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The status of black sea bass, *Centropristis striata*, as a commercially ready species for U.S. marine aquaculture

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Abstract

Black sea bass (BSB), Centropristis striata, inhabit continental shelf waters of the eastern United States and are a member of the family Serranidae comprising true sea basses and groupers. Highly sought by fishermen, BSB are sustainably managed with 2018 commercial and recreational catch quotas of 1,600 and 1,664 m.t. (3.52 and 3.66 million lb), respectively. Wild broodstock are easily caught and adapted to recirculating aquaculture systems (RASs). The initiation and duration of the spawning period are controlled by photothermal conditioning, and eggs and larvae have been produced from December through August. GnRHa $(5-10 \mu g/g bw)$ implants are effective at inducing ovulation in post-vitellogenic females (>500 µm mean oocyte diameter = MOD). Fertilized eggs (0.94 mm diameter) are obtainable by strip spawning, but volitional spawning may yield higher egg quality. Most BSB develop as females and then switch to male (protogynous hermaphrodites) after several years. Yolksac larvae (YSL, 0 day post-hatching = 0 dph, 3.0 mm total length = TL) are reared to the post-metamorphic stage in RASs using greenwater Nannochloropsis oculata and enriched rotifers (2-22 dph), Artemia nauplii (16-22 dph)

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and enriched metanauplii (23–36 dph), and co-feeding microparticulate diets (55.5–59% crude protein (CP), 10–15% crude lipid (CL)) from 15 dph, with complete weaning by 36 dph. Environmental optima for larvae are temperature (19–22°C), salinity (28–36 g/L), light intensity (1,500 lx), and photoperiod (16 L: 8 D). Survival of YSL to 50 dph (1 g) averages 12–15%. Advanced fingerlings (mean wt. = 27 g) were stocked in 16 m³ RAS tanks (103 fish/m³) at 33 g/L and 21°C and fed a commercial diet (55% CP, 15% CL) reached mean marketable sizes of 454 g (1 lb), 568 g (1.25 lb), and 682 g (1.5 lb) in 17, 20.2, and 22.9 months post-hatching, respectively, with high growth variation. Harvest biomass density was 55 kg/m³ and feed conversion ratio was 1.1–1.2. Pasteurellosis *Photobacterium damsela* infections during growout were controlled by lowering water temperature. Wholesale prices for whole-on-ice BSB (0.75 lb to >2.0 lb) are size-tiered, with higher per pound prices for larger fish.

density was 55 kg/m³ and feed conversion ratio was 1.1-1.2. Pasteurellosis Photobacterium damsela infections during growout were controlled by lowering water temperature. Wholesale prices for whole-on-ice BSB (0.75 lb to >2.0 lb) are size-tiered, with higher per pound prices for larger fish. BSB growers target niche markets for ultra-fresh product, which garner premium prices for fish of assorted sizes. Availability of BSB fingerlings from the University of North Carolina Wilmington's hatchery has enabled startup RAS farmers to grow and to market BSB, but commercial expansion will require investment in research to lower production costs. Research is needed to lower feed and fingerling costs, increase growth and minimize size variation through grading and selective breeding, maximize biomass densities in RAS, and biomitigate RAS nutrients by multitrophic aquaculture. In-depth economic modeling of BSB production in RAS incorporating the latest and untapped advances in culture technologies will be important to understand opportunities for improving profitability.

KEYWORDS

diseases, economics and marketing, growout, hatchery, larval rearing, marine finfish aquaculture, nursery, RAS, waste management

1 | INTRODUCTION

Black sea bass (BSB), *Centropristis striata*, are a high-value marine finfish that inhabit continental shelf waters from Maine to the Florida Keys and are members of the family Serranidae comprising true sea basses and groupers. Two

distinct stocks of BSB have been identified along east coast of the United States: the North Atlantic stock, ranging from Maine to Cape Hatteras, North Carolina (NC); and the South Atlantic stock, ranging from Cape Hatteras, NC, to Florida (Roy et al., 2012; McCartney et al., 2013). Both BSB populations can be found at depths of 18–45 m in the summer around hard substrate, but the northern population overwinters in deeper water ranging from 79 to 185 m (Mercer, 1989; Musick & Mercer, 1977). A third distinct population resides in the Northeastern Gulf of Mexico (Hood et al., 1994; Mercer, 1989). Recent studies using mitochondrial DNA sequences suggest very limited mixing between BSB stocks north and south of Cape Hatteras (McCartney et al., 2013). Available data also show that Atlantic and Gulf stocks of BSB are genetically distinct (Bowen & Avise, 1990). BSB are bottom-feeding carnivores, and they aggregate and find shelter in areas with structure. Adults feed mainly on crustaceans and fish, while juveniles eat amphipods, isopods, and shrimp (Mercer, 1989).

1.1 | Life history and habitat

BSB are protogynous hermaphrodites (Lavenda, 1949; Musick & Mercer, 1977); most fish develop first as females and then later sex-reverse into males at around 23–33 cm (9–13 in) and 2–5 years of age. Mature males have a dark blue nuchal hump anterior to the dorsal fin. Spawning progresses latitudinally from south to north; in the Middle Atlantic Bight, spawning occurs from March to May (Wenner et al., 1986), while off the New England coast, BSB spawn during June and July (Mercer, 1978, 1989). Fecundity varies with size and age from 17,000 eggs/female (Wenner et al., 1986) in an age of 2 year fish (108 mm standard length = SL, 140 mm total length = TL) to 1,050,0000 eggs/female in larger older females (438 mm SL, 454 mm TL), and an average female age of 2–5 years produces around 280,000 eggs (ASMFC, 2009). The pelagic eggs (diameter = 0.95 mm) are buoyant and contain a single oil globule (White, 2004).

Precise spawning locations of BSB are not known but are believed to occur at depths of 19.8–48.8 m (65– 160 ft) on the continental shelf in coastal marine waters. After spawning, fertilized pelagic eggs float in the water column and hatch within 52 hr at 19°C. The larvae drift in coastal waters 3.2–80.5 km (2–50 mi) offshore as they grow and develop, and they settle in nearshore marine waters when they reach about 1.27 cm (0.5 in). Eventually, the young juveniles migrate into estuaries, bays, and sounds and shelter in a variety of bottom habitats such as rocks, submerged reefs, aquatic vegetation, oyster reefs, and man-made structures such as wrecks, piers, pilings, and jetties (ASMFC, 2009). Older juveniles and adults prefer deeper bays and coastal waters and are most prevalent at salinities above 18 g/L.

1.2 | Management

Both commercial and recreational harvests of BSB are controlled through a management plan. The North Atlantic stock of BSB is managed by the Mid-Atlantic Fisheries Management Council (MAFMC) and the Atlantic States Marine Fisheries Commission (ASMFC), and the South Atlantic stock is managed by the South Atlantic Fisheries Management Council (SAFMC) (NOAA Fisheries, 2020a). Neither stock is currently overfished, nor undergoing over-fishing, nor undergoing stock rebuilding (MAFMC, 2019; SAFMC, 2018). However, regulations are in place to limit the ocean catch of BSB to maintain the sustainability of the stocks. In the Mid-Atlantic region, commercial and recreational BSB fisheries are heavily managed using catch and landing limits, commercial quotas, recreational harvest limits, minimum fish sizes, closed fishing seasons, gear regulations, permit requirements, and other provisions (MAFMC, 2019). Annual catch limits for both commercial and recreational fisheries have been in place since 2012. Commercial landings have fluctuated since 1980 (Figure 1a), but dockside price per pound, adjusted for inflation, has more than doubled, reflecting strong consumer demand for the species (Figure 1b) (NOAA Fisheries, 2020b). In the South Atlantic region, commercial landings have fallen by about half since 1980. Although no longer under a stock



FIGURE 1 Black sea bass commercial fishery landings (pounds, (a)) and dockside value (\$ per pound, (b)) in the Mid-Atlantic region (north of Cape Hatteras, NC) from 1980 to 2018. *Source:* Data from NOAA Fisheries (2020b)

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rebuilding plan, BSB fisheries in the South Atlantic remain heavily regulated using a variety of management measures, including season closures and, since 2006, either total allowable catch or annual catch limits (SAFMC, 2018).

BSB are an excellent food fish, similar in appearance to Pacific groupers, with firm white flesh ideal for a variety of cooking techniques that use whole fish as well as for sushi and sashimi (Dumas & Wilde, 2009; Wilde, 2008). A solid niche market for BSB exists in the state of NC (Wilde, 2008) and in large metropolitan areas such as New York City, Philadelphia, Atlanta, and San Francisco, where fish are typically marketed fresh whole or live (Dumas & Wilde, 2009). Given consistently increasing market demand and dockside prices coupled with stringent fishing regulations that limit current and future landings, complete life cycle aquaculture production techniques for BSB from the Atlantic Coast populations have been developed in the eastern United States from Florida to New Hampshire since around 2000. Long-term comprehensive research at the University of North Carolina Wilmington (UNCW) supported by various federal (USDA-NIFA, NOAA Marine Aquaculture), state (NC Fishery Resource Grant Program, NC Biotechnology Center, MARBIONC, and NC Sea Grant), and private (Cotton Inc., NC Farm Bureau) agencies have established hatchery and recirculating aquaculture system (RAS) growout technologies for the BSB. An interest is developing in commercial production of BSB among private growers in Texas, Georgia, NC, Virginia, New York, New Hampshire, and Maine). Ongoing research is comprehensive, ranging from controlled breeding to larviculture and juvenile production in hatcheries, growout in recirculating systems, and marketing and economics. The results have been promising and have shown that BSB can be bred in captivity, raised from egg to adult stages in recirculating

aquaculture systems, and that cultured product can garner lucrative niche markets. An industry stakeholder panel at the Marine Finfish Aquaculture Workshop sponsored by Marine Biotechnology Center of Innovation (MBCOI) and by NOAA Coastal Aquaculture Planning and Environmental Sustainability Program in November 2014 assessed BSB as the top candidate food fish species for commercial aquaculture in NC. BSB is one of only a few marine finfish species currently grown in commercial RAS facilities in the United States with pilot quantities of farmed product reaching consumers in metropolitan markets. In this article, the status of BSB as a commercially ready species for U.S. aquaculture is reviewed. Statements in the following sections not followed by literature citations represent established practice at UNCW.

2 | BROODSTOCK MANAGEMENT AND CONTROLLED BREEDING

2.1 | Controlled-environment broodtank system

Controlled-environment broodtank systems used for BSB at UNCW are circular tanks 2.44 m (8 ft) in diameter and 1.22 m (4 ft) deep with a volume of 4.64 m³ (1,225 gal) (Watanabe, 2011). Outdoor tanks are insulated and provided with fiberglass dome with sliding door. Tanks are supported by biological filter (bubble bead filter), foam fractionator, UV sterilizer, and heat pump for recirculation of water, which are essential for temperature control. To control photoperiod, the cover is fitted with a daylight fluorescent fixture (500 lx at water surface) controlled by a timer to simulate seasonal changes.

2.2 | Broodstock procurement

In NC coastal waters, wild-caught broodstock BSB are preferably caught during the fall when fish are still in relatively shallow waters and have not yet moved into deeper offshore areas for winter. It is important to be aware of commercial and recreational catch size restrictions (30.5 and 35.4 cm, 10 and 12 in., in NC) and that the female-to-male transition occurs at 22.9–33.0 cm (9–13 in.). Hence, a range of fish sizes including smaller fish (likely females) are collected.

2.3 | Quarantine procedures

Upon capture, broodstock are placed in the quarantine tank system under temperature and salinity conditions similar to those at the site of capture and at a density of <30 fish/m³ (11.4 kg/m³). Fish are treated with formalin (30 mg/L, indefinite bath) to kill protozoan parasites and monogenetic trematodes (Watanabe, 2011). External parasitic crustaceans (e.g., fish lice, *Argulus* and anchor worm, *Lernaea*) are treated with CuSO₄ (0.3 mg/L active Cu⁺⁺, added daily for 10 days). Newly captured BSB broodstock accept both thawed fish (e.g., Atlantic silversides, *Menidia menidia*) and artificial feeds within a week of capture and are fed a high-quality marine fish grower with a dietary protein level of 50–55% and a dietary lipid level of 15–18% (Bentley et al., 2009). Healthy fish are transferred to a controlled-environment broodtank system after 30–45 days in quarantine.

2.4 | Stocking and feeding

Each broodtank (diameter = 244 cm, 8 ft; vol = 4.7 m^3 , 1,234 gal) is stocked with around 24 fish (5.1 fish/m³) fish at a male to female ratio of 1:1. Broodstock are fed twice daily to satiation a high-quality marine fish grower supplemented once or twice weekly with thawed fish (e.g., Atlantic silverside, *Menidia menidia*).

2.5 | Photothermal conditioning

The natural spawning season of BSB in coastal waters of southeastern NC is during the spring and early summer months (April through June). Broodstock BSB at UNCW conditioned on an ambient photoperiod cycle until April and then switched to a constant spring photoperiod of 13 L:11 D and temperature of 19°C reach maturity from April through late July and are spawnable by hormone induction throughout this period (Watanabe et al., 2003; White, 2004).

Photoperiod manipulation can be used to alter the timing of reproduction in BSB to achieve out-of-season spawning (Howell et al., 2003). Broodstock BSB in Rhode Island reared on an accelerated photoperiod cycle were spawned from March to June, whereas those on a simulated natural photoperiod spawned from May through August (Howell et al., 2003). In NC, broodstock BSB reared on an accelerated photoperiod cycle were spawned from December through February, while those held on a simulated natural photoperiod spawned from April through July (Watanabe et al., 2003; Watanabe and Carroll, UNCW unpublished data).

Female broodfish undergo sex reversal to male, usually at a size range of 22.9–33.0 cm (9–13 in.) and 2–5 years of age, so senescent males must be replaced with wild-caught females, or with first-generation (F1) females. Although the factors that cause female-to-male transition are not clearly understood, the removal of large males in a broodtank may induce larger females to change sex (Benton, 2005; Benton & Berlinsky, 2006). Stocking density and environmental perturbations can also influence the rate of sex change (Fournier et al., 2007).

2.6 | Monitoring gonad stage

Using photothermal conditioning, female BSB broodstock will usually develop their gonads through the end of the vitellogenic stage when yolk deposition into oocytes is completed. However, they do not reliably undergo the final stages of maturation and ovulation, which may be induced with hormone treatment. An ovarian biopsy is performed to confirm gonadal stage, and females with a mean oocyte diameter (MOD) of at least 330 μ m, but preferably >500 μ m (Denson et al., 2007; Tucker Jr., 1989; Watanabe et al., 2003; White, 2004), are suitable for induced ovulation by hormone administration.

2.7 | Hormone-induced spawning

The synthetic neuropeptide hormone luteinizing hormone-releasing hormone analog (LHRHa) is a popular option for induced spawning of BSB. The hormone is incorporated into a cholesterol-cellulose (90:10) pellet (Berlinsky et al., 2005; Watanabe et al., 2003; White, 2004), which is implanted into the muscle near the dorsal fin, and injection of LHRHa in liquid form is equally effective at inducing ovulation (Berlinsky et al., 2005). Low-to-mid dose levels (5–10 µg/kg body weight) (Bentley et al., 2009) result in better spawning performance than higher dose levels (50–75 µg/kg body weight) (Watanabe et al., 2003; White, 2004). After treatment, the female is placed into a spawning tank along with three to four spermiating males, with spawning anticipated in approximately 48 hr. Spermiating males are identified by the release of a hydrated sperm ("milt") from the sperm duct when the abdomen is gently squeezed.

2.8 | Volitional spawning

For volitional spawning, the LHRHa-implanted female is placed in a spawning tank with several spermiating males. Spawning usually begins approximately 2 days following implant, with the female releasing eggs and the males releasing sperm in the water column where fertilization takes place. Volitional spawning is usually completed within 2-6 days but can continue for up to 10 days after hormone implantation. At a hormone dose of 5 µg/kg, a female will release around 72,000 eggs and yield around 6,000 yolksac larvae (YSL)/female. To increase egg production, multiple females can be implanted and placed in the same tank with spermiating males for "group spawning," maintaining the same ratio of approximately two to three males per female. Group spawning has a synergistic effect on both spawning performance and egg quality when compared to spawning individual females (White, 2004).

2.9 | Strip spawning

Volitional spawnings yield inconsistent egg production and eggs of different developmental stages that differ in age by up to 10 days. For more consistent egg production, the strip spawning technique may be used, but is more labor intensive and stressful on the broodstock and can cause mortality. To optimize timing of strip spawning, females are checked with a cannula (internal diameter = 1.14 mm) at 2–4 hr intervals beginning approximately 36 hr after implant. Females with egg samples containing >50% hydrated and translucent (ripe) eggs are ready to be strip-spawned. Females release around 85,000 eggs (mean diameter = 0.94 mm), or 77,000 eggs/kg body weight. Eggs are fertilized by adding approximately 1 ml of milt per 100 ml of eggs (1,345 eggs/ml).

2.10 | Egg incubation

Following strip spawning and fertilization, floating (viable) embryos are incubated at densities of no greater than 1,000/L. Diffused aeration maintains embryos and larvae in suspension. Water temperature is maintained at 19°C, water flow to the incubator is adjusted to approximately 500–700% of tank volume/day and makeup seawater is added at 30–50% of tank volume/day to maintain quality. At 19°C, eggs will hatch about 52 hr after fertilization.

3 | LARVAL REARING

3.1 | System requirements

The larval culture system consists of 1.85 m (6 ft) diameter by 0.91 m (3 ft) deep fiberglass tanks (vol = 2,500 L, working vol = 2,000 L). Tanks are supported by bubble bead filter, foam fractionator, UV sterilizer, and heat pump for recirculation of water. A mesh screen standpipe is in the center of the tank. At stocking, the standpipe is covered with 105-µm mesh screen, which is increased incrementally to 250, 500, 750, and 2,000 µm as the larvae grow. Diffused aeration is supplied to the larval rearing tank (LRT) through airstones positioned to create a circulation pattern sufficient to maintain larvae in the water column. BSB larvae need relatively high illumination (1,000–1,500 lx at the water surface) to feed efficiently, and standard fluorescent fixtures are used to provide uniform lighting (Copeland & Watanabe, 2006; Russo et al., 2017). A long photoperiod of 18 hr light and 6 hr dark is maintained to allow larvae to feed for a prolonged time each day (Russo et al., 2017).

3.2 | Hatching through metamorphosis

3.2.1 | Stocking

LRTs are filled with full strength seawater (34 g/L) and water temperature and exchange are adjusted to 19° C and 200%/day, respectively. On 1 dph, YSL (~3.0 mm TL) are stocked into the LRTs at a density of 30 larvae/L (60,000 larvae/tank). Aeration is adjusted to keep the larvae in suspension without causing excessive swimming, which can

inhibit feeding and growth (Mangino & Watanabe, 2006). Microalgae (greenwater), specifically *Nannochloropsis oculata* in condensed paste form, is added twice daily beginning on 1 dph and continuing through the rotifer feeding stage (20 dph) to maintain a density of 1,000,000 cells/ml (Berlinsky et al., 2000).

Beginning on 1 dph, tank temperature is increased by 1°C each day until a final rearing temperature of 22°C is reached around 4 dph. The water surface in each tank is cleaned continuously using an air-driven skimmer (Figure 2) to remove oil and debris and to promote oxygen exchange at the surface.

3.2.2 | Feeding and maintenance

Live feeds (rotifers and Artemia)

BSB larvae can be intensively reared through juvenile stages using standard feeding regimens for marine finfish, including enriched rotifers from 2 dph through 20 dph, weaning to *Artemia* over a 6-day period (15–21 dph), co-feeding of a formulated diet beginning on 15 dph, with complete weaning from live feed by 35 dph, coinciding with the onset of metamorphosis (Berlinsky et al., 2000; Berlinsky et al., 2001; Carrier III et al., 2011; Copeland & Watanabe, 2006; Rezek et al., 2010; Russo et al., 2017). Except for freshly hatched *Artemia*, rotifers and older *Artemia* nauplii are enriched before they are fed to the larvae to improve nutritional quality, especially the essential fatty acids docosahexaenoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, ARA) (Carrier III et al., 2011; Rezek et al., 2010; Russo et al., 2017).

At UNCW, a practical enrichment protocol used successfully for BSB includes pre-enrichment with Rotigrow Plus (Reed Mariculture Inc., Campbell, CA) to boost essential fatty acids, DHA, eicosapentaenoic acid (EPA), and ARA, followed by an 8 hr enrichment period using N-Rich (Reed Mariculture, Inc.), a blend of microalgae that provides enrichment of polyunsaturated fatty acids (PUFAs), protein, and other essential nutrients (Watanabe and Carroll, UNCW, unpublished data). Larvae are fed live prey at least twice per day (08:00 and 17:00 hr), but more frequent feedings throughout the photophase (e.g., 08:00, 12:00, 17:00, and 21:00 hr) are advantageous (Russo et al., 2017). For a prescribed total daily ration of rotifers and *Artemia*, feeding lower prey densities (smaller meals) at higher frequencies $(3 \times /day \text{ or } 4 \times /day)$ improves larval growth and vitality and may increase osmoregulatory ability, without affecting survival through pre-metamorphic stages, and therefore optimizes the efficiency of utilization of expensive planktonic prey while maximizing fingerling yield and quality (Russo et al., 2017).

Artificial microparticulate feeds

Artificial microdiets are added beginning on 15 dph and are co-fed with both rotifers and Artemia. Live prey is gradually reduced as artificial feeds are increased over a 20-day period through 35 dph. A high-quality marine microdiet (e.



FIGURE 2 Hatchery-raised black sea bass post-metamorphic stage juveniles (approximately 50 dph, 1 g weight) raised in a 2,000 L larval rearing tankat the University of North Carolina Wilmington (UNCW) Aquaculture Facility (Wrightsville Beach, NC). *Source*: Photo courtesy: UNCW/W.O. Watanabe

g., Otohime, Nisshin Feed Co., Tokyo, Japan; Gemma Micro, Skretting, Canada; or NRD, INVE, Belgium) (crude protein = 55.5–59%, crude lipid = 10–15%, 200–300 μ m particle size) is fed at least four times per day at a nominal rate (0.5 mg/fish/day) (Alam et al., 2006). Feed rate and particle size are increased based on mouth size and feeding success.

3.3 | Daily protocols

On 2 dph, rotifers are added at a density of 5/ml and then gradually increased until a maximum feeding density of 20/ml is reached by 10 dph. Beginning on 15 dph, larvae are fed newly hatched *Artemia* nauplii at a density of 0.15/ml, which is increased daily until a maximum density of 3/ml is reached around 27 dph. On 15 dph, artificial microdiet (250μ m) is co-fed with newly hatched *Artemia* two to three times per day at a nominal rate of 0.5 mg/fish/day. On 20 dph, microdiet (250μ m) is co-fed with newly hatched *Artemia* two to three times per day at a nominal rate of 0.5 mg/fish/day. On 20 dph, microdiet (250μ m) is co-fed with enriched *Artemia* metanauplii. Beginning on 25 dph, microdiet particle size is increased ($250-360 \mu$ m), and automatic feeders are used to provide continuous feeding during the photophase. On 30 dph, *Artemia* density is decreased by 50% per day so that larvae are completely weaned to artificial feed ($360-600 \mu$ m) by 35 dph. Beginning on 35 dph, particle size is increased to $600-1,000 \mu$ m as necessary. Larvae are typically transferred to nursery tanks (NTs) on 50 dph when they have attained a body weight of approximately 1 g (Figure 2).

3.4 | Environmental conditions

Eggs are incubated and hatched at 19°C, but temperature is gradually increased and maintained at 22°C after 4 dph. Salinities of 28–36 g/L are suitable for larviculture (Berlinsky et al., 2004; Copeland and Watanabe, UNCW, unpublished data), but lower salinities will reduce buoyancy, so higher levels of aeration will be needed to maintain the early larvae in suspension. Dissolved oxygen (DO) (7–8 mg/L), pH (7.5–8.5), air flow, and gas saturation (100%) are monitored daily. Water exchange is gradually increased from 200%/day at stocking to 300–500%/day by 35 dph.

4 | NURSERY

4.1 | System requirements

The nursery system is similar to the broodtank system in design, but recirculation system components are increased in size to handle greater feed loading. The basic nursery system consists of two 2.46 m (8 ft) diameter \times 1.23 m (4 ft) deep fiberglass tanks with a working volume of 4,756 L (1,256 gal). An external standpipe controls water level, and an internal center standpipe covered by a 1–2 mm mesh screen prevents fingerlings from entering the standpipe drain. Flow is adjusted to provide a daily exchange of 900–1,000%. Diffused aeration is supplied from a blower to each NT through a 30.4 cm (12 in) diffuser on the tank bottom. To illuminate the NTs, standard fluorescent fixtures are used to provide uniform levels of lighting in the range of 1,000–1,500 lx at the water surface. Optimal photoperiod for nursery rearing of BSB is 18 hr light and 6 hr dark (Carrier III et al., 2011; Russo et al., 2017).

4.2 | Stocking

Post-metamorphic stage juveniles (mean weight = \sim 1.0 g, age = \sim 50 dph) are stocked into the NTs at a density of 1.5 fish/L (5.7 fish/gal), or 7,134 fish per tank. BSB juveniles are territorial, and cannibalism is a concern during

nursery culture. Stocking densities of up to 5.0 fish/L improve survival and growth of BSB juveniles in 150-L rectangular raceways, probably by inhibiting interfish aggression and cannibalism (Watanabe & Truesdale, 2008). Recent studies at UNCW have shown that stocking early post-metamorphic stage BSB (mean wt. = 0.54 g, age = 47 dph) in NTs at higher densities of up to 6.5 fish/L does not affect survival, growth variation, or feed conversion ratio (FCR) to a transport ready stage on 60 dph (Carroll et al., 2018; Watanabe et al., 2019). Fingerling BSB at these stages feed vigorously and are tolerant of handling for grading and splitting among tanks. Higher current velocities in the range of 0.04–0.09 m/s in circular tanks also minimize cannibalism (Stuart & Smith, 2003).

4.3 | Feeding and maintenance

In NTs, fingerlings are fed a 1 mm artificial pelleted diet (crude protein = 55%, crude lipid = 16%) at around 5% body weight/day over 4-5 feedings per day, using manual or automatic feeding. At each feeding, fish are fed to satiation, usually requiring 10–15 min. On 80 dph, feeding frequency is decreased to two times per day (08:30 and 15:00 hr). Feed pellet size is increased and the daily feed rate is decreased as fish grow: 3 mm at 4% body weight/day on 75 dph (~3 g) and 5 mm at 3% body weight per day on 95 dph (~5 g).

4.4 | Environmental conditions

Based on laboratory studies, the optimal salinity for growth of juveniles was reported to be 23.4 g/L, and fish grown at salinities of 20 and 30 g/L grow at similar rates and much faster than fish at 0 g/L (Atwood et al., 2001, 2003; Cotton et al., 2003). Recent studies at UNCW have shown that juvenile BSB can be acclimated to and raised under semi-pilot scale recirculating aquaculture system conditions at a low salinity of 10.1–12.3 g/L and at temperatures of 21–23°C, with no adverse effects on long-term growth performance, plasma osmolality, or fish plasma electrolytes compared to fish raised in full-strength 34 g/L seawater (Alam et al., 2020). Fish fed diets supplemented with 2.5–7.5% sea salt showed significantly improved survival under extreme low salinity (\sim 4 g/L) challenge conditions. These findings have potentially important implications for rearing BSB in low-salinity RAS and for siting growout operations, which may consider locations where brackish water of 10.1–12.3 g/L or higher may be sourced or prepared (Alam et al., 2020).

Optimum temperature is 25.6°C (Atwood et al., 2001, 2003; Sullivan & Tomasso, 2010), and fish reared at 25°C grow faster than those reared at 16, 20, or 30°C (Cotton et al., 2003). Juvenile BSB survive exposure to 50 mg/L nitrite-N for 10 days in 12, 20, and 35 g/L salinity, but 50% mortality occurs when exposed to 0.7–0.8 mg/L un-ionized ammonia for 24 hr at 25°C and 20 g/L salinity (Atwood et al., 2004). Median lethal concentration (LC50-96 hr) of NO₂-N is 190–241.9 mg/L, while LC50-96 hr for NH₃-N is 0.46–0.54 mg/L. Hence, juvenile BSB are relatively sensitive to acute un-ionized NH₃-N exposure (>0.5 mg/L) and are highly resistant to NO₂-N exposure (190–242 mg/L nitrite-N) (Atwood et al., 2004; Weirich & Riche, 2006). Ammonia should be maintained under 0.5 mg/L and nitrite under 50 mg/L.

5 | ECONOMIC ANALYSES OF A HYPOTHETICAL COMMERCIAL HATCHERY

The price of fingerlings that can be grown to marketable size using RAS technology is a critical variable cost in the development of a commercial BSB aquaculture industry. Under support by the North Carolina Biotechnology Center (Regional Development Grant Program), a pilot hatchery was built at UNCW's Aquaculture Facility to scale up

laboratory-based research to a pilot commercial scale and provide a source of fingerlings to startup fish farmers. Based on engineering, biological, and cost data from operating the pilot marine fish hatchery, an economic analysis of a hypothetical commercial scale BSB hatchery operation was conducted (see Watanabe, 2015 for details).

5.1 | Facility site and structures

For the baseline hatchery facility, the following assumptions were used: Project life was 30 years with equipment replaced every 10 years. Hatchery capacity was 100,000 advanced (5-g) fingerlings. The facility owner owns the land, works as general manager of the facility, and receives all returns to management (profit). It assumes 6.5% annual interest rate on the facility's construction and equipment loan with a 10-year term (repayment period) and 10% annual interest on monthly operating capital.

The hatchery is sited in coastal NC on 0.202 ha (0.5 acre) of commercially zoned land with salt water access. Due to its proximity to the coast, the 0.202 ha of facility land has a relatively high market value of \$125,000/acre, or \$62,500. The land supports a single 36.6 m \times 21.3 m (780 m²), rectangular, insulated metal building (Figure 3) with concrete floor, electrical, plumbing, and HVAC at a cost of \$807/m² (Watanabe et al., 2015) or \$630,000 (Table 1). The remainder of the site supports feed bins, a waste treatment area, parking, and zoning setbacks. The interior of the building is partitioned into a broodstock tank (BT) room, live feed culture room, egg incubation room, larviculture room with LRTs, NT area, laboratory/office room, and restrooms (Figure 3).

5.2 | Rotifer and egg incubation systems

The rotifer culture system (\$2,650) consists of four 200-L polyethylene culture tanks, enrichment tanks and sump, and a seawater storage tank (Table 1). The egg incubation system (\$2,156) consists of two, 322-L plastic tanks with a 1/15-hp water pump, sump, and 1/3-hp heat pump (Table 1).

5.3 | BT system

The broodstock system (32,348) (Table 1, Figure 3) for the baseline facility consists of four 2.44 m-diameter (vol = 4,982 L, depth = 1.07 m) BTs and the following RAS equipment: one 2 hp water pump, one bead filter, a UV sterilizer, foam fractionator, filter sump, and a 3-hp heat pump to maintain water temperature. The BTs and RAS equipment support 48 male and 48 female adult BSB at a biomass density of 0.0131 kg/L. The broodstock are divided into two equal groups. The first group is spawned in April and the second group is spawned in June. Each spawning female produces 13,500 YSL per spawn, for a total of 324,000 YSL produced by 24 females per spawn, with two spawning events per year.

5.4 | LRT system

The LRT system (\$15,218) (Table 1, Figure 3) consists of six, 2,000 L fiberglass tanks (1.83 m diameter \times 0.81 m water depth) and the following RAS equipment: three 0.5 hp water pumps, foam fractionator, bubble bead filter, UV sterilizer, sump, 2 hp heat pump, and automatic feeders. The LRTs accommodate two cohorts (spawns) per year. For each cohort, YSL are transferred from the egg incubation system to the LRTs at 1 dph and grow to approximately 1 g at 51 dph. Each of the six LRTs accepts an initial 54,000 YSL per cohort at a stocking density of less than 30 larvae/L.



FIGURE 3 Plan view of a hypothetical commercial scale recirculating hatchery for production of black sea bass fingerlings (Watanabe et al., 2015)

TABLE 1Summary of total initial investment costs of a hypothetical commercial scale recirculating hatchery for
production of advanced (5-g) black sea bass fingerlings

Item	Unit	Price (\$)/upit	# L Inits	Cost (\$)
Structure (insulated metal building, concrete floor, electrical, plumbing, HVAC)	Building	75/sf	8,400	630,000
Tanks + RAS equipment				
Rotifer tanks (200-L)	Tank	662	4	2,650
Egg incubators (322-L)	Tank	1,078	2	2,156
Broodtanks (4,980 L) + RAS	Tank	3,394	4	32,348
Larval rearing tanks (2,000 L)	Tank	2,536	6	15,218
Nursery tanks (5,200 L)	Tank	5,525	10	55,242
Hatchery-wide equipment				
Seawater supply and storage				13,662
Electricity backup generator				5,000
Blowers				1,350
Liquid oxygen monitor system				5,000
Environmental monitor				491
Geotube [®] wastewater system				2,000
Office and laboratory equipment				3,701
Miscellaneous equipment				4,297
Equipment setup labor				5,412
Total initial investment cost				778,527

Abbreviation: RAS, recirculating aquaculture system. *Source:* Adapted from Watanabe et al. (2015).

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With a baseline survival of 15% from 1 to 51 dph, each cohort produces a total of 48,600 1 g fingerlings output for all six LRTs.

5.5 | NT system

The NT system (\$55,242) is partitioned into two identical but independent systems (Table 1, Figure 3). Each of the two NT systems consists of five 5,224 L (2.44 m diameter \times 1.12 m deep) fiberglass tanks and the following RAS equipment: a 2 hp water pump, foam fractionator, bead filter, UV sterilizer, sump, automatic feeders, and a 3 hp heat pump. Total initial investment costs for tanks + RAS equipment is \$107,614.

The NTs support two cohorts per year at a nominal density of 1.5 5 g fish/L. For each cohort, 1 g fingerlings are transferred from the LRTs to the NTs at 51 dph. Fish grow to approximately 5 g each at 101 dph. Each NT is stocked with 8,100 1 g fingerlings at 51 dph, and 7,695 fingerlings survive to reach 5 g by 101 dph for a survival rate of 95%. For all NTs, 46,170 5 g fingerlings are produced per cohort or 92,340 fingerlings per year.

5.6 | Hatchery-wide equipment

Seawater supply and storage (\$13,662) include a 2 hp pump, sand filter, UV sterilizer, and reservoir tanks (Table 1). The facility has an electricity backup generator (\$5,000), regenerative blowers (\$1,350), a liquid oxygen delivery and monitoring system (\$5,000), and an environmental monitor and emergency call-back system (\$491). Geotube[®] dewatering technology (TenCate, Pendergrass, GA) (\$2,000) is used to collect solid wastes for disposal in a landfill. Other hatchery-wide equipment includes office/laboratory (\$3,701) and miscellaneous equipment (\$4,297), and setup labor (\$5,412). The total investment cost for this hatchery-wide equipment is \$40,913.

5.7 | Total initial investment cost

Total initial investment costs of facility construction and RAS and hatchery-wide equipment and installation are \$778,527 (Table 1).

5.8 | Annual operating costs

5.8.1 | Variable costs

Under baseline conditions (Watanabe et al., 2015), variable operating costs total \$71,426 per year (Table 2) and include broodstock, larval (live and artificial), and nursery feeds, as well as hormones and other chemicals used for spawning and water quality analyses, electricity, liquid oxygen and freshwater, waste treatment, office overhead, and interest on bank loans for operating costs at an annual interest rate of 10%. Labor expenses include one full-time facility manager at \$30,156 per year and two part-time laborers at \$11 per hour at \$20,796 per year.

5.8.2 | Fixed costs

Fixed operating costs total \$23,162 per year (Table 2) and include the owner's forgone interest income (opportunity cost) associated with using his land for the hatchery facility, insurance (property, liability, and workers compensation),

Item	Cost (\$)
Variable costs	
Broodstock, nursery feed	2,612
Larval feeds	5,166
Hormones, chemicals	460
Electricity	6,652
Liquid oxygen and freshwater	151
Waste treatment	510
Office overhead	1,200
Interest (10%)	3,723
Manager labor (1)	30,156
Part-time labor (2)	20,796
Subtotal variable costs	71,426
Fixed costs	
Opportunity cost of land (10%)	3,125
Insurance (property, liability, work. comp.)	6,000
Electrical demand charge	4,800
Liquid oxygen storage tank rental	1,288
Property taxes (land, bldg., equip.)	6,904
Interest (10%) on fixed costs	1,104
Subtotal fixed costs	23,162
Total (variable + fixed) costs	94,588
B/E price ^a for 92,340 5 g fingerlings per year	1.67 each

TABLE 2 Annual variable and fixed operating costs of a hypothetical commercial scale recirculating hatchery for production of advanced (5 g) black sea bass fingerlings

^aBreak even (B/E) is the sales price that makes the 30-year cumulative net present value (NPV) = 0.

Source: Adapted from Watanabe et al. (2015).

electricity demand charge, liquid oxygen tank rental fee, property taxes, and interest (10%) on fixed operating costs. Total variable plus fixed annual operating costs are \$94,588. Breakeven (B/E) price per advanced 5 g fingerling is \$1.67. Sensitivity analyses showed that B/E price is highly sensitive to harvest density (Watanabe et al., 2015).

5.9 | Production of 5 g versus 1 g fingerlings

Recent research has shown that UNCW-formulated diets replacing 50% fish meal protein with poultry meal protein produced better growth performance in early juvenile BSB than two commercial diets: Otohime and Gemma Diamond (Alam et al., 2019; Carroll et al., 2018). Using the UNCW-formulated diet, it was determined that BSB postmetamorphic fry (0.54 g, 47 dph) may be raised to small juvenile stages (1.58–1.68 g mean weight, 59 dph) at high densities of 6.5 fish per L, without adverse effects on growth, survival, and feed utilization, and that early fingerlings approximately 1 g mean weight are "transport-ready" with excellent resistance to acute crowding and shipping (Carroll et al., 2018; Watanabe et al., 2018).

Based on these findings, the baseline facility that outputs 5 g fingerlings at 1.5 fish/L was compared with a facility that outputs smaller 1 g fingerlings at a density of 5.7 fish/L (Table 3). The 1 g facility is housed in the same building, but it differs greatly in the RAS equipment needed and operations. The 1 g fingerlings are produced in the LRTs,

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TABLE 3 Comparison of initial investment, annual operating costs, fingerling output, and break even (B/E) price of hypothetical 5 and 1 g fingerling production facilities	Harvest size (g)	5	1
	Harvest density (fish/L)	1.5	5.7
	Initial investment (\$)	778,527	937,447
	Total operating costs (\$	5) 94,588	166,343
	Variable costs (\$)	71,427	141,895
	Fixed costs (\$)	23,162	24,488
	Output (no. of fish)	92,340	583,200
	B/E price per fingerling	(\$) 1.67	0.43

Source: Data summarized from Watanabe et al. (2015, 2019).

which allows the NTs to be removed from the facility and creates space to increase the BTs from 4 to 24 and the LRTs from 6 to 16. Broodstock in the 1 g facility are apportioned into three groups each conditioned under a different photothermal regime to enable three spawning seasons (early, natural, and late) each year and two spawnings per season. The 1 g facility has 12% higher initial investment cost and 76% higher annual operating costs than the 5 g facility. However, because the production cycle of a 1 g facility is reduced to only 51 days (vs. 105 days), the number of crops per year increases to six (vs. two), and the number of 1 g fingerlings per year increases by 345% to 583,200, greatly outweighing the increase in costs. Hence, the B/E price per 1 g fingerling is dramatically lowered to \$0.43 each (Watanabe et al., 2018, 2019).

6 | OUTREACH: SUPPLY FINGERLINGS TO COMMERCIAL MARICULTURE COMPANIES FOR PILOT GROWOUT TRIALS

Fingerlings from the UNCW pilot hatchery were supplied to startup BSB growers in NC, VA and in ME who transported their fish from Wrightsville Beach to their private facilities on the eastern seaboard. Market-size fish grown in 4.57–7.32 m diameter tanks in RAS at these growout facilities were distributed (live, whole on ice, or filleted) to wholesale and premium-value markets on the eastern seaboard. This pilot hatchery at UNCW is enabling new farmers to access fingerlings, establish growout technology, and understand market value and demand.

7 | GROWOUT TO MARKETABLE SIZES

7.1 | Diet and nutrition

Diet tests with BSB juveniles suggested considerable flexibility in providing energy-yielding nutrients when fed a diet with crude protein levels exceeding 40% (Goff & Gatlin III, 2005). Twice daily feeding with a fish meal-based diet containing 44% protein is optimal for the growth of juvenile BSB (Alam et al., 2008). When the influence of four formulated practical diets with different protein levels (44 and 54%) and lipid levels (10 and 15%) on the growth and body composition of BSB pre-adults (mean weight = 75.5 g) was evaluated in a pilot-scale marine recirculating system for 90 days, a combination of 44% dietary protein and 15% lipid was optimal for growth. Due to protein sparing, increasing the protein level from 44 to 54% did not produce a significant effect on weight gain at a high lipid level (Alam et al., 2009).

An important characteristic of BSB for sustainable aquaculture is that they efficiently utilize a variety of plant and animal protein sources that are alternatives to expensive fish meal (Alam et al., 2012; Alam et al., 2019). Studies at UNCW have demonstrated that it is possible to replace fish meal protein at high substitution levels with alternative protein sources, including soybean meal and poultry by-product meal proteins (Alam et al., 2012; Dawson et al., 2018). Our recent studies at UNCW have shown that glandless cottonseed meal protein with supplemental amino acids can replace 100% of the fish meal protein in the diet of juvenile (~7 to 30 g) and sub-adult stage (~50 to 150 g) BSB under controlled laboratory and semi-pilot scale conditions, respectively (Anderson et al., 2016; Alam et al. UNCW, unpublished data), with no diminution in growth performance and feed conversion efficiency compared to fish fed a fish meal protein-based control diet. Protein is the most expensive component in a marine finfish diet. Cottonseed flour protein is the same price as soybean meal, and if it can replace almost all of the fish meal in a 50% protein diet, feed cost may be reduced by 50%. The available data show a significant potential for reducing feed costs—a key operational expense in fish production—by using sustainable protein sources that are alternatives to fish meal in BSB growout diets.

7.2 | Growout of hatchery-reared juveniles under low salinity

Earlier studies have suggested that BSB are moderately euryhaline and that growth performances at salinities of 20 and 30 g/L were much higher than in fish raised at 10 g/L (Atwood et al., 2001, 2003, 2004; Cotton et al., 2003; Young et al., 2006). However, studies at UNCW have found that when hatchery-reared juvenile BSB were raised under controlled laboratory conditions at low salinities of 15 and 10 g/L and fed isolipidic and isonitrogenous diets formulated with graded levels of sea salt (0, 2.5, 5, 7.5, 10, 12.5%) for 10 weeks (9-39 g), optimum growth and survival were maintained in fish fed 5% sea salt (Alam et al., 2015). In a recent study conducted in a semi-pilot scale RAS at low salinity (10.8 ± 0.9 g/L), hatchery-reared BSB juveniles fed isolipidic and isonitrogenous diets with graded levels of salt (0, 2.5, 5, and 7.5%) for 8 months (19.8-182 g) showed no treatment differences in growth performance, plasma osmolality and electrolytes, and biochemical composition of body tissues compared to fish raised in full-strength 34 g/L seawater (Alam et al., 2020). When salinity was further reduced from 10.1 to 4 g/L, fish fed 0% salt showed poor survival (29%), whereas fish fed 7.5% salt showed highest survival (67%), and a significant linear relationship between dietary salt and survival was observed. The results suggest that BSB juveniles can be raised at a low salinity of 10.1–12.3 g/L with no negative effects on long-term growth performance. Salt-incorporated diets, however, improved survival under extreme low salinity ($\sim 4 \text{ g/L}$) challenge conditions. These findings have important implications for rearing BSB in low salinity RAS and for expanding siting options for RAS growout operations, which may consider locations where brackish water of 10.1-12.3 g/L or higher may be sourced.

7.3 | Growout of hatchery-reared juveniles to marketable stages in a pilot-scale RAS

Growth and feed utilization of hatchery-reared juvenile BSB were studied at UNCW in outdoor recirculating tanks consisting of two 16 m³ (diameter = 4.57 m) units with conical lids (Figure 4). The insulated fiberglass tanks were illuminated with timer-controlled fluorescent fixtures and were supplied with sand-filtered and UV-treated natural seawater (32–35 g/L) at an exchange rate of approximately 10% total system volume per day. Tanks had a double drain system and swirl separator to remove solids from the effluent before it was processed by a 60 μ m drum screen mechanical filter, a subgrade biological filter containing air-circulated Kaldness biomedia, foam fractionator, and UV sterilizer. Temperature was controlled using a heat pump, and water was passed through an oxygen cone before it was returned to the fish tank. Details of RAS system design and components are provided in previous publications (Carroll et al., 2005; Gibson et al., 2020).

Fingerlings (mean wt. = 27 g, age = 125 dph, N = 3,300) were stocked into two tanks at 1,650 fish per tank (103/m³). Fish were grown under 33 g/L salinity and 21°C and under ambient photoperiod conditions. A commercial diet (Skretting, Vancouver, BC, Canada) containing 55% protein and 18% lipid was fed to satiation daily for 570 days. Mean temperature, salinity, DO, and pH for the duration of the trial were 21.0°C, 33 g/L, 8.5 mg/L, and 6.9, respectively. On 695 dph, mean weight was 682 g (range = 328–1,350 g) (Figure 4), FCR ranged from 1.12 to 1.19, survival

FIGURE 4 Growout of hatcheryreared black sea bass in recirculating aquaculture systems (RAS) at the University of North Carolina Wilmington (UNCW) Aquaculture Facility (Wrightsville Beach, NC). Two 16 m³ tanks were each stocked with 1,650 fish (initial weight = 27 g; age = 125 dph) and fed a commercial pelleted diet for 570 days at an average temperature of 21°C. Plotted points represent means and vertical bars denote ranges. *Source:* Photo courtesy: UNCW/W.O. Watanabe





ranged from 75 to 79%, and final biomass density reached 52 kg/m³. For the duration of the trial, water quality in the culture tanks averaged 1.12 mg/L total ammonia-nitrogen and 0.25 mg/L nitrite-nitrogen. In this trial, BSB were grown from egg to an average size of 568 g (range = 270-1,100 g) in 20 months without selective grading. The wide range in individual body weights among fish during this trial suggests that periodic grading and culling of slow-growing fish, control of sexual maturation, and selective breeding may be practical approaches to reduce growth variation and average growout time and increase productivity during practical culture.

BSB are very hardy in RAS culture from nursery through marketable stages and studies in progress at UNCW are showing that biomass densities as high as 65 kg/m^3 may be safely reached without a reduction in performance; however, as biomass densities reached 67 kg/m^3 , a reduction in fish feed consumption became evident in association with declining water quality (i.e., increasing suspended solids and CO₂), suggesting that RAS-specific filtration and degassing were limiting. This suggests that BSB may tolerate even higher biomass densities during growut in RAS without compromising growth performance if RAS biofiltration and degassing components are adequately sized to maintain optimal water quality conditions under high biomass loading and feeding rates. Studies in progress are also showing that fish are relatively tolerant to handling stress during periodic grading and transfer among tanks during the growout process as well as during harvesting (Figure 5) and live hauling, with overall survival of graded fish (~84%) to full marketable sizes appreciably lower than in ungraded control fish (~91%) (Carroll et al. unpublished data). Other investigators have likewise noted that the RAS production of BSB shows excellent potential (Perry et al., 2007).

8 | DISEASES

8.1 | Eye lesions (cataracts and exopthalmia or "popeye")

Eye lesions including cataracts (opacities in the eye lens) and exopthalmia (popeye) have been observed in BSB during growout from juvenile through marketable stages in RAS. Lesions usually start with damage to the cornea causing cataracts, which are followed by popeye characterized by a bulging of one or both eyes from the socket due to the 558



FIGURE 5 Harvesting hatcheryreared black sea bass grown from fingerling to marketable stages in a pilot growout recirculating aquaculture system at the University of North Carolina Wilmington (UNCW) Aquaculture Facility (Wrightsville Beach, NC). *Source:* Photo courtesy: UNCW/Bradley Pearce

accumulation of fluid behind or within the eye. Usually the formation of cataracts precedes exopthalmia, which eventually leads to the rupture of the eye lens and loss of the eyeball. Fish that lose only one eye often heal and resume active feeding, but fish with bilateral popeye are euthanized.

Eye lesions in BSB can occur in one eye (unilateral) or both eyes (bilateral). Unilateral eye lesions may be related to abrasions from nets, collisions with hard objects in the tank or with other fish during frenzied feeding, or interfish aggression. Bilateral popeye (without cataract formation) sometimes develops within 24 h and is likely related to release of gas from supersaturated water-producing embolisms in the fish's eyes and bloodstream. Bilateral eye lesions often indicate systemic effects of suboptimal water quality conditions, including suspended solids, dissolved CO_2 , pH, and dissolved metabolites (ammonia). Multiple interacting factors involving nutrition, pH, temperature, and DO and CO_2 affecting acid-base balance in the blood and the osmoregulatory properties of the eye lens may be involved (Bjerkås & Sveier, 2004). In Atlantic cod, exposure to CO_2 levels within the range of 6.99–19.77 mg/L over a period of 5 months was found to cause eye lesions (Moran et al., 2012; Neves & Brown, 2015).

Eye problems can be effectively managed by preventing eye irritation from suspended solids and collisions with solid objects in the tank. Careful control of gas saturation, including DO (\sim 150%), nitrogen saturation (\sim 100%), and CO₂ (<10 mg/L) by strategic placement of degassing towers, diffused aeration, or in-tank aerators is important in controlling dissolved gas saturation under high-density rearing conditions. In addition, pump intake lines and water sump tanks are checked frequently to avoid air entering the pump intake under high pressure resulting in water gas supersaturation (Vinci et al., personal communication; Summerfelt et al., 2000; Moran, 2010a, 2010b). Maintaining stable water temperature conditions in growout tanks prevents embolisms caused by release of gas bubbles from solution from gas-saturated water (offgassing) during rapid warming. Multiple or continuous feedings of the daily ration over a 10–16 hr period inhibit feeding frenzies and prevent a rapid decrease in DO concentrations following a heavy meal. This obviates the need to maintain highly supersaturated DO levels to compensate.

8.2 | Bacterial pathogen (pasteurellosis)

Photobacterium damsela subspecies piscicida is a gram-negative rod bacteria, which causes a disease in fish known as pseudotuberculosis or fish pasteurellosis. At UNCW, pasteurellosis has been observed primarily among larger BSB, including broodstock and subadult fish in growout tanks, and outbreaks occur from late spring or early summer until mid-autumn when water temperatures rise above 23°C (Watanabe, 2011). The disease is characterized by lethargy and skin ulcers and fin erosions, which are covered with mucus, so the lesions appear as small white nodules and

ulcerations on the head and gill covers of the affected fish. If detected early and water temperatures are lowered to 19–21°C, only a small percentage of the population is affected and the symptoms gradually disappear. Inactivated autogenous vaccines for *P. damsela* are available commercially (Kennebec River Biosciences, Richmond, ME) and have been developed specifically for BSB at UNCW. The vaccine is easily applied to small juveniles using a simple dipping station, but controlled studies are needed to determine the frequency of treatments needed during the growout period and cost-effectivity. Since a lowering of water temperature to a suboptimal range of 19–21°C is used to treat fish for pasteurellosis, vaccination may potentially enable BSB to be reared continuously at temperatures close to the reported optimum of 25.6°C (Atwood et al., 2001, 2003; Sullivan & Tomasso, 2010) to maximize growth and productivity.

9 | WASTE MANAGEMENT

A major pollutant in the effluent of RAS is total suspended solids (TSS), consisting of uneaten feed, fish feces, algae, and biofloc sloughed from biological filters (Ebeling et al., 2005; Summerfelt & Vinci, 2008). These biosolids are concentrated as they are sequestered by solids removal components (particle traps, swirl separators, bead filters, microscreen filters, foam fractionators) from the process water (Losordo & Westers, 1994; Cripps & Bergheim, 2000; Ebeling et al., 2005; Gibson et al., 2020), producing a high-strength low-volume effluent that requires significant treatment before discharge (Losordo & Westers, 1994). The importance of minimizing solids concentration in effluent discharge is emphasized by the Environmental Protection Agency's regulations, which limit TSS, while allowing state or regional programs to address regional and site-specific conditions through the National Pollutant Discharge Elimination System (NPDES) discharge permit requirements (Ebeling et al., 2005; Summerfelt & Vinci, 2008).

Recent studies in our laboratory have demonstrated that Geotube[®] filtration systems are highly effective at removing suspended solids from effluent from a marine finfish RAS and can aid production facilities in meeting government discharge compliance as well as reducing the impact of aquaculture on local waters (Gibson et al., 2020). When the organic polyacrylamide polymer Drewfloc 2449 (Ashland, Inc., Covington, KY), generally regarded as safe for use in systems producing fish for human consumption, was applied at 10 mg/L to a commercial-scale Geotube[®] system growing BSB, the TSS of the Geotube[®] filtrate was negligible, averaging 16.8 mg/L over the 24 hr study period, or 4.66% of the TSS in raw effluent with a mean daily TSS of 360.2 mg/L. The commercial-scale Geotube[®] system removed 92.1% of the TSS in 33 g/L seawater effluent from the RAS. Polymer treatment had little or no effect on dissolved nutrient (N and P) concentrations in the effluent, and effective methods for reducing these dissolved components will be important for discharge of the Geotube[®] filtrate to the environment, or its reuse in the RAS. Laboratory studies at UNCW have shown that the salt-tolerant halophyte *Salicornia virginica* planted in drainage lysimeters is an effective biofilter for dissolved N and P in effluent from a RAS for marine finfish (Watanabe & Farnell, 2018). Pilot-scale studies are needed to evaluate the integration of a Geotube[®] and *Salicornia* to remediate wastes from a marine finfish RAS.

10 | MARKETING

BSB offer distinct marketing advantages for aquaculturists on the eastern U.S. seaboard; they are well known to consumers and have an established, high-value market comparable to high-value snapper and grouper species (Wilde, 2008). BSB can be raised in RAS on the eastern U.S. seaboard where offshore aquaculture is logistically difficult and where major metropolitan markets are in close proximity to minimize shipping costs and a facility's environmental footprint. Aquaculture facilities can avail higher prices when the natural fishery is closed or availability of wild-caught fish is poor. With proper documentation (i.e., bill of lading), they can currently provide fish of any size on World Aquaculture Society



FIGURE 6 Hatchery raised black sea bass grown from fingerling through marketable stages in a pilot growout recirculating aquaculture system at the University of North Carolina Wilmington (UNCW) Aquaculture Facility (Wrightsville Beach, NC). *Source*: Photo courtesy: UNCW/W.O. Watanabe

demand, including below the minimum regulated limits for commercial fishermen. BSB can be sold for premium farm gate prices at small sizes of 1.25 lb and above in whole-on-ice or live forms (Figure 6) to eliminate processing and by-product wastes and to maximize farm production value. They are indigenous to U.S. coastal waters and can be produced to meet the demands of the ecologically conscious American and the global consumer who are discerning about what they eat and will pay a premium price for products grown in an environmentally sustainable manner. RAS production systems are more biosecure and will facilitate production of genetically improved stocks with higher growth performance in the future.

10.1 | Wholesale marketing

In the traditional wholesale marketing process in NC, ocean-caught BSB are sent from dockside to a wholesale seafood distributor (i.e., packing house) where fish are sorted and graded by the half pound and packed with ice into waxed cartons with drains in the bottom corners. Waxed cartons generally holding ice and 50 lb of fresh fish are stacked on pallets and loaded by forklifts into refrigerated trucks for delivery to distributors, restaurants, and retail seafood markets on the eastern seaboard (T. Davis, personal communication). Wholesale prices (whole on ice) are size-tiered and range from \$2.00 to 3.50 per lb for small fish from 0.5 to 1.0 lb, from \$4.00 to \$6.50 per lb for medium fish from 1 to 2 lb, and from \$7 to 8 per lb for jumbo fish >2 lb. By participating in this well-established distribution chain for wild-caught BSB, a grower can easily access northeastern metro markets, which purchase fresh finfish on a weekly basis and seek a steady source of high-quality product. Startup aquaculturists, however, must compete with commercial fishermen for this traditional wholesale market.

10.2 | High-value niche marketing

High-value retail markets provide prices that could be much more profitable for startup growers. The traditional high-value retail market for BSB is characterized as a "niche" market of upscale, gourmet, white table-cloth seafood, and sushi restaurants (Berlinsky et al., 2000; Copeland et al., 2005). A demand analysis for farm-raised BSB was conducted in the upscale niche restaurant market of NC via surveys of restaurants drawn at random from the population of all NC restaurants (Wilde, 2008). The product was delivered to the restaurant where the chef prepared the product and completed a survey. Restaurant demand was determined assuming that BSB price (\$7.50 per lb) was the

same as grouper, a comparable substitute species. Individual restaurant demand was then extrapolated to estimate an aggregate statewide niche market demand for farm-raised BSB in NC of 179,077 kg (394,798 lb) per year, representing an annual statewide value (\$2,960,985), 57% higher than the commercial landing value for BSB in NC (\$1.7 million) (NCDMF, 2009) and indicating a strong, established demand for BSB and a limited supply (Wilde, 2008). A preference for whole fish exceeding 908 g (2.0 lb) was considered a potential industry constraint (Wilde, 2008).

A similar demand analysis was used for the high-value, niche market for farmed BSB in four metropolitan areas: New York City, Philadelphia, Atlanta, and San Francisco (Dumas & Wilde, 2009). Assuming that farm-raised BSB were sold whole, fresh/chilled, 1.5–2.5 lb in size, and at prices equal to the average prices of comparable fish (e.g., snapper, grouper, and striped bass) in each city, aggregate niche market demand ranged from 97,066 lb per year for New York City to 21,972 lb per year for Atlanta, totaling over 218,000 lb per year for these four cities. A majority of chefs preferred fresh/chilled fish instead of live fish or frozen fish fillets, and no off flavor was reported by participants. The preferred preparation methods were sautéed (37%) and baked (13%), and sushi, sashimi, and "other" (11% each). These studies demonstrated a significant demand for farm-raised BSB in upscale niche U.S. metropolitan markets at prices that may be profitable to startup growers. The logistics of delivery and invoicing to multiple independent establishments are challenges to niche marketing.

10.3 | Live marketing

Anecdotal information from collaborating commercial growers at UNCW indicate that premium niche market prices (\sim \$7–9 per lb for fish \geq 1.25 lb) may also be obtainable from live seafood dealers on the eastern U.S. seaboard, a market logistically difficult to supply by fishermen that can potentially be supplied on demand from aquaculture facilities (T. Davis, personal communication). Live markets pay farm gate prices from \$7 to \$8.50 per lb for whole fish in the 1.0–1.5 lb range, but transport costs may lower the farm gate value by \$0.50–0.75 per pound. Live seafood dealers arrange live-hauling at farm gate, which avoids a producer's packing and distribution costs (ice, transport, labor, invoicing) associated with niche marketing. Live fish dealers on the eastern U.S. seaboard, however, require large (e.g., 6,500 lb minimum per haul) and consistent quantities of product year-round to offset the costs of live-hauling and distribution to metropolitan centers (e.g., Atlanta, DC, NYC, Philadelphia, and Boston) of the U.S. east coast.

11 | FUTURE RESEARCH NEEDS

Through comprehensive research supported by federal, state, and private agencies since 2000, hatchery methods for BSB, including broodstock husbandry and controlled breeding and larval and nursery culture methods for BSB are well developed. A not-for-profit research hatchery (UNCW) has provided startup growers access to fingerlings and conduct pilot growout and marketing trials to develop RAS growout technology, understand market value and demand, and develop sound business strategies for scale up (Watanabe et al., 2019). Hatchery-reared BSB juve-niles are hardy and can be grown to premium marketable sizes in intensive recirculating aquaculture systems on low fish meal protein diets under relatively high densities with little or no disease. Fish pasteurellosis may be prevented during growout by lowering rearing temperatures below optimum, but improved biosecurity and vaccination protocols can maximize safe rearing temperatures and growth rates. High growth (and size) variation during the growout stages suggests that periodic grading, control of early sexual maturation, and selective breeding are potentially important research avenues to increase growth rates and production efficiency. Pilot-scale research on the integration of a Geotube[®] and *Salicornia* is needed to demonstrate waste remediation technology that can permit future RAS facilities to operate sustainably in both inland and coastal areas with access to salt water. Comprehensive, in-

depth economic analyses of BSB growout production in RAS are needed to assess the financial implications of faster fish growth resulting from selective breeding, more sustainable feeds that incorporate cheaper protein sources in replacement of fish meal, alternative grading practices, and higher safe stocking densities. Commercial scale demonstration of a viable business model to grow and market BSB will be critical to farmers, lenders, policy makers, and investors. Multi-stakeholder projects' cost shared between public and private groups will minimize risk to prospective farmers and provide experiential opportunities for students, technicians, and a model for educating the public.

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