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**REVIEW ARTICLE** 

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# The status of spotted seatrout (*Cynoscion nebulosus*) as a technologically feasible species for U.S. marine aquaculture

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# Abstract

Culture models and facilities for large-scale, commercial production of popular Gulf of Mexico species are unavailable. The spotted seatrout (Cynoscion nebulosus) is one of the most popular recreational fishes in the Gulf of Mexico. Seatrout culture techniques were adapted from red drum (Sciaenops ocellatus) protocols developed in the 1970s. Broodstock husbandry, spawning, and extensive pond rearing techniques using fertilized and bloomed brackish ponds were well-established by the 1980s. By 2018, approximately 80 million 25-30-day old seatrout had been produced, mainly for stock enhancement. Cannibalism and poor nutrition hindered intensive tank culture. Between 2005 and 2015, an intensive tank-rearing protocol that reduced cannibalism and intracohort variability and increased average survival to almost 50% was developed using algal concentrate, rotifers, brine shrimp (Artemia sp.), and microencapsulated feeds. Preliminary results suggested that a 500 g fish could be produced in approximately 10 months. Nevertheless, interest in commercialization has remained low. Zootechnical performance throughout the latter stages of culture, the economics of production, consumer preferences/perceptions, and market capacity must be documented to complete the assessment of the spotted

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seatrout as a species for commercial aquaculture. The optimization of aquafeeds specific for seatrout and a domestication program is warranted to further facilitate industry growth.

#### KEYWORDS

aquaculture, Cynoscion nebulosus, history, spotted seatrout

# 1 | INTRODUCTION

Fish currently accounts for almost 20% of the protein consumed by humans (FAO, 2018). Most agree that fish production must double over the next 30 years to meet the demands of a growing human population and the expected increase in the per capita consumption of fish. Aquaculture, which has increased its share of overall fisheries production from 9% in 1980 to more than 50% today, has the potential to meet the growing demand. However, in the US and most developed countries, the majority (75% in the US) of fisheries products consumed is of marine origin. In comparison, only about 30% of worldwide aquaculture production comprises marine species (FAO, 2018). Simultaneously, almost 90% of US seafood is imported, and most of that is farm-raised. Indeed, US marine aquaculture's lack of capacity has contributed to an annual seafood trade deficit of over \$16 billion (NMFS, 2018).

The development of commercial marine aquaculture in the US has been constrained by concerns over competition among user groups for space and resources, the use of nonindigenous species, genetic impacts of escapees on wild stocks, the spread of disease to wild stocks, and environmental issues such as the impacts of fishmeal use, wastes, chemicals, hormones, and effluents. Over the last 20–30 years, advances in material technology and system engineering have provided various production models ranging from offshore cage systems to land-based recirculating systems capable of alleviating most of the concerns and serving as a foundation for a vibrant, diversified industry. However, in the Gulf of Mexico region, the historical supply of popular marine species from fisheries has been adequate. As a result, interest in developing large-scale culture methods for Gulf of Mexico species is recent. Culture models and facilities capable of capitalizing on the advancements in aquaculture technologies to costeffectively produce these species at a commercial scale are still lacking.

The spotted seatrout (*Cynoscion nebulosus*) is a euryhaline, estuarine-dependent marine fish that is a candidate for aquaculture in the southeastern United States. The spotted seatrout is most abundant in the south Atlantic and the Gulf of Mexico, where it is commonly associated with brackish habitats. Adults tend to prefer higher salinities for spawning (≥25 psu). Spotted seatrout are highly sedentary and typically remain within a single bay or estuary throughout their life (GSMFC, 2006). Seatrout are carnivorous, voracious, and generalist feeders. Indeed, in many estuaries, the adult seatrout represents the primary piscine predator. On average, seatrout mature at approximately 1 year (30 cm), live 3–5 years, and reach an adult size of 40–50 cm. The spawning season is protracted and spans most of spring and summer.

The spotted seatrout is one of the most popular recreational fishes in the Gulf of Mexico and among the top five harvested in the US (NMFS, 2020). In 2018, recreational fishing in the Gulf of Mexico generated over 56 million angler trips and around 6.5 m.t. of landed seatrout (NMFS, 2020). The Texas saltwater recreational fishery alone generates approximately \$2.0 billion per year in economic impact (NMFS, 2020). Yearly landings in Mississippi have risen fivefold since the early 1990s (Leaf et al., 2016). In Texas, spotted seatrout account for 42% of landings from private boats and 73% of landings from party boats (Green & Campbell, 2010). Although regulations vary by state, landings have shifted over time from the commercial fishery to the recreational fishery due to changing regulations and an increase in the recreational fishery's importance. Today, approximately 98% of the seatrout harvest is in the recreational fishery (NMFS, 2020).

Management of the fishery focuses on maintaining spawning biomass through creel, season, and size restrictions. The spawning potential ratio (SPR) is the number of eggs that could be produced by an average recruit in a fished stock divided by the number of eggs that an average recruit could produce in an unfished stock and serves as a measure of the impact of fishing on the potential productivity of a stock (Goodyear, 1990). As the seatrout is not a federally managed species, individual states set the recommended minimum SPR for their jurisdictions. While these recommendations vary, SPRs below those minimums have been observed in Mississippi (Leaf et al., 2016) and Louisiana (West, Xinan, & Adriance, 2019) in recent years. In response to concerns about low SPRs and/or increasingly heavy fishing pressure, several states including Mississippi, Texas, and South Carolina have initiated aquaculturebased stock enhancement programs.

The spotted seatrout's generalist feeding habits, its inshore habitats that make it easily accessible to anglers, and the pleasant texture and taste of its flesh not only make it one of the most popular recreational fishes in the Gulf of Mexico (NMFS, 2020) but also one of the most important species in the cuisines of the northern Gulf coast. With a recreational fishery that is heavily exploiting the stocks and no significant commercial fishery, the supply of seatrout in the market is seasonal and variable. Without a dependable commercial supply of spotted seatrout, regional restaurants and fishmongers fill the gap with other imported, often farmed species that are consistently available. Commercial aquaculture could mitigate these supply irregularities and maintain the spotted seatrout's prominence on seafood menus.

This article reviews the status of culture technology for the spotted seatrout (*Cynoscion nebulosus*), demonstrates current capabilities for seatrout production, and examines the research and marketing needs that could facilitate the rapid expansion of commercial-scale aquaculture in the Gulf of Mexico region.

# 2 | CULTURE HISTORY

The Spotted Seatrout is cultured using methodology patterned on techniques developed for the closely related Red Drum. Initial trials to spawn spotted seatrout were conducted during the 1970s by university and state fisheries researchers who spawned captive red drum and spotted seatrout using hormone injections and conditioning using photoperiod and temperature. Initial spotted seatrout captive spawning and larval rearing investigations include those of Colura (1974) and Arnold, Lasswell, Bailey, Williams, and Fable Jr. (1976). Colura (1974) induced spawning of spotted seatrout during their natural spawning season by injecting human chorionic gonadotropin and observed hatch after 15 hr. Arnold et al. (1976) developed methods to maintain adult seatrout in captivity and obtained spontaneous spawning in response to a photoperiod-temperature regime. They also established techniques to culture the larvae. Adult spotted seatrout were maintained in an indoor tank (dimensions  $6 \times 3.3 \times 1.5$  m<sup>3</sup>, volume 30,000 L) with the salinity ranging between 25 and 30 psu. Temperature and light were adjusted to simulate a spawning season. When the light regime equaled 15 hr light, 9 hr dark, and water temperature 26°C, the spotted seatrout began to spawn. The fish continued to spawn during each of 13 consecutive months for a total of 82 spawns. Eggs were collected with hand nets from the filter box, counted in glass beakers, and placed in 74 L aquaria. The eggs hatched after 18 hr and newly hatched seatrout were fed rotifers (Brachionis plicatilis) and brine shrimp (Artemia sp.) (see Figure 1). These early successful spawning and rearing trials furthered an interest in rearing spotted seatrout for aquaculture and fisheries stock enhancement purposes. Currently, two established aquaculture methods (intensive recirculating tank systems and outdoor rearing ponds) are used to rear spotted seatrout larvae to 30 days post-hatch and beyond.

Arnold et al. (1976) developed the original intensive tank-rearing protocol for seatrout. One to two day old larvae were stocked at up to 5/L into indoor (with fluorescent lighting) or outdoor tanks previously stocked with algae (50,000–75,000 cells/ml), fed rotifers (20/ml), and transitioned to brine shrimp (3–5/ml) by the eighth day (Figure 1). Arnold et al. (1976) achieved up to 30% survival through 30 days. Low survival was attributed to cannibalism and nutrient deficiencies. Taniguchi (1981) concluded that intensive seatrout culture was feasible at a low stocking

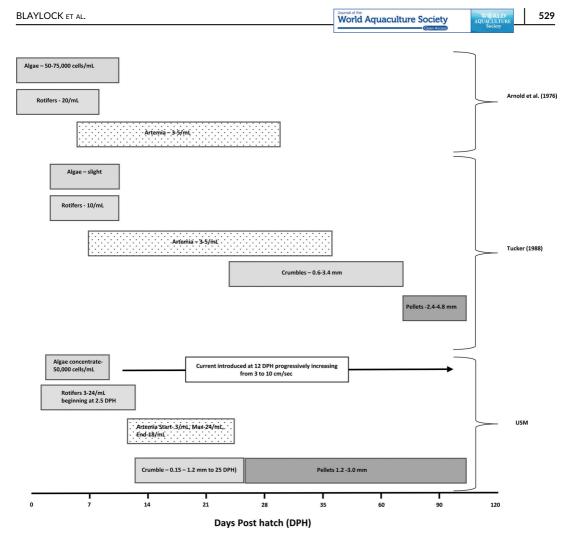


FIGURE 1 Comparison of protocols for intensive production of spotted seatrout, Cynoscion nebulosus

density (0.5-5/L) with a moderate density (100-1,000/L) of rotifers or copepods, but he did not report survival rates. He further determined that the inclusion of copepod nauplii into the diet with rotifers improved the growth rate over 12 days but did not improve survival. Tucker (1988) incorporated dry, pelleted feed into a seatrout rearing protocol between days 19 and 24 (Figure 1). He found that the fish most readily accepted feed after day 33 and that they could be reared past 100 days with good food conversion (FCR = 0.78-1.55) if they were reared at low density and with diets high in protein and fat and low in carbohydrate. Tucker (1988) stocked between 4 and 200 fish of varying ages in various trials but noted survival through 50 days from only one trial (12.5%). Except for Taniguchi (1981), who used copepod nauplii in early rearing diets, others used the standard fare of rotifers and brine shrimp without commercial enrichment. Salinity was ambient (25-35 psu), but Taniguchi (1981) determined that the optimum salinity for larval rearing was approximately 28.1 psu. While stocking densities varied widely during the above-mentioned trials, cannibalism and low survival were consistently observed, especially at high density. Consequently, subsequent protocol development at the University of Southern Mississippi (USM) used low density culture. Briefly, newly hatched seatrout larvae were stocked at a density of 10 larvae/L, offered ss-type enriched rotifers, and transitioned to enriched Instar-2 brine shrimp nauplii beginning at 5 days post-hatch (DPH) until day 22. Dry feed was co-fed during the brine shrimp feeding period, and larvae were weaned at 25 DPH (Figure 1). Early runs on the initial protocol averaged 20% survival (range 7%-28%) through 30 DPH. This protocol and subsequent versions (detailed in the

following) were applied at USM to produce more than 1.9 million seatrout between 2006 and 2018. Although cannibalism remained an obstacle, average survival had increased to 48% by 2018.

Initial trials to evaluate the feasibility of culturing spotted seatrout larvae in outdoor rearing ponds were conducted during the 1970s–1980s (Colura, King, Gray, & Bumguardner, 1992). First efforts to culture hatcheryspawned spotted seatrout in ponds were reported by Colura, Hysmith, and Stevens (1976). Spotted seatrout larvae were stocked in nine 0.1–0.4 ha ponds between May and August. Stocking rates varied between 50,000 and 359,000 larvae/ha. Six ponds received 2 DPH larvae, while the other three ponds received 4–7 DPH larvae. Pond temperatures ranged from 28 to 34°C, with a salinity of approximately 20 psu. Ponds were initially fertilized with cottonseed meal at 170.5 kg/ha, followed by cottonseed meal applications at 56.8 kg ha<sup>-1</sup> week<sup>-1</sup>. A commercial salmonid diet was used to feed the ponds starting 14 days after stocking at the rate of 2.5 kg/ha five days per week. The ponds were harvested when samples of fingerlings averaged 40 mm total length (TL), 29–32 days after stocking. Overall survival was 3% ranging from 0 to 18.6%. Copepods comprised 55.6% of the diet of spotted seatrout less than 25 mm TL. Spotted seatrout 25 mm TL or greater fed primarily on polychaetes (40.5%) and secondarily on palaemonid shrimp (34.2%). Poor survival was attributed to a mismatch in the timing of nauplii or adult copepod availability as prey during the fingerlings' developmental stages. Lack of properly sized prey for feeding presumably can compound the cannibalistic tendencies of larval and juvenile spotted seatrout (Arnold et al., 1976). Therefore, the timing of pond fertilization is essential to providing adequate prey for the fingerlings at the appropriate time.

Porter and Maciorowski (1984) improved spotted seatrout fingerling production in ponds by combining organic and liquid inorganic fertilizer (568 kg/ha cottonseed meal, 3.3 L/ha phosphoric acid, 1.8 kg/ha urea) applications and increasing the time interval between pond preparation and larvae stocking. After 22 days, six ponds yielded more than 50,000 spotted seatrout fingerlings with an average recovery of 8.4% and a mean production of 46.9 kg/ha. Salinities in the ponds ranged from 14.0–21.4 psu. Harvest yields were improved using pond management strategies designed to increase zooplankton forage by increasing fertilization rates and combined organic and inorganic fertilizer types. Monitoring zooplankton and sampling water quality were identified as essential components of rearing spotted seatrout fingerlings in ponds (Colura et al., 1992). Zooplankton was sampled two to three times weekly. Mean densities per liter of rotifers, copepod nauplii, adult copepods, and polychaete larvae were calculated each week. Water quality including dissolved oxygen, temperature, and salinity were measured daily at each pond drain-box between sunrise and 8:00 a.m. These early pond studies and subsequent revisions (detailed in the following) led to the development of a large-scale stock enhancement program in Texas. To date, over 133 million spotted seatrout juveniles have been produced at Texas Parks and Wildlife Department (TPWD) hatcheries and released into Texas coastal waters.

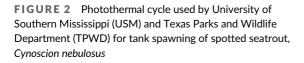
# 3 | CULTURE STATUS AND PROTOCOLS

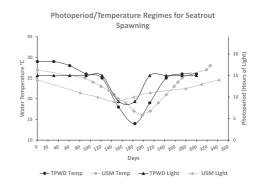
# 3.1 | Broodstock

# 3.1.1 | Volitional

Tanks used to spawn captive spotted seatrout are typically operated as part of a recirculating aquaculture system (RAS) and utilize spawning technology developed for red drum (McCarty, 1987). Because open, continuous flow systems lack sufficient biosecurity and control over water quality and temperature, this discussion focuses on recirculating aquaculture systems used to spawn captive spotted seatrout (broodfish) (Colura, Bumguardner, Gray, & King, 1991).

Spawning systems for spotted seatrout are typically located in enclosed hatchery buildings to control the water temperature and light duration. Spawning tanks are almost always constructed of fiberglass. The "standard" tank found in existing hatcheries measures 3.7 m in diameter and holds approximately 12,870 L of water. Spawning





systems can comprise single tanks with independent filtration systems or a series of tanks (most frequently 2) with a shared biofiltration system. Typical systems include mechanical filtration, biofiltration, protein skimming, UV/ozone disinfection, and temperature/light controls.

Spotted seatrout broodfish are maintained in groups of 15–30 fish targeting a 1:1 sex ratio per standard 12,870 L tank. Spotted seatrout broodfish used for spawning typically range in size from 0.5 to 1.5 kg. TPWD hatchery staff feeds the broodfish shrimp, squid, mackerel, and beef liver at a rate of 2–3% of body weight three times per week. USM feeds shrimp, squid, and cigar minnows at 2–3% body weight three times per week and adds a weekly vitamin supplement (Sea Tabs<sup>®</sup> Vitamin supplement for marine animals at a dosage of 1 pill animal<sup>-1</sup> week<sup>-1</sup>).

Spotted seatrout broodfish are subjected to photoperiod-temperature cycles to induce gamete maturation and spawning. TPWD uses a 120- or 150-day cycle, and USM uses a 153-day cycle. Spawning occurs when the water temperature is between 25 and 28°C (Figure 2), salinity is between 20 and 38 psu, and the photoperiod exceeds 12 hr of light. At USM, if no spawning occurs within 30 days of achieving the appropriate light and temperature conditions, fish are "shocked" by increasing the temperature by one degree and adding an hour of daylight to the photoperiod. Twenty-four hours after the temperature increase, the temperature is decreased by 3° for 24 hr and then increased again by 3-4° to initiate spawning. At TPWD, brooders will spawn intermittently (every 2-5 days) for 6 months, with periodic rests, depending on how many eggs are needed to meet stock enhancement goals and the logistical constraints of the facility. The brooders are rested by a controlled water temperature drop  $(2-5^{\circ}C)$  for a brief period before resuming spawning activities at the preferred spawning temperatures (26-28°C). On average, 300,000 eggs per tank are collected per spawning event. A tank of spotted seatrout broodfish is typically spawned from May through October and then recycled through the next spawning year's photoperiod-maturation regime. At USM, broodfish are allowed to spawn for a period of up to 4 months. Spawning frequency varies by tank and size of fish. The average spawning frequency is 18 spawns per month (30 days), and the average fecundity per spawn is 150,000 eggs. Because both TPWD and USM produce seatrout for stock enhancement purposes, 25% of the broodfish are exchanged with wild fish annually to maintain genetic diversity.

# 3.1.2 | Hormonal induction of spawning and in vitro fertilization

Because spotted seatrout spawn volitionally in tanks, efforts to induce ovulation and spawning have been limited. Initial attempts employed chorionic gonadotropin (Colura, 1974). Colura, Maciorowski, and Henderson-Arzapalo (1990) tested three dosages and various pituitary extracts. Gonadotropin treatments induced ovulation with all tested hormone preparations between 26 and 32 hr post-injection. The highest ovulation rate was achieved using the highest dose of chorionic gonadotropin (1,100 IU/kg) but with a low fertilization rate (average 17.2%). The intermediate dose with this hormone achieved 91.8% ovulation with a slightly higher fertilization rate (27.3%). The other pituitary preparations were less successful, averaging 25.8% ovulation (range 0–75%) and low fertilization

(0–6.3%). Induction with chorionic gonadotropin was revisited recently by Bardon-Albaret and Saillant (unpublished results) on seven females induced at 1,100 IU/kg using protocols described in Bardon-Albaret and Saillant (2017). The trial led to ovulation in four females 26–29 hr post-induction. Three spawns showed high fertilization rates (71–78%) and fecundity over 345,000 eggs/kg suggesting that the protocol is compatible with the production of large viable spawns.

Induction methods using luteinizing hormone-releasing hormone (LHRH) were attempted via intramuscular injection (Thomas & Boyd, 1988) and oral administration (Thomas & Boyd, 1989). Both approaches led to ovulation and production of viable eggs, although spotted seatrout brooders appeared sensitive to stress, which impacted the response to injection treatments. Oral administration successfully induced ovulation and spawning with high fertilization and hatch rates (93% and 75%, respectively), suggesting the protocol could be used to produce viable spawns. This approach has the advantage of avoiding fish handling but requires large amounts of hormone (>1 mg/kg). Results are difficult to predict unless fish are handled to determine the maturation stage of oocytes prior to administration. Another promising approach is the use of slow-release LHRH implants (Mylonas & Zohar, 2000), which could also help to mitigate handling stress effects when spawning in tanks is desired.

If strip spawning and in vitro fertilization are desired when developing a domestication program, recent results with gonadotropin listed earlier appear as a possible option. LHRH injection also should be considered as a means to potentially improve egg quality. The first single injection attempts employed a high dose (100 ug/kg), which may have impacted egg quality. Lower doses may improve results, possibly in combination with an antidopaminergic to mitigate the effects of handling stress.

# 3.2 | Larval rearing

# 3.2.1 | Extensive culture

Pond culture methods are modifications of procedures established by Colura et al. (1991) for rearing spotted seatrout larvae to fingerling size (25–30 mm total length). Ponds used for production are usually from 0.2 to 0.8 ha in size and 0.9 to 1.2 m deep with a catch basin. Both earthen and plastic-lined ponds have been used successfully to rear spotted seatrout fingerlings. Filling outdoor rearing ponds with seawater should start 5–10 days before anticipated larval stockings. The salinity range of incoming water should be 10–45 psu, ideally between 20 and 35 psu. The water temperature should be at least 23°C. Seawater should be filtered with a 0.5 mm filter bag or drum filter. Ponds are fertilized with a combination of chemical inorganic and organic fertilizers (Table 1). Commonly used fertilizers (ammonium nitrate–45% N, phosphoric acid–55%  $P_2O_5$ , and cottonseed meal–44% protein) produce a rapid phytoplankton bloom that stimulates copepod production, a primary food for larval spotted seatrout. Larvae are stocked into ponds when zooplankton densities reach 250 organisms/L.

In a hatchery setting, fertilized embryos are transferred to 500-945-L incubators at a maximum stocking rate of 3,000 larvae/L. Favorable water quality is maintained in the incubators by providing seawater flow-through at a rate of 6 L/min (17 turnovers/day for the 500-L tank, 9 for the 945-L tank) and providing aeration. Temperature and salinity of the flow-through water source should be maintained at levels comparable to the brood tanks where the eggs were spawned. Larvae hatch within 24 hr. Within 36-40 hr post-hatch, larvae have developed mouthparts, distinct eye pigmentation, and a complete digestive tract. These first-feeding larvae, which average 2.1 mm total length (Fable Jr., Williams, & Arnold, 1978), are transported from an incubator tank to rearing ponds for stocking via fiber-glass transport boxes. Larvae are acclimated with pond water at the pond site. When larvae are being shipped from a hatchery to ponds at a commercial growout facility, larvae are transported in standard plastic fish shipping bags with styrofoam-lined cardboard boxes. For short trips of less than 6-8 hr, 100,000 larvae per bag are typical. Oxygen is added to the bags, and the bags are opened at the pond site. The bag is floated in the pond and acclimated for 15-30 min with pond water. This allows the temperature within the bag to equal that of the water at the stocking site.

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Day	Procedure
-6	Start filling pond (0.2 ha)
-5	Add 15 L/ha ammonium nitrate and 6.25 L/ha phosphoric acid
-3	Add 227.5 kg/ha cottonseed meal
-2	Add 15 L/ha ammonium nitrate and 6.25 L/ha phosphoric acid
0	Stock larvae
2	Add 28.5 kg/ha cottonseed meal
4	Add 15 L/ha ammonium nitrate and 6.25 L/ha phosphoric acid
7	Add 28.5 kg/ha cottonseed meal
9	Add 28.5 kg/ha cottonseed meal
12	Add 15 L/ha ammonium nitrate and 6.25 L/ha phosphoric acid
15	Add 28.5 kg/ha cottonseed meal
17	Add 28.5 kg/ha cottonseed meal
20	Add 15 L/ha ammonium nitrate and 6.25 L/ha phosphoric acid
23	Add 28.5 kg/ha cottonseed meal
25	Add 28.5 kg/ha cottonseed meal
30	Harvest

 TABLE 1
 Pond fertilization schedule utilized by Texas Parks and Wildlife Department's Coastal Fish Hatcheries to culture spotted seatrout fingerlings

Note: Negative values represent days prior to stocking.

Stockings should be conducted before noon as water temperatures and light intensity increase during the day. Pond stocking densities range from approximately 500,000–700,000 larvae/ha.

Water quality parameters (dissolved oxygen, temperature, and salinity) are monitored daily at the pond-drain box during early morning and late evening hours with a multiparameter meter. Zooplankton densities in the ponds and fish growth rates are monitored two to three times per week. Zooplankton presence is assessed using standardized zooplankton tows, pulled vertically at the deepest end of the pond. Mean densities per liter of rotifers, copepod nauplii, and copepods (adults and copepod) are calculated each week. A density of greater than 250 zooplankton organisms/L provides a suitable food supply for the larvae as they grow. As the larvae develop to the fingerling stage, supplemental dry feed (40–50% protein) can be provided beginning 6–14 days (8–21 mm TL) after stocking to increase the survival and growth of spotted seatrout fingerlings by reducing starvation and cannibalism. Supplemental feeding rates of approximately 4 kg/ha daily have been used successfully (Colura et al., 1992). Feed rates and feed size are increased as fingerlings grow and zooplankton densities are diminished. Fingerlings remain in the ponds for 30 days or until they reach a target size of 30–35 mm total length. Once they reach the target size, the ponds are drained, and the fish are harvested.

Most hatcheries harvest fingerlings by draining the pond into the catch basins, but seines and nets can be used. During harvest, fish are sampled to assess survival and growth. The fish are transferred to portable tanks for transport to advanced growout (market size) facilities or coastal stocking locations for stock enhancement purposes. Survival from stocking to fingerling (25–30 mm TL) harvest averages 30–40%.

Because spotted seatrout do not survive sudden temperature drops and are susceptible to cold winter weather, the growout of fingerlings (25–30 mm) to market size (35–38 cm) is generally conducted at indoor closed recirculation facilities. This involves overwintering the fish indoors to reach marketable size in one growing season. Fingerling sized fish are moved in standard fish hauling tanks equipped with an oxygen system. Maintaining water quality in tanks while transporting fingerlings is critical. The tank water should be cleaned by filtration (i.e., sand filter) and free of organic debris. A salinity similar to the harvested pond's water is preferred for hauling, but 20–40 psu has been used successfully. The temperature of the water while hauling fingerlings should be between 20 and 26°C. Transport water temperatures below 15°C or above 27°C should be avoided as they can cause excessive stress on the fish. The water's condition should be hard (preferably over 300 ppm) while slightly alkaline (pH 7.5–8.5).

Oxygen concentration in the hauling tanks is held above saturation while fish are being loaded to account for increased respiration rates due to handling stress. As the fish become acclimated to hauling tank conditions, dissolved oxygen levels should be adjusted to near saturation levels.

# 3.2.2 | Intensive culture

Eggs collected from volitional spawning are incubated in a separate controlled environment at a stocking density of 1/ml. Saltwater is exchanged at a rate of 0.3 L/min (4.3 turnovers/day). After hatching, the conical bottom incubators are siphoned to remove debris and eggs that ceased to develop and hatch. The larvae are maintained in the incubation system for an additional 24 hr. Water temperature in the incubator is kept between 26 and 28°C under continuous photoperiod. The exchange rate is maintained at 0.3 L/min (4.3 turnovers/day). Aeration (~50 ml/min) is through a medium pore diffuser placed in the center of the incubator to distribute air evenly from the center to the incubation tank's perimeter, allowing the yolk-sac larvae to upwell and downwell gently and slowly through the water column. By 2 DPH, the larvae are counted in the incubators and are moved volumetrically from the incubation system to the conical bottom culture tanks.

Although some specific components have varied across the years, intensive larval rearing systems at the University of Southern Mississippi typically consist of 2,000-L culture tanks (3–5 per system), which drain to a sump. Conical bottoms are preferred for various technical reasons including ease of cleaning and drain harvesting, but flatbottomed tanks have been used successfully. A bead filter (Bubble-Bead<sup>®</sup> or Polygeyser<sup>®</sup>) serves as both a particle and biological filter. A series of cartridge filters removes fine particles and residual prey (10–50  $\mu$ m), and a UV sterilizer sanitizes water for return to the culture tanks. An in-line titanium water heater is also included to raise the temperature as needed. Pure oxygen is provided when needed through a diffuser from a central liquid oxygen system. Culture systems are designed to accommodate a high (20% body weight per day) feeding rate with a relatively low (up to 15 L/min or 11.5 turnovers/day) water exchange rate at the culture density used (20 larvae/L).

#### Feeding

Early culture efforts involved adding cultured live microalgae (*Isochrysis galbana*, *T-Iso*, *Nannochloropsis occulata*) as background in the culture tanks to maintain some minimal nutrition level in the prey beyond their initial enrichment and stabilize water quality. As the larviculture protocols evolved, we transitioned to an algal concentrate due to logistical and budget constraints and consistency issues associated with live algae. Our initial product (RotiGrow Nanno<sup>®</sup> from Reed Mariculture, Inc.) is a high-yield rotifer food comprising a concentrate of a single algal species (*Nannochloropsis occulata*) designed to produce phospholipid-rich rotifers with high levels of polyunsaturated fatty acids.

Nauplii of calanoid copepods, which are considered superior to rotifers and brine shrimp, were offered during early feeding (through day 6) of seatrout larvae by Lemus, Blaylock, Apeitos, and Lotz (2010). The inclusion of copepod nauplii did not increase survival, but larvae fed either copepods alone or in combination with rotifers were larger and in better condition than those fed rotifers alone, perhaps because copepods provided a nutritional supplement. Larvae fed copepods also tended to be more uniform in size than those fed rotifers. Despite the positive effects of copepods on early growth, Lemus et al. (2010) concluded that the benefits of copepods are currently outweighed by the cost and difficulty in providing a sufficient number of copepod nauplii to support large-scale culture.

#### Management of cannibalism

Cannibalism is a major issue in spotted seatrout. As reported in Arnold et al. (1976), cannibalism begins on or around 10 DPH and continues throughout the species' life stages. USM has sought to decrease cannibalism and increase survival by improving feeding strategies, stocking density, water turnover rates/flow, and salinity. These advancements, made through a combination of experimentation and ad hoc adjustments, allowed reducing cannibalism-associated mortality to less than 15% (based on the difference between the total accounted mortality and the number of larvae harvested).

Optimization of stocking density and feeding frequency involved ad hoc adjustments to protocols and behavior studies completed by Manley, Rakocinski, Lee, and Blaylock (2014, 2015). Manley et al. (2014) found that aggressive behaviors were generally higher at lower stocking densities (15/L and 30/L) than at a high stocking density (60/L). Survival did not differ among stocking densities, but larvae at high stocking densities (30/L and 60/L) grew more slowly than those at 15/L. Manley et al. (2015) showed that a 2-hr feeding frequency (compared to 1-, 4-, and 8-hr frequencies) elicited the fewest aggressive behavior. Hunger, perhaps driven by gut evacuation time and food availability, was the main influence on aggressive behavior. Thus, while we were unable to adequately test the combination of optimal density and feeding frequency, the studies suggest that seatrout may be cultured for at least 13 days at a significantly higher density (>30/L) than previously demonstrated as long as adequate levels of food can be maintained.

Ostrowski and Molnar (1998), Chambers, Apeitos, and Zimmerman (2001), and Callan, Laidley, Ostrowski, and Molnar (2012) showed that a high-flow environment and an increased exchange rate of water through the tank decreased cannibalism. Our initial assessment of the appropriate flow (flow rate, current speeds, aeration, and exchange rates) in the seatrout system was by trial and error. The energy was controlled by adjusting the angle of spray bars returning water to the tanks to create sufficient current to maintain the majority of the fish stationary and swimming against the current. Current speed increases progressively from 3 cm/s or 2 body lengths/s (12 DPH) to 10 cm/s or 4 body lengths/s (23 DPH) as larvae develop.

#### Culture at low salinity

Currently, larvae are cultured in approximately 25 psu or greater artificial saltwater (Crystal Sea<sup>®</sup> Marinemix - bioassay formula). Because of the cost (a minimum of  $1.08 \times 10^5$  gal is used annually in the USM hatchery at \$0.10-0.11/gal at 30 psu), we investigated the feasibility of using low salinity culture methods for rearing spotted seatrout larvae and juveniles. Gigli (2019) found that the hatch rate of eggs spawned at 25–35 psu is reduced significantly if embryos are incubated at 12.5 psu, but not at 18.75 psu. However, the embryos that did hatch at 12.5 psu survived as well as those maintained at 18.75 psu or 25 psu. Thus, potential techniques for mitigating hatch-related mortality could include lowering the salinity of the broodstock to decrease the osmotic shock of lower incubation salinity, increasing stocking density at 12.5 psu to account for the higher initial loss, or stocking at high salinity followed by a progressive reduction of salinity to avoid the osmotic shock incurred by a direct transfer. The feeding phase of larval culture remains to be formally evaluated at low salinity.

#### Current protocol at USM

Our current approach involves using conical bottom tanks stocked with fifteen 2 DPH seatrout larvae per liter. Enriched (INVE Aquaculture Selco<sup>®</sup> S-presso) s-type rotifers are fed at 3 rotifers  $ml^{-1} day^{-1}$  beginning at 60 hr post-hatch and slowly increased to 24  $ml^{-1} day^{-1}$  through approximately day 11. Water flow is initiated at 1 L/min (0.7 turnovers/day) at 60 hr post-hatch and increased to 12 L/min (9 turnovers/day) at the end of the 25-day larviculture period. A background algal concentrate (RotiGrow Nanno–Reed Mariculture) is also added twice daily for a five-day period beginning at 3 DPH. Beginning at 10 DPH, or when >80% of the fish are at least 5.0 mm TL, enriched (INVE Aquaculture, Selco<sup>®</sup> easy DHA) Instar-2 brine shrimp nauplii are offered at 3 nauplii  $ml^{-1} day^{-1}$ . Dry feed beginning with a 0.15 mm pellet is co-fed in small amounts with brine shrimp beginning after 1–2 days of the transition to brine shrimp. The amount of brine shrimp is increased to 24 ml/day through 14 DPH and remains at those levels through

approximately 19 DPH. Pellets are increased in size and amount to a 0.8 mm pellet fed at 280 g daily on day 12 and a 1.2 mm pellet fed at 345 g daily at 22 DPH. Flow is also increased to 4 L/min (3 turnovers/day) at 12 DPH to 14 L/min (10.5 turnovers/day) at 23 DPH. After 19 DPH, the proportion of brine shrimp in the ration decreases to 18 and 16 ml/day over a 2 day period, and the proportion of dry feed increases so that the fish are completely weaned to pellets on approximately 25 DPH, at which point fish are approximately 25–30 mm. The 25–30 mm juve-niles can be either released or transferred to nursery systems for further culture. Adaptive management of feed transitions, larval density, feeding frequency, and general husbandry resulted in increased survival over early runs. Since 2016, survival has averaged 48%.

# 4 | NURSERY AND GROWOUT

Nursery and growout are accomplished in temperature-controlled systems equipped with propeller-washed bead filters, moving bed bioreactors, protein fractionators equipped with ozone, and oxygen cones supplied from a central liquid oxygen tank. Each system, which may range from a series of 1.5 m<sup>3</sup> tanks linked together to single 40 m<sup>3</sup> units, is designed to accommodate bioloads in excess of 40 kg/m<sup>3</sup>.

USM historically has grown fish to only approximately 100 mm for tagging and releasing. Initial attempts included stocking fish directly from larval rearing tanks into large (5–40 m<sup>3</sup>) tanks, but survival was low due to the inability to precisely manage the flow and feed distribution. As such, we transitioned to stocking from larval rearing into smaller (1.5–2 m<sup>3</sup>) nursery tanks for approximately 70 days of pre-growout culture prior to stocking into the larger tanks.

# 4.1 | Nursery and pre-growout

Early nursery trials in the small tanks investigated the effect of density on survival through the early nursery period (25–50 DPH). Survival ranged from 93% at the low stocking density (0.8 fish/L) to 82% for the high stocking density (3.5 fish/L) under a high exchange rate (40 L/min) and a strong current (15–20 cm/s) with feeding rate adjusted to 8–10% body weight/day. FCRs ranged from 0.95 to 1.1 (Blaylock and Apeitos, unpublished results).

Cannibalism is also controlled via size grading, another strategy widely used in finfish culture. The current USM protocol involves separating the largest fish in the population, currently estimated at approximately 5% of the population, every 20–25 days (Blaylock and Apeitos, Unpublished data) into separate tanks and achieves survival greater than 85% through the 70-day nursery phase.

The current process involves stocking 25-DPH larvae into 1.6 m<sup>3</sup> nursery tanks at  $3-5 \times 10^3$  fingerlings/m<sup>3</sup> density and feeding them initially at 5% body weight/day and decreasing to 3% over 30 days. Fish grow approximately 1 mm/day and achieve approximately 90 mm (15 g) by 90 DPH. The FCR during this phase is typically 1.8–2.3.

# 4.2 | Growout to market size

Information on growout of fish past the pre-growout phase (90 DPH) is limited. Preliminary trials were conducted in 10 m<sup>3</sup> tanks and 35 m<sup>3</sup> tanks. Fish were cultured under constant light (24-hr light period) at 25–27°C and 25 psu. Fish were graded monthly to remove the largest fish (about 5% of the population) into separate tanks as mentioned earlier. Market size seatrout (35–38 cm TL, approximately 450 g) were harvested after approximately 10 months (Blaylock and Apeitos, unpublished data). Gigli (2019) reported faster growth at 10 psu than at 20 or 25 psu during a 267 day growout trial suggesting potential advantageous outcomes of low salinity culture.

# 5 | LIMITATIONS/NEEDS

# 5.1 | Nutrition and feeds

There are currently no specific data on the nutritional requirements of spotted seatrout and, to the best of our knowledge, no specific diet has been developed for any stage of this species. Seatrout are routinely grown on diets developed for other marine species, including Skretting Gemma, Rangen Salmon Starter, and Ziegler marine diets. Nutritional requirements need to be formally studied. Such studies could result in lowering the currently known FCRs. Studies on the closely related red drum could be used to orient research, particularly recent success incorporating soybean meal at high rates (70%) without detrimental effects on production traits (Rossi Jr., Newcomb, & Gatlin III, 2016).

# 5.2 | Breeding/domestication/genetics

To date, aquaculture programs for spotted seatrout have been developed by state agencies in Texas, Mississippi, Florida, and South Carolina only for stock enhancement purposes. All programs involved breeding wild-caught spotted seatrout to prevent domestication and minimize genetic impacts on wild populations. To the best of our knowledge, there have been no attempts to domesticate the species. Domestication would benefit a developing industry by increasing the predictability of production performance and identifying traits for use in selective breeding programs. The high survival rate during larval rearing and growout of spotted seatrout discussed earlier would be compatible with common-garden breeding designs where families are mixed in the same tanks for evaluation in the same conditions, and parentage information is recovered at the end of the culture period using molecular pedigrees (e.g., Saillant, Ma, Wang, Gatlin, & Gold, 2007). The relative success of producing viable embryos during strip spawning reported earlier also suggests that large mating designs could be created for genetic testing and estimation of breeding values in family breeding programs (e.g., Dupont-Nivet et al., 2007).

Understanding the structure of natural populations is a prerequisite to a domestication program. It allows determining the geographic areas where broodstock can be sourced to stock culture units (e.g., net pens) with minimal expected impacts on surrounding wild populations if fish escape and reproduce with local wild fish. The population structure of spotted seatrout was investigated in several studies using mitochondrial DNA (Gold, Richardson, & Furman, 1999), microsatellites (Seyoum et al., 2018; Somerset & Saillant, 2014; Ward, Bowers, Hensley, Mobley, & Belouski, 2007), and allozymes (e.g., King & Zimmerman, 1993; Ramsey & Wakeman, 1987). All studies were consistent with an isolation by distance pattern. The survey of Seyoum et al. (2018), who sampled most of the range in the US, revealed two discontinuities separating a western group (west of the Apalachicola River), the Florida Gulf coast east of Apalachicola, and populations of the eastern US coast north of Palm Beach, respectively. Allozymes studies also suggested adaptive variation occurring on a latitudinal gradient in Texas (King & Zimmerman, 1993). Further analysis of genomic variation may reveal other subdivisions reflecting local adaptation. This information will prove useful to design relevant units for the management of culture systems where interaction with wild stocks is a concern, and to sample adequately for genetic variation to build strains with maximum genetic variability and initiate selective breeding.

# 5.3 | Marketing

Despite the fact that spotted seatrout meat is pleasantly textured and mildly flavored and that juveniles can be produced in large numbers as seeds for growers, the species is not widely known beyond the coastal southeastern US.

The economic contribution of commercial fishing of spotted seatrout was estimated based on 2015 landing values using the IMPLAN software at a modest \$216,000 total impact of which \$106,669 was the landing value

(Posadas, 2017). This low number may underestimate consumption in restaurants in particular. Commercial landings are clearly seasonal with low values during winter indicating a potential niche for cultured products to supply the market.

An aquaculture industry that provides a consistent supply to local markets is possible, but a long-term, sustainable strategy requires expansion beyond local markets. Several issues must be addressed to establish seatrout culture as a viable industry, including the economics of production, consumer perceptions, and producer impediments.

USM has determined that a 25-mm fish can be produced in a RAS system for \$0.35 -\$0.75/fish depending on the number produced (the labor required for a production run is largely independent of the size of the run, so producing 150,000 as opposed to 50,000, for example, decreases the cost per fish). Production data at USM suggest that a 90-mm fish would cost \$1.50 to \$2.00 per juvenile depending on production numbers per unit volume. The cost of production beyond that stage is unknown. A detailed cost analysis that includes production/processing, fixed, capital, and start-up costs is required.

A market evaluation needs to assess regional and national consumer preferences/perceptions. Consumer attitudes with respect to wild vs. farmed, local vs. nonlocal or imported, spotted seatrout vs. other mild-flavored white fish, price, and cut would inform potential farmers and processors in the development of a viable business plan. The supply-side also should be engaged to assess market capacity, producer/processor interest, and potential participation barriers.

# 6 | SUMMARY

In summary, work to date showed that large-scale production of spotted seatrout larvae to support an industry is feasible. Extensive production in ponds is constrained by low yields per unit area, high variability and low predictability, and the limited availability and extent of coastal and/or saline ponds. In addition, pond production limits the ability to manage cannibalism, a significant issue in seatrout culture. Closed-system intensive culture alleviates these constraints and maximizes control over the environment, facilitating the high production per unit area desired in commercial operations. Survival rates of spotted seatrout approach 50% in these systems, comparable to those in mature marine hatchery industries. The next step for seatrout aquaculture is to determine this industry's economic viability through cost and market analyses and perform formal commercial scale growout trials to determine zootechnical parameters needed to assess growout production costs. Further work on nutrition and domestication would likely benefit the industry through improved performance and product quality.

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#### CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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