengths of acclimation during which salinity was increased by 1 ppt per day (Table 2). Larvae (0 dph; n=100 per tank) were stocked into 66.2 L (60 L working volume) experimental tanks (n=16; 4 treatments x 4 replicates). Salinity in all treatments was maintained at 35 ppt until 3 dph. The salinity of the control group (Group A) was increased to 50 ppt at 3 dph to simulate stocking into a hypersaline environment per TPWD protocols. All salinity increases were performed at a rate not exceeding 10 ppt/hour, as this is the acclimation protocol for TPWD when stocking into environments with different salinities (McDonald et al. 2016). Salinity was maintained at 50 ppt for this group for the remainder of the study (i.e., day 17). In Group B, salinity was increased by 1 ppt/day starting at 3 dph and continuing until 6 dph at which time salinity was increased from 38 ppt to 50 ppt to simulate stocking into a hypersaline environment. Salinity was maintained at 50 ppt for this group for the remainder of the study. In Group C, salinity was increased by 1 ppt/day starting at 3 dph and continuing until 9 dph at which time salinity was increased from 41 ppt to 50 ppt to simulate stocking into a hypersaline environment. Salinity was maintained at 50 ppt for this group for the remainder of the study. In Group D, salinity was increased by 1 ppt/day starting at 3 dph and continuing until 12 dph at which time salinity was increased from 44 ppt to 50 ppt to simulate stocking into a hypersaline environment. Salinity was maintained at 50 ppt for this group for the remainder of the study.

TABLE 2. Daily target salinities (ppt) for each treatment group in the salinity study. Actual salinities were within ±0.5 ppt of targets. Groups A, B, C, and D were subjected to hypersaline (50 ppt) conditions at 3, 6, 9, and 12 dph, respectively.

Days Post Hatch	Group A	Group B	Group C	Group D
1	35	35	35	35
2	35	35	35	35
3	50	36	36	36
4	50	37	37	37
5	50	38	38	38
6	50	50	39	39
7	50	50	40	40
8	50	50	41	41
9	50	50	50	42
10	50	50	50	43
11	50	50	50	44
12	50	50	50	50
13	50	50	50	50
14	50	50	50	50
15	50	50	50	50
16	50	50	50	50
17	50	50	50	50

Feeding protocols for fish larvae in this study were similar to those used in the feeding study, however, only AlgaMac-3050® was used as the rotifer enrichment in this experiment. Rotifers were cultured using Nanno 3600® and enriched for 8 hours using AlgaMac-3050® before feeding. Enriched rotifers were fed to larvae beginning at 2 dph and continued until 12 dph. After 12 dph, no additional rotifers were added to the experimental tanks and rotifer densities were allowed to decline as they were consumed. Rotifer densities in the experimental tanks were maintained at or above 6 rotifers/mL until 12 dph. *Artemia* nauplii were hatched and enriched following the same procedures described in the feeding studies above. *Artemia* were fed to larvae beginning at 5 dph and continued until the end of the experiment at 17 dph. *Artemia* densities in the experimental tanks were maintained at or above 2 nauplii/mL. Dry feed (Rangen

#0, <0.6 mm) was fed to larvae beginning at 7 dph and continued until 17 dph. Dry feed was given at a rate of 0.5g twice daily at 0800 and 1600 hours. A larger dry feed (Rangen #1, 0.6-1.0 mm) was introduced at 14 dph and continued until 17 dph. At 14 dph feeding was increased to 1.0g (0.5g #0 plus 0.5g #1) twice daily. All treatments received the same feeding regime. The salinity study concluded when larvae were 17 dph.

At the conclusion of the salinity study, fish were euthanized via rapid chilling in an ice bath (AVMA Panel on Euthanasia, 2013), counted to determine survival, weighed, and measured (standard length) to calculate growth and body condition. Thirty (n=30) individuals from each tank were randomly selected for growth measurements. If there were fewer than 30 survivors in a tank, all fish collected from that tank were used.

Growth Indices

In both the feeding and salinity studies, larval success in different treatment conditions was compared by measuring survival and various growth indices. Percent survival was determined by counting the number of surviving fish in each treatment at the conclusion of the experiment and calculated using the formula 100*(final number of fish / initial number of fish). Thirty individuals (n=30) were randomly selected from each tank at the conclusion of the experiments for growth measurements. If there were fewer than 30 survivors in a tank, all fish collected from that tank were used. Weight (mg) and standard length (mm) were measured (as noted below) and recorded. These measurements were used to calculate Fulton's condition factor (K), specific growth rate (SGR, Ricker 1979), and percent weight gain (PWG). Weight was measured by weighing each fish individually (wet weight) using an analytical laboratory balance (Ohaus, PA124C) to 0.1 mg. To obtain wet weight, a fish was placed on a tared microscope slide

and blotted with filter paper to remove excess water. Blotting continued until filter paper came back dry and all excess water was removed. The slide and fish were then quickly weighed to minimize desiccation from air exposure. Standard length was measured using a microscope (Labomed, Lx400) and a ruled slide with 0.1 mm subdivisions. Fulton's condition factor (K) was calculated using the formula $K = (W*10^5)/L^3$, where W is weight in g, and L is standard length in mm (Anderson and Neumann 1996). Specific growth rate (%/day) was calculated using the equation $SGR = 100\% (\ln W_2 - \ln W_1)/(t_2 - t_1)$, where W_1 and W_2 represent the initial and final weights of fish sampled at t_1 and t_2 (days), respectively (Ricker 1979). Percent weight gain was calculated using the equation $100*(\text{Weight}_{\text{final}} - \text{Weight}_{\text{initial}}) / \text{Weight}_{\text{initial}}$. As fish were too small at hatching to be handled without causing mortality, measurements of initial weight needed for the calculation of specific growth rate and percent weight gain were determined by measuring the weight of 20 randomly selected individuals that were not stocked into experimental tanks. Upon hatching, these fish (n=20) were euthanized by rapid chilling in an ice bath, individual weights measured, and an average initial weight calculated.

Statistical Analyses

A one-way analysis of variance (ANOVA) was used to test for differences in water parameters and live feed densities among treatments in each study. A one-way analysis of variance (ANOVA) was performed to test for differences in survival (%), final standard length (mm), final weight (mg), Fulton's condition factor (K), specific growth rate (SGR), and percent weight gain (PWG) among treatments in each study. If a significant difference ($P<0.05$) in any of these factors was indicated by ANOVA, post hoc testing was performed using Westfall's adjustment of the Tukey HSD procedure (Westfall 1997). Statistical analyses were performed using the statistical software R (Version 3.3.2) © R Foundation.

Results

Feeding Study: Cool Water

Water parameters were measured once daily (n=12). There were no significant differences in temperature (P=0.991), dissolved oxygen (P=0.878), salinity (P=0.992), or pH (P=0.969) between treatments (Table 3). Live feed densities were determined once daily prior to performing water exchanges and feeding. There were no significant differences in rotifer (P=0.992) or *Artemia* (P=0.513) densities between treatments (Table 4).

TABLE 3. Water parameter data for the cool water (avg. temp = 21.4°C) feeding study. Parameters were measured once daily for the 12-day duration of the study.

Treatment		Algamac-3050	Easy Dry Selco	Ori-one	RotiGrow Plus	Study Average
Temperature	Mean	21.4	21.4	21.4	21.5	21.4
(°C)	SD	0.8	0.7	0.7	0.7	0.7
	Range	20.2 - 23.1	20.2 - 23.0	20.2 - 23.1	20.2 - 23.1	20.2 - 23.1
DO	Mean	6.3	6.2	6.2	6.3	6.3
(ppm)	SD	0.5	0.5	0.5	0.5	0.5
	Range	5.3 - 7.1	5.1 - 7.1	5.2 - 7.2	5.2 - 7.1	5.1 - 7.2
Salinity	Mean	35.1	35.1	35.1	35.1	35.1
(ppt)	SD	0.4	0.4	0.4	0.4	0.4
	Range	34.4 - 35.9	34.1 - 36.0	34.3 - 35.8	34.4 - 36.0	34.1 - 36.0
pH	Mean	8.1	8.1	8.1	8.1	8.1
	SD	0.2	0.2	0.2	0.2	0.2
	Range	7.8 - 8.3	7.8 - 8.3	7.8 - 8.3	7.8 - 8.3	7.8 - 8.3

TABLE 4. Feed density data for the cool water (avg. temp = 21.4°C) feeding study. Live feed densities were determined once daily (n=12) prior to performing water exchanges and feeding.

Treatment		Algamac-3050	Easy Dry Selco	Ori-one	RotiGrow Plus	Study Average
Rotifer	Mean	8.47	8.52	8.43	8.41	8.46
Density	SD	1.58	1.54	1.65	1.64	1.59
(rot./mL)	Range	6.67 - 9.67	7.00 - 9.67	6.67 - 9.67	6.33 - 9.67	6.33 - 9.67
Artemia	Mean	3.09	3.05	2.96	3.13	3.06
Density	SD	0.48	0.41	0.43	0.41	0.43
(*Art* ./mL)	Range	2.00 - 4.00	2.33 - 3.67	2.33 - 3.67	2.33 - 4.00	2.00 - 4.00

Survival (%) and growth (i.e., standard length (mm), wet weight (mg), Fulton's condition factor (K), specific growth rate, and percent weight gain) were significantly ($P<0.05$) affected by feeding treatment. Larvae fed rotifers enriched with Algamac-3050® had significantly higher ($P<0.05$) survival than larvae fed rotifers enriched with any of the other three treatments (Fig. 2).

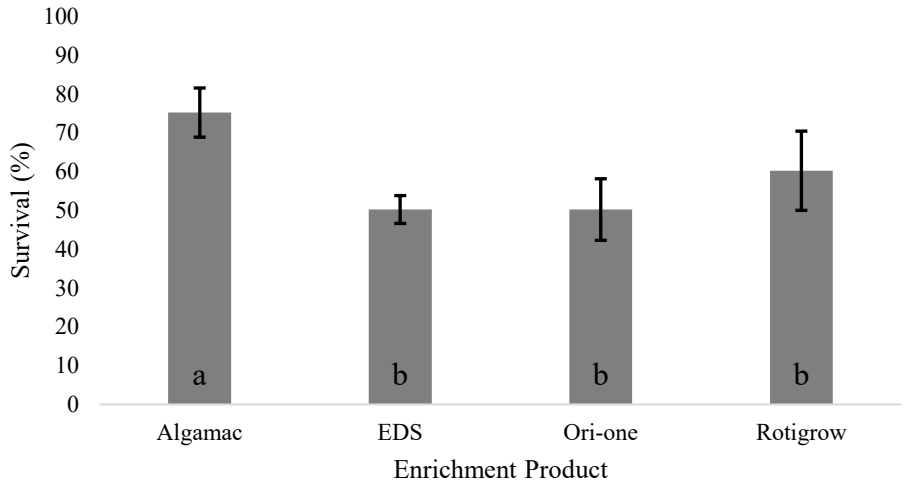

FIGURE 2. Mean (n=4; ± SD) survival (%) of larval spotted seatrout after 12 days fed rotifers enriched with different products, as well as Artemia and dry feed, in the cool water (avg. temp = 21.4°C) feeding study. Means with different letters are significantly different ($P<0.05$).

Larvae fed Algamac-3050® were significantly ($P<0.05$) longer (3.60±0.25 mm), heavier (0.92±0.18 mg), faster growing (16.1±1.9 % weight gain/day), and gained more weight (607±141 %) than any other treatment group (Table 5). There were no significant ($P<0.05$) differences in Fulton's condition factor (K, Table 5).

TABLE 5. Growth indices of larval spotted seatrout fed rotifers enriched using different commercial enrichment products in the cool water (avg. temp =21.4°C) experiment. Superscripts indicate statistical differences between treatments as indicated by Tukey's HSD contrasts.

Enrichment Product		Algamac-3050	Easy Dry Selco	Ori-one	RotiGrow Plus
Standard Length (mm)	Mean	3.6[a]	3.5[b]	3.1[c]	3.2[d]
	SD	0.25	0.27	0.19	0.22
	Range	3.1 - 4.1	3.0 - 4.1	2.8 - 3.7	2.8 - 3.8
Wet Weight (mg)	Mean	0.92[a]	0.83[b]	0.57[c]	0.68[d]
	SD	0.18	0.22	0.18	0.19
	Range	0.4 -1.3	0.4 - 1.3	0.3 - 1.0	0.3 - 1.1
Fulton's Condition Factor (K)	Mean	1.95	1.91	1.83	1.94
	SD	0.17	0.20	0.29	0.24
	Range	1.34 - 2.33	1.34 - 2.33	1.23 - 2.29	1.37 - 2.33
Specific Growth Rate	Mean	16.1[a]	15.2[b]	11.9[c]	13.4[d]
	SD	1.9	2.4	2.8	2.5
	Range	9.4 - 19.2	9.4 - 19.2	7.0 - 17.0	7.0 - 17.8
Weight Gain (%)	Mean	607.1[a]	541.7[b]	337.8[c]	421.2[d]
	SD	140.8	166.4	138.1	148.4
	Range	207.7 - 900.0	207.7 - 900.9	130.8 - 669.2	130.8 - 746.2

Feeding Study: Warm Water

Water quality parameters were measured once daily (n=12). There were no significant differences in temperature ($P=0.230$), dissolved oxygen ($P=0.457$), salinity ($P=0.241$), or pH

(P=0.999) between treatments (Table 6). Live feed densities were determined once daily prior to performing water exchanges and feeding. There were no significant differences in rotifer (P=0.990) or *Artemia* (P=0.987) densities between treatments (Table 7).

TABLE 6. Water parameter data for the warm water (avg. temp = 26.2°C) feeding study. Parameters were measured once daily for the 12-day duration of the study.

Treatment		Algamac-3050	Easy Dry Selco	Ori-one	RotiGrow Plus	Study Average
Temperature	Mean	26.2	26.2	26.3	26.1	26.2
(°C)	SD	0.5	0.5	0.5	0.5	0.5
	Range	25.2 - 27.0	25.1 - 27.1	25.0 - 27.1	25.0 - 27.1	25.0 - 27.1
DO	Mean	5.3	5.3	5.3	5.4	5.3
(ppm)	SD	0.5	0.4	0.5	0.5	0.5
	Range	4.1 - 6.1	4.3 - 6.0	3.9 - 6.0	4.1 - 6.4	3.9 - 6.4
Salinity	Mean	35.1	35.0	34.9	35.0	35.0
(ppt)	SD	0.3	0.3	0.3	0.3	0.3
	Range	34.6 - 35.7	34.5 - 35.8	34.4 - 35.7	34.5 - 35.37/	34.4 - 35.8
pH	Mean	8.2	8.2	8.2	8.2	8.2
	SD	0.3	0.3	0.3	0.3	0.3
	Range	7.9 - 8.8	7.9 - 8.8	7.8 - 8.8	7.8 - 8.8	7.8 - 8.8

TABLE 7. Feed density data for the warm water (avg. temp = 26.2°C) feeding study. Live feed densities were determined once daily (n=12) prior to performing water exchanges and feeding.

Treatment		Algamac-3050	Easy Dry Selco	Ori-one	RotiGrow Plus	Study Average
Rotifer	Mean	8.36	8.36	8.42	8.42	8.39
Density	SD	1.15	1.14	1.24	1.22	1.18
(rot./mL)	Range	7.00 - 8.67	7.00 - 9.00	7.00 - 9.00	7.33 - 9.00	7.00 - 9.00
Artemia	Mean	1.98	2.00	1.93	1.93	1.96
Density	SD	0.85	0.93	0.83	0.86	0.86
(*Art* ./mL)	Range	0.67 - 4.00	0.67 - 4.00	0.67 - 3.67	0.67 - 3.67	0.67 - 4.00

Survival (%) and growth (i.e., standard length (mm), wet weight (mg), Fulton's condition factor (K), specific growth rate, and percent weight gain) were significantly ($P<0.0001$) affected by feeding treatment. Larvae fed rotifers enriched with Algamac-3050® (83.3±2.8%) or Easy Dry Selco® (79.8±6.0%) had significantly higher ($P<0.05$) survival than larvae fed rotifers enriched with Ori-one® (61.5±6.7%) or RotiGrow Plus® (28.3±7.5%) (Fig. 3).

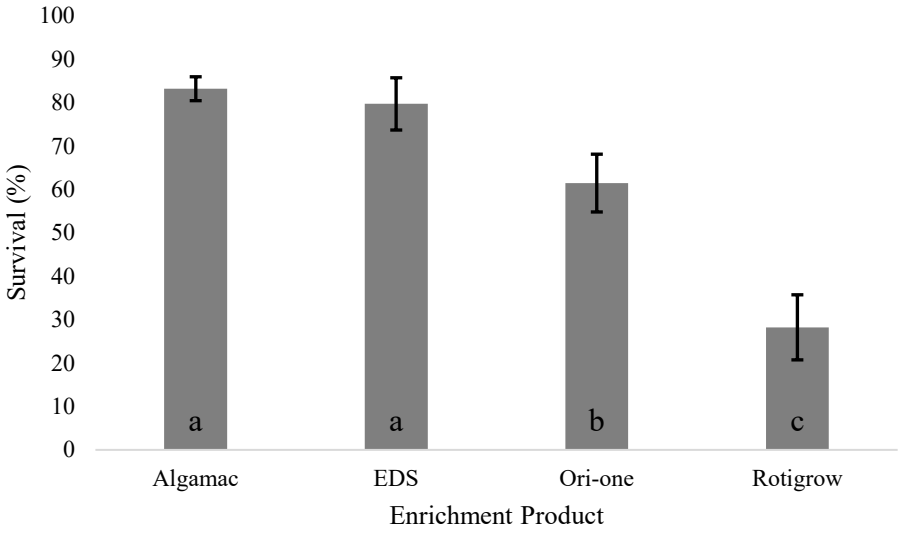

FIGURE 3. Mean (n=4; ± SD) survival (%) of larval spotted seatrout after 12 days fed rotifers enriched with different products, as well as Artemia and dry feed, in the warm water (avg. temp = 26.2°C) feeding study. Means with different letters are significantly different ($P<0.05$).

Larvae fed Algamac-3050® were significantly ($P<0.05$) longer (8.24±0.90 mm), heavier (8.35±1.52 mg), faster growing (35.2±1.6 % weight gain/day), and gained more weight (6,854±1,263 %) than any other treatment group (Fig. 4). There were significant differences ($P<0.0001$) in Fulton's condition factor (K) between treatments. The two treatments that exhibited the least growth, Ori-one® (K=1.99±0.29) and RotiGrow Plus® (K=1.95±0.24), had the highest condition factor, which were not significantly ($P<0.05$) different from each other.

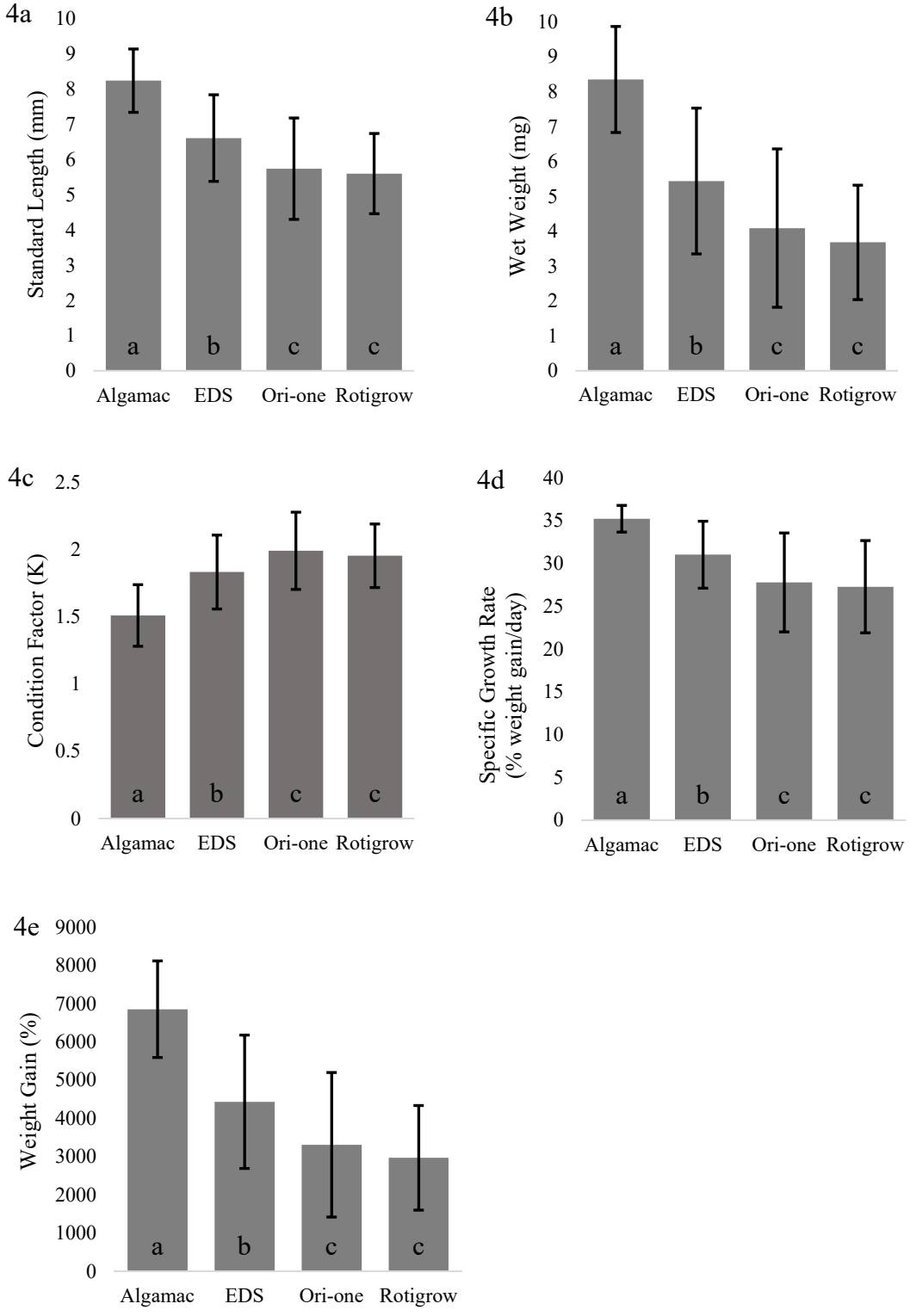

FIGURE 4. Mean (n=4; ± SD) standard length(mm)(4a), wet weight (mg)(4b), condition factor (K)(4c), specific growth rate (4d), and percent weight gain (4e) of larval spotted seatrout in the warm water (avg. temp = 26.2°C) feeding study. Means with different letters are significantly different ($P<0.05$).

The Easy Dry Selco® treatment produced intermediate growth, and a corresponding intermediate condition factor (K=1.83±0.27) that was significantly different from all other treatments. Although Algamac-3050® produced the greatest growth, larvae in this group had a significantly ($P<0.05$) lower condition factor (K=1.51±0.23) than all other treatments.

Salinity Study

Water quality parameters were measured once daily (n=17). There were no significant differences in temperature (P=0.296), dissolved oxygen (P=0.214), or pH (P=0.822) between treatments (Table 8). Salinity was adjusted over time (days post hatch) according to treatment (Fig. 5). Live feed densities were determined once daily prior to performing water exchanges and feeding. There were no significant differences in rotifer (P=0.998) or *Artemia* (P=0.976) densities between treatments (Table 9).

TABLE 8. Water parameter data for the salinity study. Parameters were measured once daily for the 17-day duration of the study. Groups A, B, C, and D were subjected to hypersaline (50 ppt) conditions at 3, 6, 9, and 12 dph, respectively.

Treatment		Group A	Group B	Group C	Group D	Study Average
Temperature	Mean	26.4	26.5	26.5	26.5	26.5
(°C)	SD	0.3	0.3	0.3	0.3	0.3
	Range	25.4 - 26.9	25.6 - 27.0	25.9 - 27.1	25.4 - 27.0	25.4 - 27.1
DO	Mean	4.9	5.0	5.1	5.2	5.1
(ppm)	SD	0.4	0.5	0.5	0.5	0.5
	Range	4.4 - 6.1	4.4 - 5.9	4.4 - 5.9	4.4 - 6.0	4.4 - 6.1
pH	Mean	8.3	8.3	8.3	8.3	8.3
	SD	0.1	0.1	0.1	0.1	0.1
	Range	8.1 - 8.5	8.1 - 8.5	8.1 - 8.5	8.2 - 8.5	8.1 - 8.5

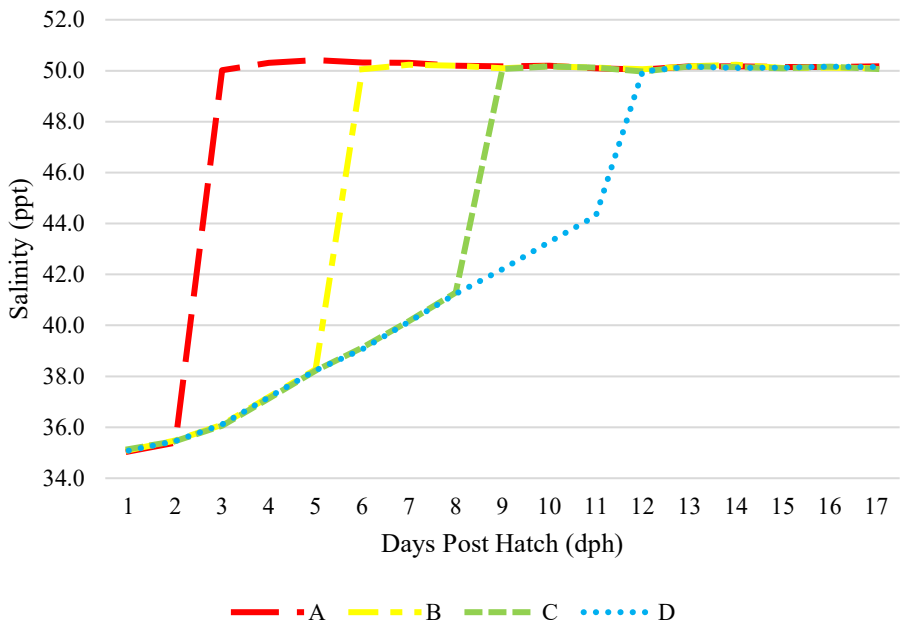

FIGURE 5. Mean (n=4) daily salinity for treatment groups A, B, C, and D in the salinity study. Actual salinities were within ±0.5 ppt of target salinities. Salinity was increased by 1 ppt per day starting at 3 dph for each treatment group prior to a rapid increase (≈10 ppt/hr) to hypersaline (50 ppt) conditions to simulate being stocked into a hypersaline outdoor pond. Groups A, B, C, and D were subjected to hypersaline conditions at 3, 6, 9, and 12 dph, respectively.

TABLE 9. Feed density data for the salinity study. Live feed densities were determined once daily (n=17) prior to performing water exchanges and feeding. Groups A, B, C, and D were subjected to hypersaline (50 ppt) conditions at 3, 6, 9, and 12 dph, respectively.

Treatment		Group A	Group B	Group C	Group D	Study Average
Rotifer	Mean	7.17	7.21	7.20	7.19	7.19
Density	SD	1.53	1.45	1.52	1.52	1.50
(rot./mL)	Range	3.33 - 9.00	3.00 - 9.00	2.67 - 9.00	2.67 - 9.00	2.67 - 9.00
Artemia	Mean	2.20	2.25	2.24	2.19	2.22
Density	SD	0.76	0.86	0.80	0.78	0.80
(*Art* ./mL)	Range	0.67 - 3.33	0.67 - 3.67	1.00 - 3.67	0.67 - 3.67	0.67 - 3.67

Survival (%) and growth (i.e., standard length (mm), wet weight (mg), Fulton's condition factor (K), specific growth rate, and percent weight gain) were significantly ($P<0.0001$) affected by treatment. Group A had significantly ($P<0.0001$) lower survival than all other treatment groups. Survival ranged from 2-15%, with a mean of 8.3±5.9 %. Survival was higher in Group B, with a range of 46-62% and a mean of 52.5±7.0 %. The greatest survival was seen in Groups C (mean of 65.0±7.7 %, range of 57-73%) and D (mean of 65.3±7.1 %, range of 59-75%); there was no significant difference between these two groups (Fig. 6).

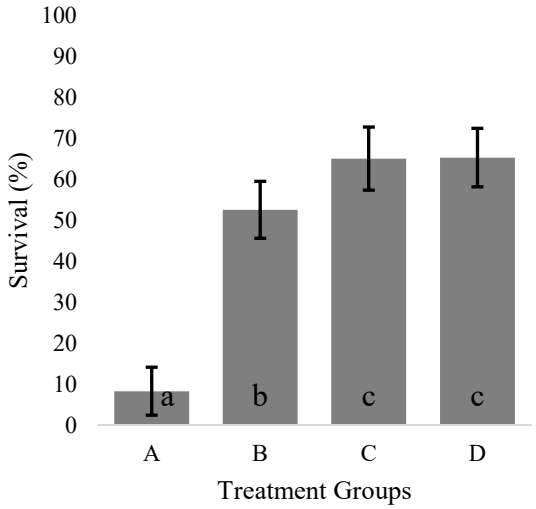

FIGURE 6. Mean (n=4; ± SD) survival (%) of larval spotted seatrout exposed to hypersaline (i.e., 50 ppt) conditions after various acclimation periods in the salinity study. Salinity was increased by 1 ppt per day starting at 3 dph for each treatment group prior to a rapid increase (≈10 ppt/hr) to hypersaline (50 ppt) conditions to simulate being stocked into a hypersaline outdoor pond. Groups A, B, C, and D were subjected to hypersaline conditions at 3, 6, 9, and 12 dph, respectively. Means with different lower-case letters are significantly different ($P<0.05$).

Growth followed a similar trend. Larvae in Group A were significantly ($P<0.05$) shorter, weighed less, had a lower specific growth rate, and lower percent weight gain than any other treatment group (Fig. 7). These growth indices were significantly higher in Group B larvae, and significantly higher in both Groups C and D (Fig. 7). There was no significant difference in growth indices between Groups C and D (Fig. 7). There were significant differences ($P<0.0001$) in Fulton's condition factor (K) between treatments (Fig. 7). The two treatments that exhibited the greatest growth, Group C ($K=1.32\pm0.14$) and Group D ($K=1.31\pm0.10$), had the lowest condition factors and were not significantly ($P<0.05$) different from each other. Group B experienced intermediate growth, and a corresponding intermediate condition factor ($K=1.49\pm0.21$) that was significantly different from all other groups. Group A experienced the least growth, but larvae in this group had a significantly ($P<0.05$) higher condition factor ($K=2.30\pm0.38$) than all other treatments.

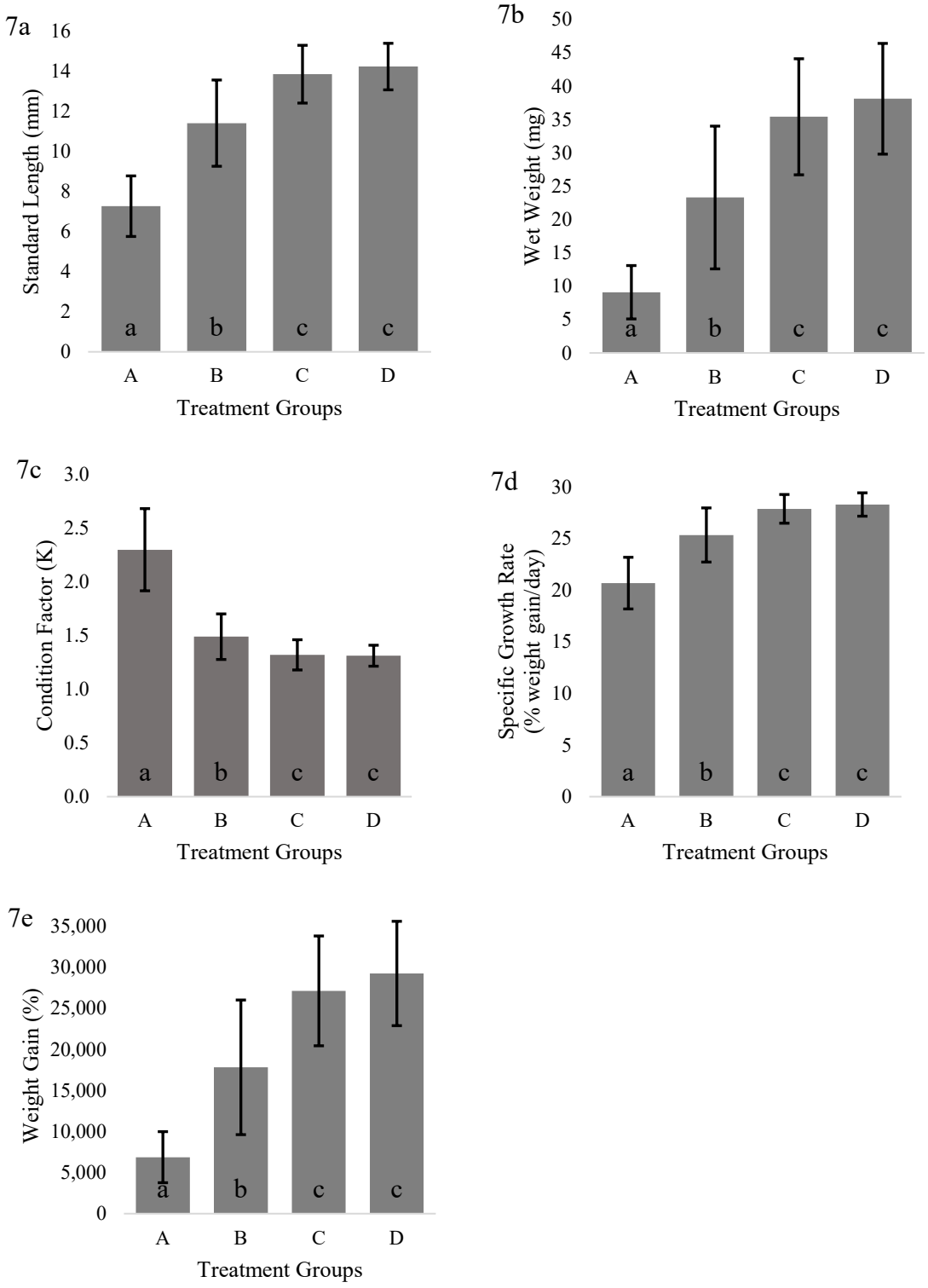

FIGURE 7. Mean (n=4; ± SD) standard length(mm)(7a), wet weight (mg)(7b), condition factor (K)(7c), specific growth rate (7d), and percent weight gain (7e) of larval spotted seatrout in the salinity study. Means with different lower-case letters are significantly different ($P<0.05$).

Discussion

Feeding Studies

Spotted seatrout larvae obtain their first nutrients from their yolk-sac, which is supplied with nutrients derived primarily from the diet of the female broodstock (Fernandez-Palacios et al. 1997; Hilton et al. 2008). Once all yolk-sac reserves have been depleted, larvae must obtain the majority of their nutrients from external food sources. Extrinsic feeding begins at 3 dph in larval spotted seatrout (Banks et al. 1991; Kucera et al. 2002a). The present study found that when larval spotted seatrout are incubated beyond the point of first extrinsic feeding (3 dph), rotifers cultured on Nanno 3600® and enriched with Algamac-3050® produced greater survival and growth than enrichment using Easy Dry Selco®, Ori-one®, or RotiGrow Plus®. In both the cool and warm water feeding trials, Algamac-3050® produced higher survival than all other treatments, although there was no significant difference between Algamac-3050® and Easy Dry Selco® in the warm water trial. Similarly for growth, in both the cool and warm water trials, Algamac-3050® produced significantly longer, heavier, and faster growing fish than all other treatments.

Fulton's condition factor in the cool water trial was not significantly different among treatments. In the warm water trial, the treatment that produced the most growth (Algamac-3050®), resulted in the lowest condition factor, while the treatments that produced the least growth (Ori-one® and RotiGrow Plus®) had the highest condition factors. This may have been due to differences in allometric and isometric growth between treatments based on varying degrees of larval ontogenic developments. According to Wuenschel et al. (2004), spotted seatrout larvae undergo notochord flexion at 4.9 mm standard length and the hypural plate begins forming at 5.4 mm standard length. During hypural plate formation, there is a rapid increase in

tail size as a proportion of total length, as well as changes in the type and relative sizes of muscles used for swimming. This means that between 4.9 – 5.4 mm standard length, larvae are undergoing allometric growth as their bodies change in proportion and dimension. Larvae in the warm water trial ranged from 2.8 – 9.9 mm standard length, meaning that some larvae had undergone ontogenic changes while others had not, making a comparison between these larvae on the basis of condition factor impractical and not relevant. This is supported by a study by Froese (2006) that explained that during early stages, fish grow in length in a greater ratio than they grow in other dimensions, meaning allometric growth is occurring. That study also noted that condition factors should only be compared directly if the specimens are similar in length. As there were significant differences in length between treatments, the comparisons made in the present study are not relevant.

Despite the differences in temperature, total survival was similar between the cool (59.0±7.0%) and warm (63.2±5.7%) feeding trials. Although Algamac-3050® produced the best results in both feeding trials, there were pronounced differences in overall growth between the cool and warm water trials. For all treatments, fish in the cool water trials experienced less growth than fish in the warm water trials. As ectotherms, the growth and metabolism of spotted seatrout is heavily influenced by the temperature of their environment. For most fish species, increasing temperatures result in increased metabolic rates, which increases their ability to exploit the energy in the food they consume, leading to faster growth (Fontaine et al. 2007). If fish remain within their optimum temperature range, baseline metabolic costs remain low, allowing energy to be devoted to growth, thus leading to high growth rates (Sandersfeld et al. 2017). In a study by Wuenschel et al. (2004), wild spotted seatrout larvae were collected at temperatures ranging from 20-35°C, however, the majority of collections occurred at 26-33°C,

suggesting that this represents an optimum range for spotted seatrout larvae. In the cool water study, water temperatures (21.4±0.73°C) were well below this optimum range, possibly leading to thermal stress. Environmental stressors such as this may reduce metabolic rates, as well as decrease activity and food consumption, further reducing growth rates (Weunschel et al. 2004). Neuheimer and Taggart (2007) suggested that the differences in fish growth observed at different temperatures may be so pronounced, that it is more appropriate to use "degree-days" than calendar days when evaluating growth rates and predicting future growth. This approach involves quantifying the thermal opportunity for growth in immature fish by integrating both time and temperature, thus giving a more accurate representation of growth than using instantaneous metrics such as mean temperature over a given time period (Honsey et al. 2019). In the case of the present study, the degree-day approach to analyzing growth may have yielded a better understanding of the differences in growth between the cool and warm water trials, instead of reporting mean temperatures over the duration of the trials.

In addition to appropriate temperature requirements, there are many vital nutrients required for the growth, proper development, and survival of marine fish. Many studies suggest that the most important of these nutrients are the essential fatty acids (EFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Palmtag et al. 2006; Seychelles et al. 2009; Kotani et al. 2010; Maehre et al. 2013; Ma and Qin 2014; Hache et al. 2017). Most marine fish larvae are unable to convert short chain fatty acids into long chain fatty acids such as DHA and EPA in sufficient quantities to meet the physiological demands of rapid growth taking place at this stage (Palmtag et al. 2006; Ma and Qin 2014). Therefore, these EFAs must be supplied in sufficient quantities in the diet. In the case of early stage larvae that are fed rotifers, preformed DHA and EPA are supplied by enriching the rotifers with either microalgae or commercially

prepared enrichment products like those evaluated in the present study. Enrichment is necessary to increase rotifer fatty acid profiles as they are naturally low in DHA and EPA (Palmtag et al. 2006; Hache and Plante 2011; Hache et al. 2017).

DHA and EPA are important for larval growth, development, survival, and stress resistance. In addition to providing energy for metabolism, these EFAs are involved in the structure of membrane phospholipids, development of visual functions, and production of eicosanoids, which are involved in a variety of physiological functions, including those related to stress resistance (Seychelles et al. 2009; Hache and Plante 2011; Hache et al. 2017). Studies have shown the DHA and EPA requirements for larval fish to be both species and stage specific (Hache et al. 2017), as well as highly variable (0.3 g kg^{-1} to 39 g kg^{-1}) (Maehre et al. 2013). Some studies have suggested that the DHA/EPA ratio is more important for growth and development than absolute amounts (Kotani et al. 2010; Hache and Plante 2011; Ma and Qin 2014). Although specific recommendations vary by species, optimal DHA/EPA ratios for marine fish are generally around 2:1 (Sargent et al. 1995; Seychelles et al. 2009; Hache and Plante 2011; Ma and Qin 2014; Hache et al. 2017). When choosing an enrichment product for rotifers it is also important to consider that the nutritional characteristics of the enriched rotifers may be different from those of the enrichment because of the metabolism or bioconversion of nutrients during the enrichment period (Palmtag et al. 2006). For these reasons, it is necessary to determine larval feeding protocols on a species by species basis.

This was the purpose of the feeding studies in the present study. The four treatments used in the present study had the following DHA/EPA ratios: Easy Dry Selco® (19:1), Algamac-3050® (7.5:1), Ori-one® (5:1), and RotiGrow Plus® (2.3:1). The Nanno 3600® used as a culture diet for the Easy Dry Selco® and Algamac-3050® rotifers contained no DHA, only EPA

according to the manufacturer's technical data sheets (Reed Mariculture Inc., Campbell, California, USA). As previously discussed, larvae fed rotifers enriched with Algamac-3050® performed better than those receiving any other enrichment in both the cool and warm water feeding trials. This was likely due to Algamac-3050® rotifers containing adequate absolute amounts of DHA and EPA and an optimal DHA/EPA ratio for larval spotted seatrout. The reduced growth seen in the Ori-one® and RotiGrow Plus® treatments in both feeding trials may have been due to deficits in absolute DHA and EPA levels or the DHA/EPA ratio being too low to support the physiological processes occurring during the rapid growth typically seen during larval development. Although Easy Dry Selco® contained the highest DHA/EPA ratio, it did not perform as well as Algamac-3050®. Ma and Qin (2014) found that higher DHA concentrations and DHA/EPA ratios may actually reduce growth and survival. They hypothesized this may be due to an unbalance in lipid class composition, which can affect the digestion and absorption of fatty acids. There was no lab analysis of fish tissues performed in the present study to examine fatty acid profiles of larvae, which would have allowed a more thorough examination of DHA and EPA content in different treatments. Based on experimental results of the enrichments tested, Algamac-3050® provided the most appropriate nutritional profile for the extended culture of larval spotted seatrout.

Salinity Study

As an estuarine fish, spotted seatrout are euryhaline, however, exposure to salinity outside of the optimum range imparts environmental stress that can affect survival, growth, metabolism, and other physiological processes. Results of the present study indicate that under hypersaline (50 ppt) conditions, extended incubation with gradual acclimation significantly increased larval spotted seatrout survival and growth. Survival and growth improved

significantly when fish were acclimated until 6 dph and improved further when acclimation lasted until 9 dph. Additional acclimation until 12 dph did not significantly improve growth or survival from that of the 9 dph group.

Fulton's condition factor was significantly different between treatments in the salinity study. Fish that were subjected to hypersaline (50 ppt) conditions at 3 dph exhibited the least growth and had a much higher Fulton's condition factor than all other groups, which grew significantly more. Fish that were subjected to hypersaline conditions (50ppt) at 9 dph or at 12 dph grew the most yet had the lowest Fulton's condition factor. Although the previously described ontogenic changes in body shape that occur between 4.9 – 5.4 mm standard length should be less likely to affect condition factor in this trial as most fish were beyond the length threshold (mean standard length = 7.26 – 14.22 mm range for all treatments), length was significantly different depending on treatment. Significant differences in length between treatments indicates that direct comparisons of condition factor between these groups should not be made (Froese 2006).

The results of the salinity study indicate that the ability of larval spotted seatrout to cope with high salinity improves with age. It has been suggested that fish in early life stages may respond differently to changes in salinity than adults (Wuenschel et al. 2004). For most adult teleost fish, blood osmolality is maintained within a range of 270-450 mOsmo·kg^{-1} (Yang et al. 2009). This corresponds to a salinity of around 10-13 ppt, meaning that this salinity range is close to the isosmotic point for most teleost fish. When fish encounter salinity above this point, they must hypo-osmoregulate to cope with the hyperosmotic environment (Varsamos et al. 2005). When fish encounter a hyperosmotic environment, they experience water loss to the environment and an excess uptake of electrolytes, particularly Na^{+}, Cl^{-}, Mg^{+2}, Ca^{+2}, and SO_4^{-2}

(Laverty and Skadhuage 2012). Euryhaline fish have developed several physiological mechanisms to combat these issues and maintain homeostasis when exposed to changes in salinity.

In adult fish, the kidney, intestine, and gills are the primary organs involved in osmoregulation (Varsamos et al. 2005). In freshwater fish, the kidneys are important in ridding the body of excess water that is absorbed osmotically from the environment by producing large volumes of dilute urine. As this is not an issue in hyperosmotic environments, the kidneys of saltwater fish instead secrete divalent ions (mainly Mg^{+2} and SO_4^{-2}) and produce small volumes of urine that are isotonic to the blood (Varsamos et al. 2005). In a hyperosmotic environment fish will drink seawater to replace water lost to the environment through osmosis (Lin et al. 2004). As environmental salinity increases, drinking rate increases as well for most species (Laverty and Skadhauge 2012). When fish ingest seawater, it is first partially desalinated in the esophagus before passing to the intestine where most water absorption occurs, facilitated by the active transport of NaCl (Varsamos et al. 2005). The primary mechanisms for NaCl transport and thus water absorption are intestinal epithelial cells containing Na^+–K^+-ATPase (NKA; also called sodium-potassium pumps), which uses ATP to move sodium and potassium ions in opposite directions, each against its concentration gradient. This results in the active transport of Na^+ out of and K^+ into the cells of the intestinal epithelium. The Na^+ gradient created by this process is used to drive $Na^+/K^+/2Cl^-$ (NKCC) co-transporter proteins, which results in the secondary active transport of Na^+, K^+, and Cl^- into the cells of the intestinal epithelium (Gregorio et al. 2013). This process results in a high concentration of ions in the intestinal epithelium, leading to a passive osmotic gain of water from the intestinal lumen and into the blood (Laverty and Skadhauge 2012). The salts that are absorbed by the intestine to facilitate water absorption must

then be expelled from the body. This occurs primarily via NaCl secretion by the gills in adult fish (Varsamos et al. 2005). Chloride cells (CCs; also called ionocytes or mitochondrion-rich cells) in the branchial epithelium are responsible for active ion secretion (Yang et al. 2009). Chloride cells secrete ions via three major channels and transporters: cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel, NKA, and NKCC (Hirose et al. 2003). CFTR and NKCC are responsible for the removal of Cl^-, while NKA and NKCC facilitate Na^+ removal (Hwang and Lee 2007). In order to supply the energy necessary to fuel these ion excreting processes, CCs have a large number of mitochondria. ATP from these mitochondria are used largely to power the NKA pump, which in turn provides the electrochemical gradients necessary for the function of secondary processes such as the NKCC co-transporter (Hirose et al. 2003). By absorbing water in the intestine and excreting salts through the gills, adult teleost fish are able to maintain internal salt and water balance, and thus osmoregulate in different salinities. Osmoregulatory processes differ, however, depending on the age, size, and developmental stage of the fish (Ruiz-Jarabo et al. 2015).

According to Varsamos et al. (2005), in early life stages of fish osmoregulation occurs primarily at the tegument, then shifts primarily to the gills as they become more developed; further osmoregulation by the kidney and intestine increases later as these organs become more developed. In adult fish, chloride cells responsible for ion excretion are found on the gills, however, early-stage larvae may not have fully formed or functioning gills. At this stage, chloride cells are instead distributed on the yolk-sac and the skin. As gill development progresses, chloride cell densities in the skin decrease, while simultaneously increasing in the gills (Hirose et al. 2003; Bodinier et al. 2010; Perez-Robles et al. 2015; Oguz 2018). Chloride cells begin developing on the gills shortly after the gills begin to form (Brauner and Rombough

2012). Studies have indicated that developing gills become important first as an osmoregulatory organ and later as a respiratory organ (Rombough 1999; Brauner and Rombough 2012). This reasoning is based on the appearance of chloride cells on gill arches and filaments well before the development of secondary lamellae, which are the primary site of gas exchange within the gill (Rombough 1999; Brauner and Rombough 2012). Although the density of chloride cells on gill filaments remains relatively constant throughout development, the total number of chloride cells on the gills increases as filaments grow longer (Rombough 1999). Until the gills, digestive tract, and kidneys become fully functional, larval fish may be primarily dependent on cutaneous chloride cells for maintaining osmotic balance (Oguz 2018). In the present study, more advanced gill development may have contributed to better growth and survival in larvae exposed to 50 ppt at 6, 9, and 12 dph. When cultured at 26.5°C, red drum (*Sciaenops ocellatus*) larvae begin developing gill arches and filaments around 4-5 dph (Fuiman et al. 1998). As this is the same temperature as the salinity study and red drum are phylogenetically similar to spotted seatrout, larvae in the present study likely began developing gill filaments around 4-5 dph. Older larvae were likely able to osmoregulate better as gill development progressed and numbers of chloride cells on the gills increased. Osmoregulatory ability in early-stage fish may also be dependent on the ability to increase the expression of NKA proteins in chloride cells (Perez-Robles et al. 2015). As not all osmoregulatory organs are fully developed in early-stage larvae, they are more susceptible than older fish to changes in salinity (Ruiz-Jarabo et al. 2015).

The results of the present study agree with studies by Banks et al. (1991) and Kucera et al. (2002a), which determined that salinity tolerance in larval spotted seatrout is narrowest at 3 days post hatch (dph) and increases with age beyond this point. Both studies suggest this narrow range of tolerance at 3 dph is due to exposures related to the onset of extrinsic feeding. Although

studies by Banks et al. (1991) and Kucera et al. (2002a) have demonstrated that spotted seatrout larvae can tolerate a wide range of salinities (5-50 ppt), the range of tolerable salinity is variable and is related to both the parental bay of origin (Kucera et al. 2002a) and the salinity at which spawning occurs (Banks et al. 1991; Kucera et al. 2002a). Kucera et al. (2002a) found that larvae spawned from Upper Laguna Madre broodstock were better able to survive higher salinities than larvae originating from other Texas bay systems. As the present study involved hypersaline acclimation, eggs spawned by broodstock originating from the Laguna Madre were used.

Salinity tolerance of larval fishes is influenced by many metabolic factors in addition to their age and developmental stage. This is because the energetic cost of osmoregulation is increased as salinity deviates from the isosmotic point (Boeuf and Payan 2001; Varsamos et al. 2005; Tseng and Hwang 2008; Ruiz-Jarabo et al. 2015). In isosmotic environments (~10-13 ppt) the net ion and water flux between the fish and the surrounding environment are nearly null, so a minimal amount of energy is used for osmoregulation. This "saved" energy can then be used for other processes such as growth (Ruiz-Jarabo et al. 2015). Conversely, growth and survival are often reduced at high salinity (Holt and Holt 2003; Weunschel et al. 2004; Varsamos et al. 2005). When fish encounter high salinity, they increase their drinking rate to increase water absorption to combat increased water loss to the environment. High drinking rates, however, result in a high net influx of salts that must be excreted via active ion transport, which uses energy (Varsamos et al. 2005). The NKA pump which is responsible for directly or indirectly driving most of the water and ion movements of osmoregulation requires ATP (energy) (Boeuf and Payan 2001; Hwang and Lee 2007; Tseng and Hwang 2008; Urbina and Glover 2015). This energy is believed to come mostly from carbohydrate metabolism, which is supplied in the form of glucose derived from the liver in adult fish (Tseng and Hwang 2008). According to Boeuf and Payan

(2001), numerous studies have shown that 20 to >50% of the total fish energy budget may be dedicated to osmoregulation, although other studies indicate it may be closer to 10% (Kirschner 1993; Morgan 1998). Regardless of the exact metabolic cost, the increased energy requirements for osmoregulation leave fewer energy reserves for growth (Banks et al. 1991; Holt and Holt 2003; Wuenschel et al. 2004). Growth may also be reduced at high salinities due to a decrease in activity which may be accompanied by a decrease in feeding (Wuenschel et al. 2004). This means that early stage-larvae may not ingest enough energy or have the energy reserves to undertake the osmoregulatory processes necessary to cope with large or abrupt changes in salinity (Tseng and Hwang 2008; Perez-Robles et al. 2015).

This was likely the case in the present study. Although survival was not quantified daily in the present study, daily observations indicated that the majority of fish exposed to 50 ppt salinity at 3 dph survived the initial acclimation process. Numbers of larvae in the 3 dph group were noticeably reduced, however, by 5 dph (2 days after hypersaline exposure). Although larvae were able to initially survive the change to 50 ppt, they likely lacked the energy reserves necessary to maintain osmoregulation in this environment over an extended period of time. Differences in growth between treatments in the present study were also likely a result of the energetic costs associated with osmoregulation and its impact on growth. The earlier larvae were exposed to high salinity, the more energy they had to devote to osmoregulation instead of growth, resulting in early exposure groups exhibiting less growth compared to later exposure groups. These results agree with a study by Norris (2016), that indicated that red drum (*Sciaenops ocellatus*) exposed to high (40 ppt) salinity had significantly reduced growth compared to fish at 30 ppt. Growth parameters in the present study indicated that early exposure to high salinity negatively affected larval growth.

Gradual acclimation may improve survival and growth of spotted seatrout larvae subjected to stressful salinity changes. Studies have shown that gradual acclimation rather than abrupt changes in salinity can increase tolerance to salinity changes. For example, Persian sturgeon (*Acipenser persicus*) larvae that were reared in fresh water until 20 dph, then acclimated to a salinity of 12.5 ppt over a period of 5 days had greater weights and higher survival compared to those subjected to an abrupt salinity change from fresh water to 12.5 ppt (Khatooni et al. 2011). Larvae that were acclimated gradually had a mean weight of 353.75±10.60 mg compared to 299.66±23.13 mg for abruptly transferred fish. There were no mortalities when larvae were acclimated gradually, however, abrupt transfer resulted in survival rates of only 23.3-26.7% (Khatooni et al. 2011). Tadpoles of the Indian rice frog (*Fejervarya limnocharis*) that had longer acclimation periods to brackish water (3ppt to 7 ppt for 24 or 48 hours, then to 11 ppt vs. directly from 3 ppt to 11 ppt) resulted in higher survival at the end of a 48-hour period. Tadpoles did not survive over 12 hours when transferred directly to 11 ppt, compared to 10-20% survival at the end of the study for the 24-hour acclimation group and 90% survival for the 48-hour acclimation group (Wu et al. 2014). Both studies hypothesized that longer acclimation resulted in greater survival because time is needed in order to activate osmoregulatory mechanisms.

When fish are subjected to high salinity resulting in osmotic stress, they undergo a crisis period during which they must overcome dehydration, followed by a regulatory period in which osmoregulatory mechanisms are activated and blood osmolality is stabilized (Lai et al. 2019). In fish larvae, experimental evidence suggests ~250-600 $mOsmo \cdot kg^{-1}$ is the blood osmolality range compatible with survival (Varsamos et al. 2005). In hypertonic environments, fish use several mechanisms to keep blood osmolality from increasing beyond survivable limits. Within minutes of exposure, the size of the chloride cells increases and the activity of the NKA in them increases

markedly (Hirose et al. 2003; Lema et al. 2018). This is followed by an increase in chloride cell numbers, an increase in the number of NKA units within the chloride cells, and changes in gene expression in the gills, liver, and intestine (Hirose et al. 2003; Maryoung et al. 2015; Chourasia et al. 2018; Lema et al. 2018). These secondary changes may take hours or days depending on the species, developmental stage of the fish, and the severity of the osmotic challenge. A metabolic reorganization must occur to meet the energetic demands associated with these physiological changes, as well as to cope with the increased energy needed to fuel the more numerous and more active NKA pumps (Jarvis and Ballantyne 2003). Gradual acclimation allows time for these less rapid physiological changes to occur, which can better prepare fish for exposures to high salinity. This may explain why larvae in the present study that experienced a longer acclimation period performed better in terms of survival and growth than larvae that received a shorter acclimation period.

Management Implications

The results of this study indicate that when hypersaline (50 ppt) conditions are present in outdoor rearing ponds, larval spotted seatrout survival and growth can be increased by increasing incubation time and using acclimation procedures. This allows larvae more time to develop the biological mechanisms necessary for osmoregulation and accumulate the energy reserves necessary to osmoregulate over extended periods of time. Gradual acclimation during this extended incubation period may promote improved function of osmoregulatory organs and processes. Rotifers enriched with Algamac-3050® yielded positive growth and survival results as a feed source for larval spotted seatrout during the extended incubation trials.

Cannibalism is a potential issue with extended incubation, as cannibalism is known to be a major cause of mortality when spotted seatrout are raised at high densities (Manley et al. 2015).

The present study indicated that acclimation to 6 or 9 dph was sufficient to significantly increase survival and growth. Cannibalism typically increases in frequency once larvae reach 10 dph as size differences among larvae become more pronounced (Manley et al. 2014). Daily observation in the salinity study indicated that cannibalism did not become an issue until 14 dph and beyond. This indicates that incubation could be extended to 6 or 9 dph without significant losses due to cannibalism.

While the current study suggests that stock enhancement hatchery operations could benefit from extended incubation and gradual acclimation of larval spotted seatrout when outdoor rearing ponds have hypersaline conditions, additional studies are needed to refine this procedure. Improvements in larval survival and growth should be investigated over shorter increments of time (e.g., 4, 5, 6, dph) and compared to additional costs associated with longer incubation periods (e.g., feed costs, labor hours) to develop a cost-benefit analysis. This could be used to determine an optimal extended incubation period in which returns are maximized and costs are minimized. Future studies should examine and ascertain at what hypersaline concentration (i.e., >45 ppt) can the extended incubation procedure be best utilized. Finally, the extended incubation procedure used in this study should be investigated at scale (i.e., hatchery production trial) to determine if the increases in larval growth and survival observed in the laboratory equate to improved returns from outdoor rearing ponds under hypersaline conditions.

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LIST OF APPENDICES

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APPENDIX 1. Survival data and summary of growth data: cool water feeding study.

Treatment	Tank	Percent Survival (%)
Algamac-3050	4	84
	5	73
	10	75
	16	69
Easy Dry Selco	1	53
	6	45
	9	52
	14	51
Ori-one	2	41
	8	60
	11	52
	15	48
Rotigrow	3	70
	7	46
	12	61
	13	64

Length (mm)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	3.1 - 4.1	3.0 - 4.1	2.8 - 3.7	2.8 - 3.8
Mean	3.60	3.50	3.11	3.24
Median	3.6	3.5	3.1	3.2
Std. dev.	0.248	0.265	0.193	0.220
Variance	0.061	0.070	0.037	0.048
IQR	0.4	0.4	0.3	0.3

Weight (mg)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	0.4 - 1.3	0.4 - 1.3	0.3 - 1.0	0.3 - 1.1
Mean	0.92	0.83	0.57	0.68
Median	0.90	0.85	0.55	0.70
Std. dev.	0.18	0.22	0.18	0.19
Variance	3.35E-05	4.68E-05	3.22E-05	3.72E-05
IQR	0.30	0.30	0.30	0.30

Condition Factor (K)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	1.34 - 2.33	1.34 - 2.33	1.23 - 2.29	1.37 - 2.33
Mean	1.95	1.91	1.83	1.94
Median	1.985	1.929	1.852	1.948
Std. dev.	0.168	0.201	0.295	0.237
Variance	0.0282	0.0403	0.0870	0.0560
IQR	0.181	0.204	0.379	0.305

SGR (% wt. gain/day)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	9.37 - 19.19	9.37 - 19.19	6.97 - 17.00	6.97 - 17.80
Mean	16.11	15.18	11.87	13.40
Median	16.124	15.633	11.985	14.030
Std. dev.	1.85	2.35	2.78	2.50
Variance	3.422	5.539	7.703	6.232
IQR	2.654	2.972	4.663	3.917

Weight Gain (%)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	207.7-900.0	207.7-900.9	130.8-669.2	130.8-746.2
Mean	607.1	541.7	337.8	421.2
Median	592.3	553.8	323.1	438.5
Std. dev.	140.8	166.4	138.1	148.4
Variance	19,819.6	27,695.9	19,073.7	22,024.0
IQR	230.8	230.8	230.8	230.8

APPENDIX 2. Survival data and summary of growth data: warm water feeding study.

Treatment	Tank	Percent Survival (%)
Algamac-3050	2	86
	5	80
	12	82
	15	85
Easy Dry Selco	3	84
	8	72
	9	85
	14	78
Ori-one	4	71
	7	58
	10	56
	13	61
Rotigrow	1	38
	6	26
	11	29
	16	20

Length (mm)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	6.6 – 9.9	3.2 – 9.2	2.9 – 8.4	2.8 – 7.7
Mean	8.24	6.61	5.74	5.6
Median	8.2	6.8	5.7	5.8
Std. dev.	0.899	1.231	1.444	1.139
Variance	0.808	1.514	2.086	1.298
IQR	1.7	1.5	2.3	1.6

Weight (mg)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	5.2 - 11.4	0.9 - 10.0	0.4 - 8.6	0.3 - 7.5
Mean	8.35	5.44	4.09	3.68
Median	8.20	5.60	3.70	3.80
Std. dev.	1.52	2.09	2.27	1.64
Variance	2.30E-03	4.38E-03	5.14E-03	2.70E-03
IQR	2.50	3.10	3.90	2.30

Condition Factor (K)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	1.18 - 1.92	1.28 - 2.80	1.45 - 2.78	1.23 - 2.57
Mean	1.51	1.83	1.99	1.95
Median	1.469	1.805	1.948	1.920
Std. dev.	0.229	0.275	0.287	0.236
Variance	0.0524	0.0756	0.0824	0.0560
IQR	0.433	0.232	0.394	0.311

SGR (% wt. gain/day)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	31.41 - 37.95	16.79 - 36.86	10.03 - 35.60	7.64 - 34.46
Mean	35.21	31.01	27.77	27.27
Median	35.20	32.03	28.46	28.79
Std. dev.	1.56	3.91	5.78	5.39
Variance	2.45	15.32	33.37	29.05
IQR	2.470	4.746	8.031	5.436

Weight Gain (%)	Algamac-3050	Easy Dry Selco	Ori-one	Rotigrow Plus
Range	4,233 - 9,400	650 - 8,233	233 - 7,067	150 - 6,150
Mean	6,854	4,433	3,308	2,967
Median	9,733	4,567	2,942	3,067
Std. dev.	1,263	1,744	1,890	1,368
Variance	1,596,271	3,042,168	3,571,709	1,871,808
IQR	2,042	2,542	3,208	1,917

APPENDIX 3. Survival data and summary of growth data: salinity study.

Treatment	Tank	Percent Survival (%)
A	1	2
	7	15
	9	5
	16	11
B	4	62
	8	49
	11	46
	14	53
C	3	60
	5	73
	12	57
	13	70
D	2	75
	6	59
	10	61
	15	66

Length (mm)	A	B	C	D
Range	4.8 - 10.5	5.7 - 15.3	10.0 - 16.5	11.5 - 16.9
Mean	7.26	11.40	13.84	14.22
Median	7.5	11.7	14.1	14.3
Std. dev.	1.509	2.150	1.439	1.158
Variance	2.278	4.623	2.069	1.341
IQR	2.3	2.9	1.9	1.6

Weight (mg)	A	B	C	D
Range	2.6 - 18.8	3.7 - 53.3	15.3 - 58.2	19.4 - 62.4
Mean	9.10	23.30	35.40	38.10
Median	9.60	23.40	35.80	37.60
Std. dev.	4.04	10.65	8.69	8.25
Variance	0.0163	0.1130	0.0755	0.0681
IQR	5.70	14.95	11.00	9.83

Condition Factor (K)	A	B	C	D
Range	1.62 - 3.20	1.14 - 2.26	1.02 - 1.69	1.05 - 1.58
Mean	2.30	1.49	1.32	1.31
Median	2.224	1.446	1.306	1.301
Std. dev.	0.382	0.212	0.141	0.098
Variance	0.1460	0.0449	0.0198	0.0095
IQR	0.517	0.224	0.198	0.124

SGR (% wt. gain/day)	A	B	C	D
Range	14.98 - 24.87	16.74 - 30.08	23.84 - 30.52	25.03 - 30.87
Mean	20.67	25.33	27.86	28.28
Median	21.51	25.96	28.09	28.34
Std. dev.	2.50	2.62	1.39	1.13
Variance	6.242	6.875	1.920	1.270
IQR	3.422	3.429	1.532	1.308

Weight Gain (%)	A	B	C	D
Range	1,900 - 14,362	2,746 - 40,900	11,669 - 44,669	14,823 - 47,900
Mean	6,874	17,808	27,110	29,228
Median	7,285	17,900	27,438	28,823
Std. dev.	3,108	8,192	6,682	6,349
Variance	9,657,421	67,113,052	44,646,088	40,315,499
IQR	4,385	11,500	8,462	7,558

APPENDIX 4. ANOVA results for survival and growth indices for cool water (avg. temp = 21.4°C) feeding trial, warm water (avg. temp = 26.2°C) feeding trial, and salinity trial.

Source	*df*	*F*	*P*
Cool water feeding trial			
Survival	3	10.14	0.00131
Length	3	59.68	<0.0001
Weight	3	47.00	<0.0001
Condition factor (K)	3	2.59	0.1011
Specific growth rate	3	46.59	<0.0001
Percent weight gain	3	47.00	<0.0001
Warm water feeding trial			
Survival	3	70.18	<0.0001
Length	3	43.24	<0.0001
Weight	3	50.90	<0.0001
Condition factor (K)	3	27.34	<0.0001
Specific growth rate	3	38.04	<0.0001
Percent weight gain	3	50.90	<0.0001
Salinity trial			
Survival	3	60.48	<0.0001
Length	3	55.49	<0.0001
Weight	3	37.17	<0.0001
Condition factor (K)	3	89.87	<0.0001
Specific growth rate	3	50.29	<0.0001
Percent weight gain	3	37.17	<0.0001