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First Record of Captive Larval Culture and Metamorphosis of the Pacific Blue Tang, *Paracanthurus hepatus*

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Abstract

The Pacific blue tang, *Paracanthurus hepatus*, is consistently among the top 20 marine ornamental species imported into the USA, with all specimens presently sourced from wild stocks. Captive culture of this species through metamorphosis has not been previously documented and fundamental information regarding reproduction, larval culture, and production techniques is scarce. This study aimed to elucidate methods that would advance our understanding and success with captive propagation of this species. A total of 50,000 eggs were collected from a single broodstock population and stocked in a 1000-L tank. Beginning at 3 d posthatch (DPH), larvae were fed three times daily a diet comprised exclusively of copepod nauplii. At 12 DPH, enriched rotifers were first fed followed by powdered feed (20 DPH) and first instar *Artemia* (21 DPH). Large mortality events were observed at 7 and 20 DPH, corresponding with starvation and flexion, respectively. By 41 DPH, the majority of the remaining larvae began associating with the bottom of the culture tank. On Day 50, the first signs of blue pigmentation marked the beginning of metamorphosis. A total of 27 juvenile blue tangs were cultured during this trial. This effort represents the first successful culture of this species in captivity.

KEYWORDS

marine ornamental, pacific blue tang

The ornamental fish trade is a valuable sector of global aquaculture production with an estimated export value of US$366 million in 2011 (FAO 2011). Global retail value of the marine ornamental trade has been estimated at US$200–330 million (Wabnitz et al. 2003) with one-half to two-thirds of that value ascribed to

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the US market (Cato and Brown 2003; Wabnitz et al. 2003). Unlike freshwater ornamental fishes, of which approximately 90% of the species and varieties are produced through aquaculture (Dawes 1999), only up to 10% of marine ornamental species are estimated to be cultured commercially (Wabnitz et al. 2003). It is estimated that 16–24 million marine fish, representing approximately 1800 unique species, are sold annually, with the preponderance of specimens resulting from wild capture (Wabnitz et al. 2003; Rhyne et al. 2012). US aquaculture production of ornamental species is dominated by Florida growers with a reported farm-gate value of US$27.3 million in 2012 (USDA 2013). However, pressures from fluctuating market demand, competition with foreign importation, and increasing regulatory compliance threaten the continued growth and resiliency of this valuable segment of agriculture.

Growing interest in marine ornamental species has resulted in approximately 700,000 US households that currently maintain marine aquaria (APPA 2012). Wild collection of ornamental species may not be sustainable in all circumstances and captive propagation may help to alleviate collection pressures on wild stocks. Accordingly, research to diversify the species cultured for the marine ornamental industry is of great interest to aquaculture producers in the USA and worldwide (Tlusty 2002). Commercial production of new commodities may enhance the resiliency of producers through enterprise diversification while addressing vacillations in purchasing preference of the ornamental hobbyist.

Development of effective culture protocols is essential for the commercialization of new aquaculture species. While vast quantities of ornamental fishes are currently cultured in captivity, empirical evidence to support management decisions and production goals is limited. Fundamental information regarding reproduction, larval culture, and production techniques is critical when evaluating a species for commercial propagation and these bottlenecks will ultimately dictate the success of domestication and cultivation efforts. Historically, efforts to develop commercial protocols for marine ornamental aquaculture production have primarily focused on species that spawn large demersal eggs, exhibit some degree of parental care, and have large precocial larvae capable of consuming rotifers (*Brachionus* spp.) and brine shrimp (*Artemia* spp.). Culture of species that spawn small pelagic eggs and have underdeveloped altricial larvae has been largely unsuccessful owing to bottlenecks mainly associated with captive spawning and larval nutrition (Olivotto et al. 2017).

The Pacific blue tang, *Paracanthurus hepatus*, is a member of the family Acanthuridae and is consistently among the top 20 species imported into the USA by volume, with all specimens currently sourced from wild stocks (Wabnitz et al. 2003; Rhyne et al. 2012, 2017). An aquaculture gap analysis performed by Murray and Watson (2014) characterized the Acanthurids as "red," indicating this family to have a high market demand with no functional culture initiatives, emphasizing the need for research to develop commercial production protocols. Fundamental information regarding reproduction, larval culture, and production techniques for the Pacific blue tang is scarce. Ho et al. (2013) reported some success with the culture of Pacific blue tang, but larvae only survived to 26 d posthatch (DPH) in this trial. Culture of this species through metamorphosis has not been previously documented. This study aimed to elucidate methods that would advance our understanding of captive propagation of this species and demonstrate culture feasibility through the larval stage.

Materials and Methods

Sixteen Pacific blue tang broodstock (ca. 15–20 cm total length [TL] and 200–600 g) are currently located at the University of Florida's Tropical Aquaculture Laboratory (UF-TAL) in Ruskin, Florida. These fish were sourced from wild stocks through commercial ornamental wholesalers. Prior to introduction into established recirculating systems, all fish were subjected to a 30-d quarantine period. A prophylactic treatment regime consisting of a chloroquine phosphate bath (10 mg/L for 30 d), levamisole hydrochloride feed (4 g/kg feed, three doses), and praziquantel bath (6 mg/L for

24 h, two doses) was administered to reduce the potential for transmission of parasites from the wild broodstock into the culture environment.

Upon completion of the quarantine period, broodstock were transferred to a recirculating aquaculture system located within a greenhouse at the UF-TAL. Broodfish were maintained in 2660-L tanks within a 35,000-L recirculating system equipped with mechanical and biological filtration, ultraviolet (UV) sterilization, foam fractionation, and supplemental aeration. Water temperature was maintained at 27 ± 1 C using three heat pumps outfitted with titanium heat exchangers (Aqua Logic, Inc., San Diego, CA, USA) and photoperiod varied seasonally due to ambient lighting in the greenhouse. Each broodstock tank was outfitted with an external egg collector, which consist of a 75-L fiberglass tank into which a 20-L plastic bucket with nylon mesh panels (200 µm) was placed. The screen collection bucket received the overflow from the surface of the broodstock tank, passively collecting and concentrating buoyant eggs while allowing the water to continue to flow through the system. The water source for both the broodstock and larval systems was reverse osmosis water mixed with artificial sea salts (Instant Ocean Spectrum Brands, Blacksburg, VA, USA) to achieve a final salinity of approximately 35 g/L. Pacific blue tang broodstock were fed a varied diet to apparent satiation three to five times daily. The diet consisted of a mixture of a commercially prepared seafood blend (LRS Fertility Frenzy, Larry's Reef Services, Advance, NC, USA); fish eggs (LRS Fish Eggs, Larry's Reef Services); frozen mysis shrimp, *Mysis diluviana* (Piscine Energetics, Inc., Vernon, BC, Canada); and a commercially available 1.7-mm extruded pellet ([EP1 – 46% crude protein, 16% crude fat, and 2% crude fiber], TDO Chroma Boost, Reed Mariculture, Inc., Campbell, CA, USA). Egg collectors were checked daily for eggs from the previous evening's spawn. Collected eggs were volumetrically quantified prior to stocking for subsequent larval investigations.

A larval culture trial commenced on the morning of May 25, 2016. On this day, 23,000 fertilized eggs were collected from a single broodstock trio (one male, two females).

FIGURE 1. *Photograph depicting the configuration of the larval rearing tank used for culture of Pacific blue tang larvae. (A) Water inflow pipe, (B) feed bucket, (C) screened internal stand pipe, and (D) external standpipe. [Colour figure can be viewed at wileyonlinelibrary.com].*

Embryos were stocked into a 1000-L larval tank, which was part of a larger 7000-L larval recirculating system. Hatching began approximately 20 h after fertilization or approximately 6 h after stocking and this date was considered to be 1 DPH for the larval trial. Two subsequent spawns were collected over the next 2 d, 2000 and 25,000 embryos, respectively. These embryos were also stocked in the same larval tank and resulted in a total of 50,000 embryos and a stocking density of 50 embryos/L for the trial. The 7000-L larval system consisted of a 2000-L moving bed biological filter, a fluidized sand filter, a foam fractionator, a series of three bag filters (50, 25, and 10 µm), and a 120-W UV sterilizer. The configuration of the larval culture tank (Fig. 1) was largely based on the system used by Callan (2016) (C. K. Callan, personal communication) to successfully culture

the yellow tang, *Zebrasoma flavescens*. The larval tank water was supplied by gravity from a header tank with a flow rate of 10 L/min (14.4 tank turnovers/d, Fig. 1A). The outflow was a vertical length of 5-cm polyvinyl chloride pipe fitted with 250-µm nylon screening. This internal standpipe (Fig. 1C) allowed water and small feed particles to flush out while maintaining the larvae inside the tank. An external standpipe (Fig. 1D) controlled the water depth within the larval rearing tank. Light was supplied by an 18-W pendant light-emitting diode that was hung off-center approximately 70 cm above the surface of the water and set on a cycle of 16L : 8D. Feeding buckets (Fig. 1B) were positioned on the opposite side of the tank from the light and used for gradual delivery of live feeds and algae while providing a shaded area. This lighting regime resulted in a gradient of light availability both across the tank and down through the water column. At the surface, the light intensity reading was 5000 lux directly under the light, 1 lux under the feeding bucket, and 250 lux equidistant from these two points. At a depth of 30 cm, the light intensity was 1600 lux under the light, 75 lux under the feeding bucket, and 300 lux at the intermediate point. At the bottom of the tank, the light intensity was 420 lux under the light, 120 lux under the feeding bucket, and 300 lux at the equidistant point. Water quality parameters (temperature, salinity, pH, dissolved oxygen [DO], total ammonia nitrogen [TAN], nitrite $[NO_2^-]$, nitrate $[NO_3^-]$, and alkalinity) were monitored at least weekly with an optical handheld meter (DO and temperature, YSI, Inc., Yellow Springs, OH, USA), a refractometer (salinity), or the appropriate benchtop test (pH, TAN, NO_2^-, NO_3^-, and alkalinity, Hach, Loveland, CO, USA) and maintained within acceptable ranges for marine ornamental species (Table 1).

The feeding regime used to culture *P. hepatus* in this trial is depicted in Figure 2. Food was first offered to the larvae at 3 DPH. The larvae were fed three times per day, and total daily feed densities were delivered over three even feedings, unless otherwise stated. From 3 to 11 DPH, only *Parvocalanus crassirostris* copepod nauplii (sieved <75 µm) were offered. The tank was fed at a mean ± SD density of 4.0 ± 1.5 nauplii/mL divided among two to three feedings per day. During the first 9 d of feeding, live microalgae (ca. 3:1 of *Tetraselmis chuii* and *Symbiodinium microadriaticum*, stock culture densities ca. 4–6 million cells/mL) was added to the tank at a mean rate of 10.7 ± 4.6 L/d. Marine L-type rotifers *Brachionus plicatilis* (sieved <100 µm) were fed starting at 12 DPH. Rotifers were offered at a total density of 0.75 rotifers/mL divided between two feedings. Rotifers used for the first feeding of the day were enriched with Ori-One (Skretting, Stavanger, Norway) while rotifers used for the second feeding were enriched with Algamac 3050 (BioMarine, Hawthorne, CA, USA). Rotifer enrichment protocols followed the manufacturer's recommendations. The rotifer feeding density was gradually increased to 6 rotifers/mL on 20 DPH, with a mean daily rotifer feeding density of 3.3 ± 2.1 rotifers/mL divided between two feedings from 12 to 20 DPH. Over that same period, copepod nauplii were offered at a mean density of 7.2 ± 1.8 nauplii/mL and algae was added at a mean rate of 14.0 ± 3.4 L/d. On Day 20, a commercial microdiet (Gemma Micro 150 & 300, Skretting) was added to the larval feeding regime. The microdiet was added *ad libitum* three to six times per day to ensure sufficient access to the new feed and accelerate weaning. On Day 21, freshly hatched *Artemia* nauplii were added to the diet. Over the next 16 d (21–36 DPH) *Artemia* were fed at a mean density of 0.70 ± 0.80 *Artemia*/mL

TABLE 1. *Mean ± SD values for water quality parameters monitored in the larval tank throughout the larval culture trial.*

Temperature (C)	Salinity (g/L)	DO (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	TAN (mg/L)	pH	Alkalinity (mg/L $CaCO_3$)
27.7 ± 0.6	34.2 ± 0.8	6.25 ± 0.60	25 ± 6	0.00 ± 0.01	0.0 ± 0.0	8.5 ± 0.1	222 ± 39

DO = dissolved oxygen; TAN = total ammonia nitrogen.

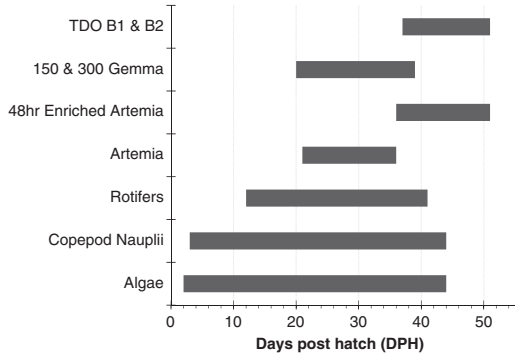

FIGURE 2. *Larval feeding regime used during the* Paracanthurus hepatus *larval trial to 51 d post hatch DPH. "TDO B1 & B2" and "150 & 300 Gemma" refer to the brands and sizes of commercial dry diets used. "48 h Enriched* Artemia*" was* Artemia *nauplii that had been enriched in Algamac 3050 for 48 h. "Artemia" refers to freshly hatched* Artemia *nauplii. "Rotifers" refers to* Brachionus plicatilis *that were sieved (<100 μm) and enriched (Algamac 3050 or Ori-One). "Copepod nauplii" were* Parvocalanus crassirostris *that were sieved (<75 μm). "Algae" refers to an approximately 3:1 mix of live* Tetraselmis chuii *and* Symbiodinium microadriaticum.

(min: 0.03 *Artemia*/mL, max: 3.00 *Artemia*/mL) divided between two daily feedings. Over this same 16-d period, copepod nauplii and rotifers were offered at a mean density of 6.2 ± 1.2 nauplii/mL and 9.4 ± 1.3 rotifers/mL, respectively, and algae (mixed as above) was added at a rate of 16.8 ± 1.3 L/d. On Day 37, larvae were fed *Artemia* enriched (Algamac 3050) for 48 h post hatch and a larger microdiet (TDO Chroma Boost B1 and B2 mix, Reed Mariculture, Inc.,). Over the next 15 d (37–51 DPH), enriched *Artemia* were fed at a density of 1.5 ± 0.2 *Artemia*/mL divided between two feedings per day. From Days 37 to 41, the larvae were weaned off rotifers and fed at a density of 5 rotifers/mL in the afternoon. From Days 37 to 44, the larvae were weaned off copepod nauplii (2.1 ± 0.62 nauplii/mL) and algae (6.9 ± 2.7 L/d). From 51 DPH onward, the larvae/juveniles were fed enriched *Artemia* and microdiet *ad libitum*. Following metamorphosis, all known developmental bottlenecks had passed and juvenile husbandry followed that typical of marine ornamental fish.

Results

The ultimate goal of this larval trial was to successfully culture the Pacific Blue Tang through the larval stage to metamorphosis and the juvenile phenotype for the first time ever in a captive setting and document an effective feeding regime. For this reason, larvae were not systematically sampled to describe morphological development. Instead, larvae were sampled intermittently for images, or when fresh mortalities were recovered from the culture tank. These periodic samples, combined with close observation, were used to document larval morphological development (Fig. 3) and identify time periods where significant mortality was observed. Two distinct periods of increased larval mortality were observed at approximately 7 and 20 DPH, which corresponded with starvation and the initiation of flexion, respectively. The mortality event observed at 7 DPH was expected, as this is a common bottleneck encountered in the culture of marine species (Olivotto et al. 2017) and especially the Pacific Blue Tang. However, the mortality observed at 20 DPH was significant as it demarcated the onset of flexion, another critical juncture that can adversely affect the success of culture efforts. Mortalities over the few days surrounding flexion were significant but hundreds of larvae still remained by 26 DPH, the age of the oldest documented cultured Blue Tang larvae prior to this study (Ho et al. 2013). Low-level mortalities continued over the next 10 d. On Day 41, a clear behavioral change was noted with the remaining larvae. On this day, the majority of the remaining larvae began to associate with the bottom of the culture tank, swimming into areas of high flow where the incoming water was deflected by the bottom of the tank. Although the larvae were still translucent (acronurus stage), behavior was indicative of settlement or the time period when marine larvae are no longer pelagic and associate with the benthos. By 50 DPH, the first signs of blue pigmentation were observed, marking the beginning of metamorphosis and the transition to the juvenile phenotype. The first larvae exhibited complete blue pigmentation (Fig. 3I) at 51 DPH, and the yellow pigmentation on the

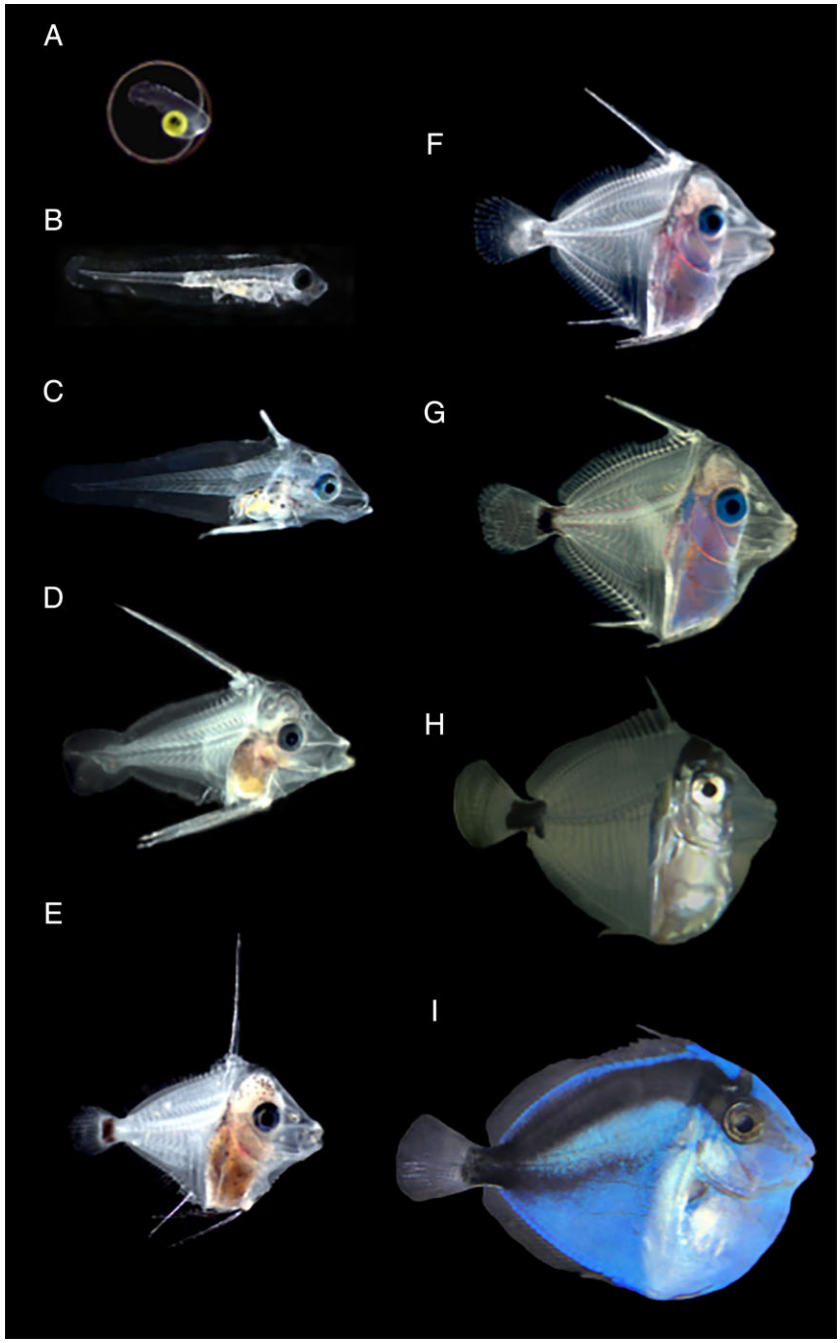

FIGURE 3. *Representative photographs of* Paracanthurus hepatus *development at (A) embryo,* D = *0.69 mm, (B) 5 d post-hatch (DPH), standard length (SL) = 2.234 mm, total length (TL) = 2.376 mm, (C) 15 DPH, SL = 3.296 mm, TL = 3.60 mm, (D) 19 DPH, SL = 5.44 mm, TL = 5.511 mm, (E) 27 DPH, SL = 5.816 mm, TL = 9.734 mm, (F) 29 DPH, SL = 7.90 mm, TL = 9.734 mm, (G) 33 DPH, SL = 8.712 mm, TL = 10.691 mm, (H) 46 DPH, SL = 17.698 mm, TL = 22.121 mm, and (I) 54 DPH, SL = 21.232 mm, TL = 25.303 mm. [Colour figure can be viewed at wileyonlinelibrary.com].*

caudal fin appeared in the coming days. By 78 DPH, all had completed metamorphosis and had adult coloration. A total of 27 Pacific blue tangs were successfully cultured for the first time in this trial with an overall survival of 0.054%.

Discussion

The successful culture of *P. hepatus* is a major breakthrough in the marine ornamental aquaculture industry. It is a very popular marine ornamental species and development of commercial culture could provide an alternative source to the wild capture fishery and help to minimize the sometimes unsustainable practices associated with wild collection (Domínguez and Botella 2014). To date, the Pacific blue tang is only the second member of the family Acanthuridae to be successfully cultured, following the yellow tang, which was successfully cultured by researchers in Hawaii (Callan 2016).

Certain aspects of general marine larval culture protocols utilized during this trial were altered compared to previously unsuccessful larval rearing attempts with this species at the UF-TAL (methods are presented in Cassiano et al. 2015). In general, a larger culture tank, higher initial stocking density, higher water flow rate, and increased feeding rate represented deviations from culture protocols previously used for this species. Since our initial successful trial, three more small cohorts of *P. hepatus* have been raised: one at UF-TAL (13 juveniles) and two at the University of Florida's Indian River Research and Education Center (UF-IRREC; 15 juveniles total). These subsequent trials were all similar with respect to feeding regimes and flow rates but were conducted in smaller culture tanks (125 L at UF-TAL and 500 L at UF-IRREC). Survival rates, however, continued to be low (0.15% at UF-TAL, 0.00012 and 0.00018% at UF-IRREC) but nonetheless prove that the general culture methods described in this article are both replicable and translatable to other aquaculture facilities.

The feeding regime used during this study (Fig. 2) was very similar to that used by Callan (2016) for the culture of yellow tang. Both Acanthurid species successfully progressed through metamorphosis using only *P. crassirostris* copepod nauplii during first feeding. Calanoid copepod nauplii are generally the preferred first feed for pelagic marine ornamental larvae, due to their small size, pelagic life cycle, nutritional content, and ability to stimulate a feeding response in marine fish larvae (Drillet et al. 2006; Schipp 2006; Baensch and Tamaru 2009; Gopakumar and Santhosh 2009; Olivotto et al. 2011). Copepod nauplii are also the most common feed item found in the gut contents of wild pelagic tropical marine larvae (Houde and Lovda 1984; Sampey et al. 2007; Olivotto et al. 2011). Previous studies evaluating *P. hepatus* culture and feeding regimes have focused on ciliates as the primary first feed item (Nagano et al. 2000; Ho et al. 2013). Nagano et al. (2000) compared initial feeding regimes that utilized two different ciliate species, the tintinnid ciliate *Amphorellopsis acuta* and the naked ciliate *Euplotes* sp. Although first feeding larvae were shown to ingest both species, no larvae from any treatment lived past 8 DPH, with the larvae fed *A. acuta* performing slightly better. Lee et al. (University of Florida, Gainesville, unpublished data) investigated prey preference of first feeding larval *P. hepatus* and recorded incidences of the larvae consuming ciliates, rotifers, and copepods, with rotifers consumed at a more frequent rate. Despite these results, rotifers have not been used successfully as an exclusive first feed to date. Further research is needed to investigate different feeding regimes and prey organisms at periods of increased mortalities. The timing of the transition from larvae feeding on copepod nauplii to rotifers will be an important area of research in the future and may impact the potential commercial profitability of this species.

Metamorphosis to fully pigmented juveniles began at 50 DPH in the first successful cohort and was delayed in later cohorts that were cultured in smaller tanks. Furthermore, behavioral association with the benthos was noticed at 40 DPH in the 1000-L tank but was less distinguishable in subsequent trials in smaller tanks. The larval duration reported in this study is slightly longer than the reported larval duration based on daily otolith increments of 37 d ($n = 1$) (Brothers and Thresher 1985) and may be due to factors

present or absent in the culture environment. Other Acanthurid species have pelagic larval periods that range from 31 to 90 d (Brothers et al. 1983; Brothers and Thresher 1985; Wilson and McCormick 1999). Plasticity in settlement time has been reported in several species (Victor 1986; McCormick 1999), and future research should focus on improving development and settlement cues to minimize the duration of the planktonic larval stage.

The successful repeated culture of Pacific blue tang larvae described in this article provides proof of concept and further validates the effectiveness of early larval feeding regimes developed for this species. However, for successful commercialization of the Pacific blue tang, protocols will have to be improved to consistently provide higher survival rates and reduce costs associated with protracted feeding of copepod nauplii. Future research will aim to improve protocol methods by focusing on manipulation of feeding rates and rearing conditions. Live prey densities and feeding frequencies, which promote larval growth and survival, are two areas of great interest moving forward. As copepods are challenging to culture, investigations into minimizing the use of copepod nauplii and weaning larvae to rotifers at the earliest possible age are critical. Additionally, optimizing abiotic conditions such as temperature, photoperiod, and water flow is critical to providing conditions conducive to the profitable culture of this species. Although there is likely a long way to go before *P. hepatus* can be produced on a commercial scale, this initial success represents an encouraging first step toward that goal.

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