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## First Data On Aquaculture of the Tripletail, *Lobotes surinamensis*, a Promising Candidate Species For U.S. Marine Aquaculture

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## REVIEW ARTICLE



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# First data on aquaculture of the Tripletail, *Lobotes surinamensis*, a promising candidate species for U.S. marine aquaculture

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## Abstract

The Tripletail, *Lobotes surinamensis*, is a warm-water pelagic fish that is increasingly targeted by U.S. anglers. The superior quality of Tripletail flesh coupled with the lack of domestic commercial fisheries stimulated interests to develop aquaculture of this species. In this work, photothermal conditioning of captive-held broodstocks promoted maturation in females, but spontaneous spawning was not observed. GnRHa slow-release implants induced ovulation in late vitellogenic females but fertility remained below 10% when GnRHa was administered alone. However, spawns with high fertility (up to 85%) were obtained when a dopamine antagonist was administered in conjunction with GnRHa implants indicating dopamine inhibition impaired final gamete maturation, in particular sperm production in males, in aquaculture conditions. Tripletail larvae successfully initiated exogenous feeding on enriched rotifers followed by *Artemia* nauplii and were weaned to prepared feeds at 25 days post hatch, yet with low survival through the late phases of larval culture. Pilot grow-out trials at low density in recirculating systems revealed impressive growth rates averaging over 170 g/month through a market size

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above 1 kg. While protocols for hatchery culture and grow-out still need to be optimized, current data suggest that Tripletail could become a successful species for U.S. marine aquaculture.

#### KEYWORDS

aquaculture, *Lobotes surinamensis*, review, tripletail

## 1 | INTRODUCTION

Seafood consumption in the United States (U.S.) reached an average 16.1 lbs per person per year in 2018, its highest since 2007. The overwhelming majority (85–95%) of this demand was filled by imports, half of which were aquaculture products, leading to an overall seafood trade deficit of \$17.1 billions (National Marine Fisheries Service, 2020). This deficit has remained over \$10 billions during the past decade and can only be reduced by developing domestic aquaculture considering the stagnation of wild fisheries harvests. However, U.S. aquaculture only ranked 17th in the world in 2014 (Lester, Gentry, Kappel, White, & Gaines, 2018) and contributes a negligible fraction of domestic consumption. The U.S. aquaculture production is largely focused on freshwater species and salmonids. The moderate fraction (13.5%) devoted to marine species consists mostly of shellfish (Montgomery, 2019). The aquaculture of marine finfish remains negligible although these species represented 31.4% of the total volume of edible seafood products imported by the U.S. in 2018 (National Marine Fisheries Service, 2020). Development of the US marine finfish aquaculture industry is therefore much needed.

Factors that have slowed the development of marine finfish culture in the United States include a complex permitting process, the large capital investment needed to establish production units, the extended time until revenue and return on investment are generated, and the lack of technology to produce species of interest. Overcoming the latter issue requires focusing efforts on species with the highest potential for commercial-scale production and marketing. Aquaculture candidate species have typically been selected based on high selling price of wild harvests (Quemener, 2002). However, this approach to select species has its limitations because the technologies to mass-produce most candidate marine fishes are not available yet (Rexroad, Rust, Riche, Wills, & Davis, 2021) and the development of these culture processes has proved challenging for many species. The selection of aquaculture finfish species may therefore need to be based, in part, on biological and technical characteristics that are important factors of success. Characteristics to consider include the feasibility of closing the life cycle in captivity, the level of technology required to culture critical life stages (e.g., salmonids require no larval rearing), the species growth rate (with fast growth rate expected to lower production costs by shortening the rearing cycle), the achievement of sufficient survival rates through the larval culture phase, and the flesh quality and fillet yield which contribute to mass marketing potential. For example, a study by Thouard, Soletchnik, and Marion (1990) evaluated possible candidate aquaculture species in La Martinique based on experiments that assessed each species' captive reproduction, larval rearing success, and growth rate. A similar procedure was employed by Alvarez-Lajonchère and Ibarra-Castro (2013) to select candidate species for intensive culture in the Caribbean region. A recent assessment of the status of marine finfish aquaculture in the U.S. identified candidate species and evaluated their status in terms of technical feasibility and degree of development of the industry producing them (Rexroad et al., 2021). Several of the species considered in this assessment appeared promising, although with varying degrees of technical readiness or commercial development. The Tripletail (*Lobotes surinamensis*) was identified as a promising candidate aquaculture fish that requires additional research to reach technical feasibility (VanderKooy, 2016).

Bringing a new species to technical feasibility requires controlling all phases of the culture cycle from captive reproduction through grow-out to market size. The first step toward developing aquaculture of a new species is to secure a source of seed for growers. Consequently, initial efforts usually focus on developing methods for captive spawning to

establish a reliable source of high-quality embryos for culture (Mylonas & Zohar, 2001, 2008). The development of culture protocols for larval stages and then for grow-out of weaned juveniles to market size follow. There is still little known about the biology of Tripletail and its potential for aquaculture. Here, we provide a review of available information on Tripletail aquaculture obtained during preliminary trials conducted in recent years and discuss current bottlenecks to expanded culture and research needs to bring this species to technical feasibility and commercial production.

## 2 | TRIPLETAIL BIOLOGY AND FISHERIES EXPLOITATION

### 2.1 | Biology and life history

The Tripletail is a marine finfish species found in tropical and subtropical waters of all oceans. In the western Atlantic Ocean, the species is reported from Massachusetts to Argentina, although it is very infrequent north of the Carolinas in the U.S. or south of Rio de Janeiro in South America. This species maintains a pelagic lifestyle throughout its life-cycle and is often found associated with floating structures. Tripletail reach sexual maturity at one to 2 years or about 494–594 mm total length for females and 380 mm total length for males (Strelcheck, Jackson, Cowan Jr., & Shipp, 2004). The spawning season extends from June through August in the Gulf of Mexico, with peak activity in July and regression beginning in August (Brown-Peterson & Franks, 2001). Tripletail is a batch spawner with asynchronous oocyte development. Brown-Peterson and Franks (2001) reported estimates of mean relative batch fecundity of  $47.6 \pm 18.1$  eggs gram ovary-free body weight<sup>-1</sup>. Tripletail do not exhibit clear sexually dimorphic characteristics and, contrary to a number of other species of fish, cannot be easily sexed through observing the genital papilla, thereby making sex identification in the field a challenge. Mature females at advanced stages of vitellogenesis can be sexed by collecting an ovarian biopsy using a catheter and identifying developing oocytes under a microscope. However, collection of ovarian biopsies using this method is often unsuccessful in immature females and males seldom release sperm during manual stripping, even during the spawning season. Consequently, immature females and non-spermiating males are often left unidentified, yet these groups can represent a substantial fraction of Tripletail collected from the wild when attempting to source broodstock.

The location of spawning is not known but Ditty and Shaw (1994) concluded that Tripletail spawn near the outer continental shelf based on the collection of small larvae (<5.0 mm) in these areas. Tripletail larvae were collected in surface waters often in or around *Sargassum* and other floatsam and the large majority of samples (75%) were in water featuring temperature above 28.8°C and salinities above 30.3 psu, respectively. Ditty and Shaw (1994) described Tripletail larval development based on field samples and reported the occurrence of a vaulted supraoccipital crest, heavily pigmented pelvic fins and large preopercular spines. The metamorphosis leading to transition to the juvenile stage was described at about 9.0–9.5 mm standard length by Ditty and Shaw (1994). Early juveniles are common during warm months of the year in coastal shallow waters along the Gulf coast (J. Franks, unpublished data).

Franks, VanderKooy, and Garber (2003) studied the stomach contents of Tripletail caught along the Mississippi Gulf Coast and described 32 different prey types. Of the stomachs containing prey, 72.2% contained crustaceans (mostly penaeid shrimps and portunid crabs) and 65.4% contained fish prey; Atlantic Bumper (*Chloroscombrus chrysurus*) and Gulf Menhaden (*Brevoortia patronus*) were the most common fish prey items. Franks et al. (2003) concluded that the diversity of prey suggests a very versatile feeding behavior, as the prey items represented both demersal and pelagic species.

### 2.2 | Fisheries and market potential

Tripletail are a very popular recreational fish because of their excellent fight and fine flesh quality (VanderKooy, 2016) but they were not fished commercially in the U.S. until recently. The lack of a commercial fishery

can be attributed to the non-gregarious lifestyle of this species, which is incompatible with cost-effective mass harvest (Saillant, Lemus, & Franks, 2014), and the overall absence of an established market because of lack of awareness by consumers. Commercial harvests have been on the rise in recent years, although they are still limited in the U.S. with only 7,000 lbs average annual landings between 2000 and 2016 (VanderKooy, 2016). The market value of Tripletail is variable. VanderKooy (2016) reported values as high as  $\$3.65 \text{ lb}^{-1}$  in the U.S. south Atlantic region yet prices in the Gulf of Mexico tended to be lower, typically around  $\$1.25 \text{ lb}^{-1}$ . The species seems most abundant along the east coast of Florida which accounted for 62% of the total U.S. commercial landings in 2000 (VanderKooy, 2016). Abroad, the largest Tripletail fishery in the world is located in South America (Guyana, Suriname and Brazil) where landings of up to 3 million tons per year are reported by VanderKooy (2016). Although the commercial fishery for Tripletail in the US is negligible, there is now an established demand by restaurateurs and fish retailers which is currently supplied largely with imports from the eastern Pacific and South America because of the lack of a reliable domestic source (VanderKooy, 2016). Aquaculture could address this void in U.S. domestic production and provide a reliable source to retailers and consumers.

### 3 | PREVIOUS STUDIES OF TRIPLETAIL AQUACULTURE

To our knowledge, at least two groups have investigated procedures for hatchery and grow-out culture of Tripletail. However, to this date, available information on these topics is limited to a report of grow-out of wild-caught juveniles maintained at low density in recirculating systems by Franks et al. (2001), preliminary studies of maturation and spawning of captive broodstock reported by Saillant et al. (2014), and pilot studies of all phases of the culture initiated at the University of Southern Mississippi in 2017 that are still on-going. Below, we summarize earlier findings and the first results of pilot trials conducted in the past 2 years at the University of Southern Mississippi.

#### 3.1 | Captive reproduction

Efforts to develop captive reproduction focused on acclimating wild-caught brooders to captivity and conditioning them for gamete maturation under a photo-thermal cycle that simulated conditions in Mississippi coastal waters. Hormonal therapies were also evaluated to stimulate final maturation of gametes and spawning. Preliminary experiments reported by Saillant et al. (2014) utilized adult Tripletails collected in 2009 and 2010 and used in spawning trials in 2010 and 2011. Fish were conditioned under a simulated photothermal cycle (Section 3.1.2, Figure S1). Some females reached advanced stages of vitellogenesis but none of the males released sperm during manual stripping. No spontaneous spawning was observed and fish were administered GnRHa slow-release implants (Ovaplant<sup>®</sup>, Syndel administered at  $75 \mu\text{g}/\text{kg}$ , or EVAc implants described in Section 3.1.3) in two series of trials. Multiple egg releases were observed following all hormonal induction trials. However, all but one of the egg releases were unfertilized and the only spawn where some eggs were fertilized had a fertility rate of 9.8%. These early trials indicated that gamete maturation and spawning in both sexes were inhibited. Gonadal maturation and hormonal induction of captive broodstock were revisited in trials initiated in 2017 and described below.

##### 3.1.1 | Broodstock collection and acclimation

Spawning trials completed to date have utilized broodstock collected during 2017 and 2018 from Mississippi coastal waters. Fish were caught by hook and line and transported to facilities at the University of Southern Mississippi's Thad Cochran Marine Aquaculture Center where they received routine prophylactic treatments including a 5 min freshwater bath followed by a 24 hr immersion in Praziquantel ( $2.5\text{--}5 \text{ mg}/\text{L}$ ) and a 1-month quarantine period during

which copper sulfate was maintained at or above 17.5 ppm to ensure fish were free of *Amyloodinium ocellatum*. At the end of the quarantine period, fish were individually tagged with Passive Integrated Transponder (PIT) tags and brought to maturation tanks. A total of 72 fish were available for evaluation. Sex was identified at the time of capture by collecting an ovarian biopsy from mature females and manual stripping of males to observe release of milt. Because males failed to emit milt during manual stripping and collection of biopsy samples from immature Tripletail females is difficult, blood samples were taken and circulating levels of 11-ketotestosterone (11KT) and Estradiol-17 $\beta$  (E2) were assayed to assist with sex identification. 11KT is an androgen specific of males and E2 is produced by both sexes but is expected to show higher levels in maturing females (Fostier, Jalabert, Billard, Breton, & Zohar, 1983). Plasma levels of the two steroids were determined using Enzyme Linked Immunoassays (Cayman Chemicals) according to the manufacturer's protocol. The levels of these two hormones were used to assign tentative sex phenotypes as follows: fish showing ratios of 11KT/E2 above 2 were sexed as putative males and those with ratios below 0.5 as putative females. The sex of fish exhibiting intermediate ratio values was classified as undetermined. All putative assignment of sexes based on these criteria were subsequently confirmed during spawning events conducted during the study. Fish were allocated to four broodtanks, two of which were subjected to a slightly shifted photo-thermal cycle (3 weeks) to facilitate the management of trials.

### 3.1.2 | Maturation

Fish were maintained in 28-m<sup>3</sup> tanks (4.9 m diameter, 1.5 m deep) during the maturation period. For hormonal induction trials, the selected males and females (mating groups featuring one or two individuals of each sex) were moved to 32-m<sup>3</sup> spawning tanks (3 m diameter, 3 m deep). Each maturation and spawning tank was connected to an individual filtration system that featured temperature control (heater/chiller), mechanical (propeller-washed bead filter for the 28-m<sup>3</sup> maturation tanks and bubble bead filter for the 32-m<sup>3</sup> spawning tanks) and biological (moving bed bioreactors) filtration, protein skimming, Ultra Violet sterilization and supporting water pumps. Water was exchanged once weekly at a rate of 5% during backwashing of the bead filter. Light was provided by three 40-W white spectrum incandescent bulbs.

Maturation and spawning tanks were equipped with a surface egg collector connected to the side of the tank. Eggs concentrated in the surface collector were maintained in suspension by gentle aeration until harvest. A mesh bag was attached to the bottom-center drain discharge located within a sump tank in order to allow the collection and counting of sinking eggs.

Artificial seawater (Crystal Sea Marine Mix, Marine Enterprises International) was used at a salinity of 30-psu in order to match salinity experienced by Tripletail in their presumed natural spawning habitat in Mississippi offshore waters. Water quality was monitored weekly for ammonia, nitrite, nitrate, pH and hardness, and daily for temperature, salinity and dissolved oxygen.

Temperature and photoperiod were adjusted weekly and followed a cycle provided in Figure S1. Photoperiod followed natural variations in Mississippi coastal waters. The temperature cycle was developed based on the average over 20 years recorded at the National Data Buoy Center station 42,007 (22 nautical miles south of Biloxi, Mississippi) except during winter when temperature was maintained at 20°C after initial observations revealed complete cessation of feeding and lethargic behavior of Tripletail exposed to temperature below 20°C. During summer, the temperature was maintained at 27°C once it reached this value until the end of the spawning trials.

Fish were fed three times per week a mixture of fish, squid and shrimp (2:1:1) routinely used for broodstock of marine species which was offered at the rate of 4% body weight per feeding. During the spawning season, the ration was partially substituted once per week with a gelatin-based diet that consisted of lecithin, vitamin tablets (Sea Tab, Pacific Research Labs, Inc.), fish oil and fish meal prepared as described in Bardon-Albaret and Saillant (2017) and fed at 1% body weight.

As in previous experiments on captive reproduction of Tripletail, spontaneous spawning was not observed. The efficacy of hormonal therapies was therefore explored in hormonal induction trials.

### 3.1.3 | Hormonal induction trials

In all spawning induction trials, fish were anesthetized by immersion in a 100 ppm Tricaine (MS-222) bath and gonadal biopsies were obtained from females using a Frydman memory catheter (CCD International). The oocytes collected from mature females were fixed in a desopacifying fixative (Ethanol Formalin Acetic acid, EFA 6:3:1) and observed at  $\times 40$  magnification under a dissecting microscope to determine oocyte size at the time of hormonal induction.

Building upon the preliminary results (discussed above) obtained by Saillant et al. (2014), which showed that slow-release GnRH $\alpha$  implants can induce final oocyte maturation and ovulation in females, trials repeated the GnRH $\alpha$  induction and explored alternative treatments to improve fertility. Mating sets composed of one to three males and females (Table 1) were transferred to spawning tanks for treatments. Females bearing late vitellogenesis oocytes (oocyte diameter  $> 400 \mu\text{m}$ ) were selected for trials. Males were identified based on 11KT and E2 hormone levels as described in Section 3.1.1. Trials were conducted on six dates and treatments included (i) GnRH $\alpha$  Ethylene-vinyl acetate copolymer (EVAc) slow-release implants (Mylonas & Zohar, 2001; Zohar, Doering, & Langer, 1994) administered at  $75 \mu\text{g kg BW}^{-1}$  in females and  $55 \mu\text{g kg BW}^{-1}$  in males, (ii) a single injection of chorionic gonadotropin ( $1,100 \text{ IU kg BW}^{-1}$  in females,  $550 \text{ IU kg BW}^{-1}$  in males) dissolved in PBS and (iii) the combination of a GnRH $\alpha$  implant and a dopamine antagonist ( $5 \text{ mg kg BW}^{-1}$  domperidone dissolved in 0.7% NaCl, 0.1 M sodium metabisulfite and administered in a single injection in the dorsal musculature) given simultaneously.

No spawn was observed in fish treated with chorionic gonadotropin. The fish from those treatments were treated with GnRH $\alpha$  implants and domperidone on two occasions, one leading to a successful spawn (Table 1). Administration of GnRH $\alpha$  implants alone resulted in unfertilized egg releases as in several previous trials reported by

**TABLE 1** Results of hormonal induction trials conducted on Tripletail *Lobotes surinamensis* brooders at the USM Thad Cochran Marine Aquaculture Center

Induction date	Mating set	Hormonal induction	Egg release date	# Eggs	Fertilization rate
June 18, 2019	3F $\times$ 3M	GnRH EVAc	June 20	595,254	8.9%
			June 20	1,234,250	0.1%
June 26, 2019	2F $\times$ 2M	hCG injection	No egg release	-	-
July 02, 2019	2F $\times$ 2M	GnRH EVAc + domperidone*	July 04	293,250	63.06%
			July 05	324,570	83.5%
			July 06	200,000	81.5%
July 19, 2019	1M $\times$ 1F	GnRH EVAc + domperidone	July 22	276,000	37.5%
July 19, 2019	2M $\times$ 2F	GnRH EVAc + domperidone	No egg release	-	-
July 26, 2019	2F $\times$ 2M	hCG injection	No egg release	-	-
July 26, 2019	1F $\times$ 1M	hCG injection	No egg release	-	-
July 31, 2019	2F $\times$ 2M	GnRH EVAc + domperidone†	No egg release	-	-
July 31, 2019	1F $\times$ 1M	GnRH EVAc + domperidone†	No egg release	-	-
August 09, 2019	1F $\times$ 1M	GnRH EVAc + domperidone	August 11	373,675	17.5%
August 09, 2019	1F $\times$ 1M	GnRH EVAc + domperidone	August 12	622,525	23%
August 15, 2019	1F $\times$ 1M	GnRH EVAc + domperidone	August 17	93,600	72.5%
			August 18	210,600	85%
August 15, 2019	1F $\times$ 1M	GnRH EVAc	August 17	70,000	0%

Note: GnRH: Gonadotropin Releasing Hormone EVAc implants ( $75 \text{ mg/kg}$  for females,  $55 \text{ mg/kg}$  for males), hCG: chorionic gonadotropin ( $1,100 \text{ IU/kg}$  for females,  $550 \text{ IU/kg}$  for males), domperidone ( $5 \text{ mg/kg}$ ). Fish were treated with hcG (with no detected effect on gamete maturation) during trials conducted on 06/26 (\*) and 07/31 (†).

Saillant et al. (2014). However, the combined administration of GnRH $\alpha$  EVAc implants and domperidone led to substantially higher fertilization rates up to 85%, yet with variable success (Table 1).

The latter results are encouraging and indicate, in particular, that stress and dopaminergic inhibition are likely involved in the lack of spontaneous spawning through inhibition of spermiation in males and possibly inhibition of oocyte maturation in some of the females and/or spawning behaviors. The anti-dopaminergic treatment applied in conjunction with GnRH $\alpha$  implants could be a solution to produce fertilized spawns but further work on husbandry conditions is needed to identify factors inhibiting spontaneous maturation and spawning of males and female Triple-tail and develop protocols that will restore volitional captive spawning.

## 3.2 | Larval rearing

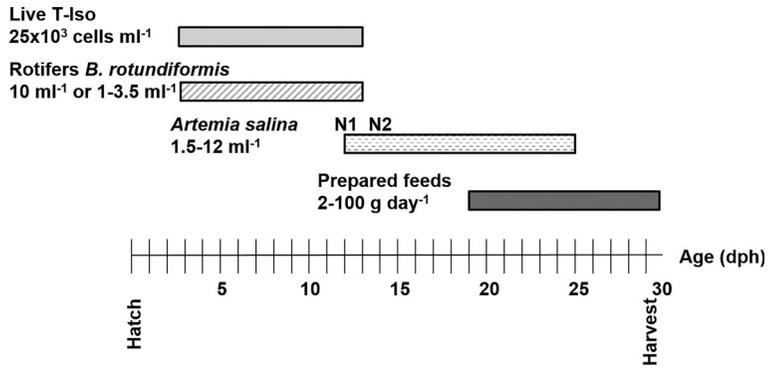
Prior to this work, viable embryos were produced during one captive spawning event that occurred in 2010 at the Thad Cochran Marine Aquaculture Center (Saillant et al., 2014). The 74,000 fertilized embryos were incubated in cylindro-conical incubators under 300 ml/min water flow and gentle aeration. The resulting larvae were stocked 24 hr post-hatch in two 1.5-m<sup>3</sup> larval culture tanks filled at half volume and offered copepods *Acartia tonsa* as an initial food item. Rotifers were offered beginning at 9 days post hatch (dph). Total mortality was observed at 11 dph suggesting initiation of feeding was mostly unsuccessful. This work evaluated alternative protocols using rotifers as an initial food item.

### 3.2.1 | Protocols tested

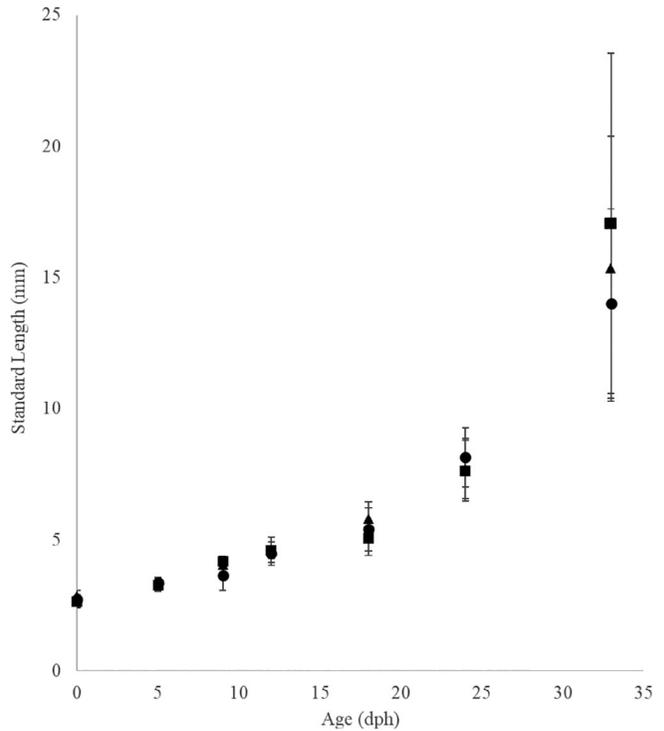
A larval rearing trial was attempted using a viable spawn produced in 2019 (Table 1). This trial was performed in an experimental unit made of two independent twin thermo-regulated recirculating aquaculture systems each composed of three cylindro-conical rearing tanks with a working volume of 1,000-L per tank stocked at an initial density of 15 larvae L<sup>-1</sup>. All tanks were located in a temperature-controlled room and connected to a water filtration unit providing biological filtration, mechanical filtration, protein skimmer, Ultra Violet sterilization and temperature control via a water heater/chiller. Experimental systems were filled with artificial seawater prepared at 30 psu using an artificial seawater mixture (Crystal Sea Marine Mix). Air stones were set to deliver a very gentle aeration in each tank. A constant artificial photoperiod of 24 hr light was applied using natural-spectra LED light-bulbs, which provided approximately 1,100 lx at the surface of rearing tanks. Tanks were isolated from external light sources using black curtains to standardize illumination. Water temperature was maintained at 27°C throughout the trial.

Enriched rotifers (S-strain enriched with Selco S-presso enrichment mixture, INVE Aquaculture) were introduced in tanks at 2.5 dph. Subsequently, tanks were fed twice daily. Prey input at each meal was determined based on the protocol target and residual prey counts determined before feeding. Tahitian strain *Isochrysis* sp. (T-iso) 25,000 cells/ml were introduced as background beginning 2.5 dph and maintained at this density until 13 dph. The target prey density was 10 ml<sup>-1</sup> in three of the tanks (group HF) and followed a progressive sequence beginning at 1 ml<sup>-1</sup> in the other three larval tanks and increasing to a maximum of 3.5 ml<sup>-1</sup> (group LF). Rotifer prey rations were reduced at 12 dph and discontinued at 13 dph. *Artemia* nauplii were introduced progressively beginning with newly hatched N1 at 12 dph followed by N2 nauplii enriched with Easy-DHA selco (INVE aquaculture) beginning at 13 dph. Dry food (Otohime diet, Reed Mariculture, sequences from A2 [150  $\mu$ m] to C [1,410  $\mu$ m] sizes was first introduced at 18 dph while *Artemia* feeding was progressively decreased beginning at 21 dph and discontinued at 25 dph (Figure 1).

**FIGURE 1** Diagram depicting the sequence of feeds during larval culture of Tripletail *Lobotes surinamensis*. Dph: days post hatch, N1: unenriched newly hatched *Artemia* Nauplii, N2: enriched *Artemia* nauplii

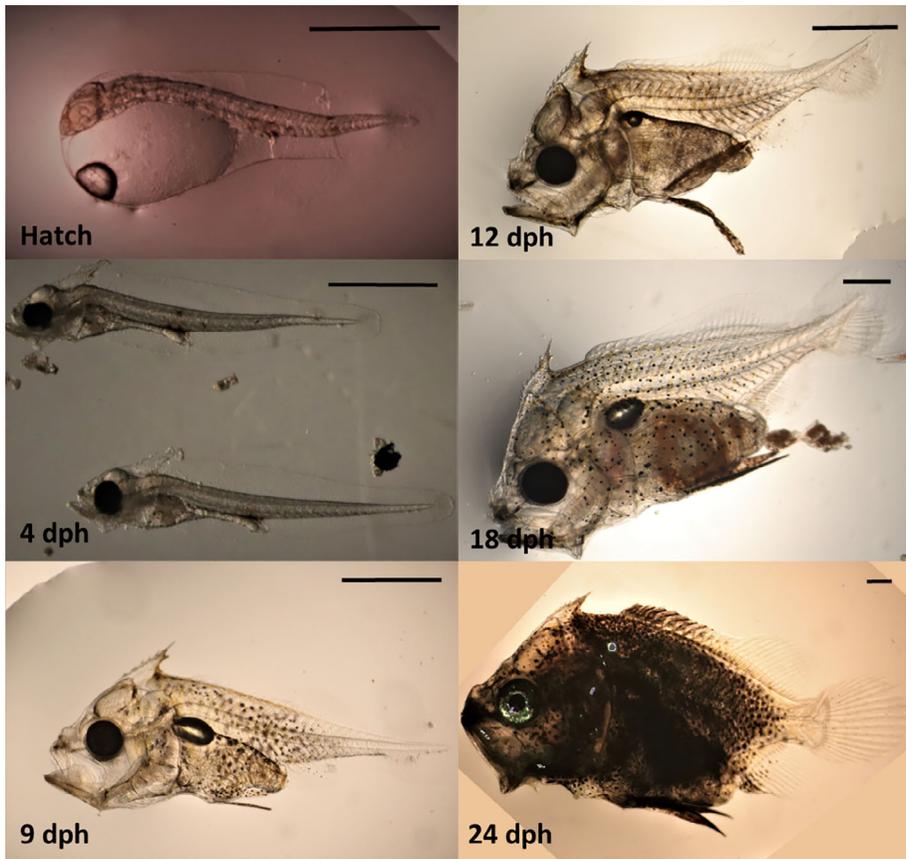


**FIGURE 2** Standard Length of Tripletail (*Lobotes surinamensis*) larvae from hatch to harvest at 33 days post-hatch (data from three larval culture tanks where larvae could be sampled through the entire larval period)



### 3.2.2 | Findings

Although larval density in culture tanks could not be quantified until harvest at 33 dph, visual observations indicated that density dropped rapidly in the first 5 days of feeding in the LF groups while larvae density remained high in the HF group until the *Artemia* transition (13 dph). Major decreases in density were recorded in both groups shortly after the transition to *Artemia* and the final survival was very low in all treatments (only 30 weaned post larvae were harvested at 33 dph). The first batches of enriched *Artemia* produced to feed larvae in this trial had low hatch rates and were hypothesized to have impacted survival of the larvae during the transition because of poor nutritional quality. Following the transition, surviving larvae grew rapidly in length but also in body depth (Figures 2 and 3).



**FIGURE 3** Pictures of Tripletail (*Lobotes surinamensis*) larvae at various stages of development from hatch to 24 dph. Newly hatched larva with yolk sac, 4 dph larvae with supraoccipital crest forming, 9, 12, and 18 dph larvae featuring abundant melanophores, spines and growth in body depth, 24 dph larva almost fully pigmented. Scale bar: 1 mm

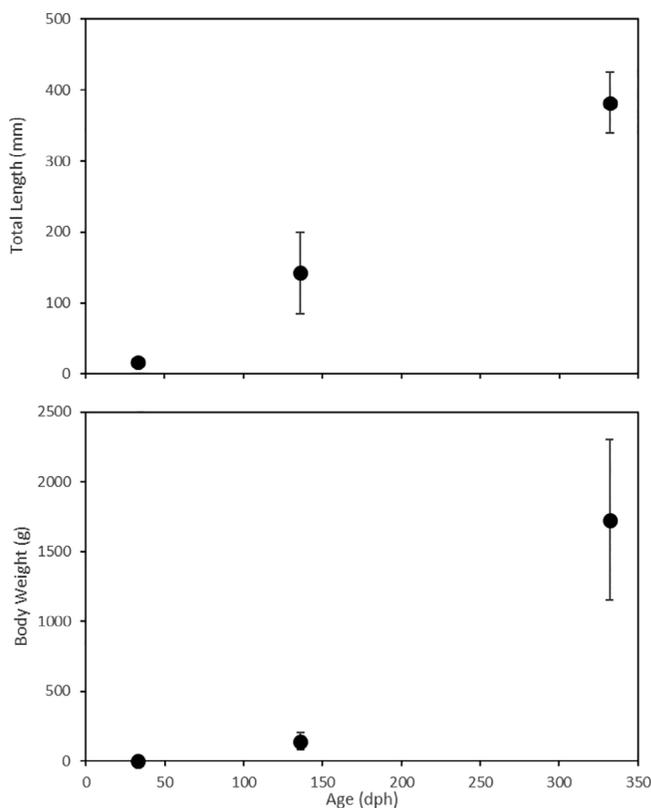
The results of this trial indicate that Tripletail larvae can be cultured using rotifers as an initial prey item, especially considering the apparent high early initial survival in the HP treatment. The transition to *Artemia* and prepared feeds will need attention during future trials to optimize growth and improve survival to weaned post-larvae.

### 3.3 | Grow-out

To date, only two preliminary Tripletail grow-out trials have been conducted at the Thad Cochran Marine Aquaculture Center. The first one was described in detail by Franks et al. (2001) and highlighted the fast growth potential of Tripletail with average weights over a kilogram reached in 7 months (from an initial weight of 12.7 g).

In 2019, the fish produced during the larval run described in Section 3.2 were cultured in closed recirculating systems similar to the spawning tanks described above (28-m<sup>3</sup> 1.5-m deep tanks) except that the tank volume was only 10-m<sup>3</sup> (tank diameter was 3 m). Fish were initially fed a commercial diet (skretting, Gemma) and transitioned to a diet of cut shrimp, fish and squid described in Section 3.1.2 at 161 dph (average weight 141.1 g). Feeding was performed by hand and the ration was adjusted to meet the demand of the fish. Growth

**FIGURE 4** Total length and body weight of Tripletail (*Lobotes surinamensis*) raised in recirculating systems at low density for a period of 10 months beginning at the end of the larval period



was monitored on three dates (Figure 4) and fish grew from 0.2 g mean weight to 1.725 kg in a period of 10 months. The growth rates of Tripletail during this low-density trials and the one conducted by Franks et al. (2001) were impressive and corresponded to an average growth rate (over the grow-out period) between 188 and 172 g/month in the study of Franks et al. and this study, respectively. These values place Tripletail among the fastest growing warm-water species candidates for aquaculture based on data reported by Benetti et al. (2010): tripletail would grow slightly slower than Cobia (250–500 g/month) and Dolphinfinch (333–667 g/month) but at a comparable or higher rate than Almaco Jack (83–250 g/month), Red Drum (75–150 g/month), and Barramundi (88–125 g/month). Yields of fillets removed from Tripletail at the end of the grow-out period in this study averaged 33%.

### 3.4 | Health and diseases

Information on diseases affecting tripletail in culture conditions is still very limited. Infestation from *Neobenedenia* parasites was reported by J. Franks in aquaculture conditions (Vanderkooy, 2016) and led to rapid mortality of infected fish. Infection by *Amyloodinium ocellatum* was reported by R. Blaylock in wild caught tripletail (Vanderkooy, 2016) and was also detected in one tank holding captive tripletail during this work, although the parasite loads in infected fish were low. Two outbreaks of *Lymphocystis* were diagnosed in tanks holding broodstock collected in the wild during the early phases of acclimation to captivity. Several fish showed symptoms in the affected tanks and a few mortality occurred but most of the fish recovered.

## 4 | BOTTLENECKS AND FUTURE RESEARCH NEEDS

This work confirmed the fast growth potential of Tripletail and suggests that closing the life cycle through control of captive maturation, spawning, and culture of the larval and juvenile stages is possible. However, a few bottlenecks must be addressed before expanded production can be considered. Captive broodstock in this and previous studies do not complete gamete maturation and spawning spontaneously. A fraction of captive female broodstocks appeared to progress to advanced stages of oocyte maturation compatible with hormonal induction of final maturation and spawning with a simple administration of GnRH $\alpha$  slow-release implants. However, spermatogenesis and/or spermiation appear inhibited in captive males and this inhibition also appears to prevent induction of spermiation using GnRH $\alpha$  administration as shown by the lack of fertility of spawns obtained when only GnRH $\alpha$  was used as a spawning agent. Preliminary results obtained in this work suggest that this inhibition may be resolved temporarily by the administration of an antidopaminergic compound, as the combined administration of GnRH $\alpha$  and domperidone produced spawns with higher fertility including some higher than 80%. Dopamine antagonists have been shown effective in reducing dopaminergic inhibition of gamete maturation during initial work on freshwater species (Chang, Peter, Nahorniak, & Sokolowska, 1984) and have since been used successfully to enable captive maturation and spawning in some marine fishes (e.g., *Mugil cephalus*, Aizen, Meiri, Tzchori, Levavi-Sivan, & Rosenfeld, 2005; *Lagodon rhomboides*, Di Maggio et al., 2010). Further work is needed, not only to optimize the induction protocols described here, but also to determine broodstock husbandry conditions that will restore spontaneous final maturation and spawning of captive brooders.

Larval culture protocols still require some development in order to achieve survival rates compatible with commercial scale grow-out but this work showed that hatchery production using mainstream live feeds (enriched rotifers and *Artemia*, Dhont et al., 2013) will likely be possible. Additional studies are needed to optimize a feeding protocol for Tripletail larvae, in particular during feed transitions. In addition, the nutritional requirements of Tripletail larvae need to be formally determined to assess the adequacy of enrichments for live feeds. Finally, the growth rates to a market size of 1 kg or greater recorded in this study suggest that a short production cycle can be expected, making Tripletail an attractive option for warm-water marine growers. Grow-out of this species still needs to be evaluated at commercial density, especially considering that this species appears to be non-gregarious in the wild and that some aggressive behavior was noted (at low density) during the early phases of grow-out in this study. Nutritional requirements also need to be studied to identify or design adequate feeds.

The U.S. market of Tripletail is still very limited with an average dockside revenue at \$34,000 between 2007 and 2014 and a peak at \$83,500 in 2014 (VanderKooy, 2016). However, indications are that a larger domestic market could be developed based on the growing demand from restaurateurs in southern coastal states and an increasing awareness of this fish and its highly desirable flesh among consumers. A formal marketing study would be valuable to assess potential market price and volume.

In conclusion, while additional data on larval culture and grow-out are needed to fully evaluate the technical feasibility of tripletail aquaculture, current data suggest that this species can be produced in hatcheries and could support a successful industry supplying the growing demand in the U.S. once protocols for culture are available.

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