



Interplay between hormonal and morphological changes throughout a critical period of larval rearing in the orbicular batfish

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ABSTRACT

Advancement and diversification of the aquaculture industry is reliant on the development of captive breeding and rearing protocols for novel fish species. Orbicular batfish (*Platax orbicularis*), a major emerging species in Polynesian aquaculture, live in brackish and marine waters around coral reefs, and are highly prized by Pacific Island communities for their high-quality meat. The present study describes the larval growth of *P. orbicularis* from hatching until 16 days after hatching (DAH) using meristics and thyroid hormone (T₃) quantification. Our study highlighted that metamorphosis of *P. orbicularis* is critical to their production in aquaculture, as found for other species. Levels of the thyroid hormone T₃ in *P. orbicularis* reached a peak at 12 DAH (i.e. hormonal metamorphosis). This peak occurred in concert with important morphological changes and increased mortality and growth between 9 and 12 DAH, clearly illustrating the sensitivity of this fish during metamorphosis. Overall, our study sheds light on the metamorphosis and larval development of a novel aquaculture species, and the interplay between hormonal and morphological changes throughout a critical period of its rearing. These findings may promote the development of protocols for mass-scale production of orbicular batfish in captivity, which may be particularly beneficial to the aquaculture industries of many Pacific Island countries.

1. Introduction

Coral reef fisheries provide food and economic revenue that support coastal communities comprising millions of people worldwide (Loper et al., 2008). Concurrently, coral reefs are facing major global and local threats (e.g. climate change, pollution, and over-fishing) that jeopardize local food security (Moritz et al., 2018). In this context, aquaculture is often seen as an alternative to the provision of coral reef fish by

small-scale fisheries to meet growing global nutritional challenges (e.g. Pomeroy et al., 2006; Liu et al., 2018; Loper et al., 2008). To date, several species have been utilized in aquaculture (e.g. representatives in the families Serranidae, Labridae, Lutjanidae and Lethrinidae; Chou and Lee, 2008). Some still rely on wild capture, whilst others have had their life cycle effectively replicated in controlled artificial systems. Although many edible reef fish have been raised experimentally from early developmental stages, only a handful of species have been successfully

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hatchery reared on a commercially sustainable basis [e.g. Leopard coral grouper (*Plectropomus leopardus*; Yoseda et al., 2008); Japanese flounder (*Paralichthys olivaceus*; Fukuhara, 1986); red sea bream (*Pagrus major*; Moteki et al., 2001)]. For most species, commercial success has proven difficult due to the fragility of fish at metamorphosis, which is the transition from the pelagic larval stage to the reef-settled juvenile stage (Dabrowski, 1984; Laudet, 2011). As mortality can reach up to 95 % during this transition from larva to juvenile in the wild (Doherty et al., 2004; Lecchini et al., 2007) and more than 60 % in aquaculture (Gasset and Remoissenet, 2011), metamorphosis is a limitation that can shape both population dynamics and aquaculture production (Dabrowski, 1984; Lecchini and Galzin, 2003). It has been recently demonstrated that larval recruitment in coral reef fish corresponds to a thyroid hormone (TH) controlled metamorphosis (Holzer et al., 2017; Besson et al., 2018). Holzer et al. (2017) have further shown that specific impairments in TH signaling lead to juveniles that feed less efficiently, potentially reducing post-metamorphosis survival.

The present study aims to better understand the metamorphosis of a coral reef fish species that is emerging as an important player in the aquaculture industry of Pacific Island countries, the orbicular batfish (*Platax orbicularis* - Forsskål, 1775; in the family Ephippidae). *Platax* species are of interest to the aquaculture industry (i.e., highly valued meat consumed by local Island communities - Gasset and Remoissenet, 2011) and some species are already cultured in Southeast Asia and the South Pacific (Holt et al., 2017; Le Maréchal, 2004; Yu, 2002). However, despite a recent study on *Platax teira* (a species from Southeast Asia; Leu et al., 2018), the early life history and identification of the planktonic stages of the orbicular batfish (*P. orbicularis*) during the hatchery phase are not fully understood (Masanet, 1995). Furthermore, despite the wealth of studies on morphological development in coral reef fishes, information on the hormonal control of their metamorphosis is sparse (Holzer et al., 2017). Thus, the present study describes the larval growth of *P. orbicularis* from hatching until 16 days after hatching (DAH) using meristics and thyroid hormone (T_3) quantification. By improving our understanding of the morphological and endocrinological changes occurring in *P. orbicularis* early life stages, this study will provide key information for larviculture methodologies in hatcheries and for successful cultivation of batfish in the future..

2. Material and methods

2.1. Larviculture

Platax orbicularis eggs were obtained in April (2016, 2018) from naturally spawned broodstock supplied by Marine Resources Department (DRM) based at the IFREMER research center in Tahiti. The broodstock comprised six females (average weight: 2.9 ± 0.3 kg) and seven males (average weight: 3.1 ± 0.4 kg). The breeders were captured from multiple locations in French Polynesia (Bora-Bora, Raiatea and Tahiti). Eggs were collected in a 700 μ m collecting basket overnight via an inlet pipe from the tank. Floating eggs were carefully harvested using a plastic beaker and transferred to a 10 L bucket. Estimations on the average number of floating eggs were taken volumetrically via six replicate counts of 2.5 mL. The 10 L bucket of eggs was then transferred into a 20 L cylindro-conical basin with pre-set mixing before transfer into the 2200 L incubation tanks. Upon hatching, usually 24 h later, the larvae were carefully transferred to the 2000 L black cylindrical composite tanks. A single batch of around 142,000 eggs was collected and eggs were stocked at approximately 71,000 eggs per tank (35.5 in. L^{-1}). After inoculation and acclimation to the rearing tanks, sunken eggs were siphoned off the tank bottom and counted, their numbers were then subtracted from the estimated stock density. Dead larvae were removed and counted twice daily (during morning and afternoon monitoring) throughout the hatchery phase. After hatching, larvae were regularly monitored for mouth opening, which is expected to occur around 36–48 h after hatching.

An open circuit aquarium system was used in this study with seawater pumped from the lagoon (5 m deep). The seawater was primarily filtered through a sand filtration unit, followed by 25 μ m and 10 μ m cartridge filters and finally purified using UV (200 mJ cm^{-2}). Water renewal rates in the rearing tanks began at 15 %/h at 0 DAH and gradually increased to 55 %/h renewal by 18 DAH. While complete darkness was maintained before mouth opening, a normal photoperiod of 12 h light and 12 h dark was applied after the opening of the mouth and for the remaining hatchery phase. Water quality parameters were measured twice daily at 0900 and 1500 using an YSI 6600 Multi-Parameter Water Quality Sonde (YSI Incorporated, Yellow Springs, Ohio, USA) and were maintained at: salinity (36 ± 0.5 PSU), pH (8.0 ± 0.1), dissolved oxygen ($9.5 \pm 0.5 \text{ mg L}^{-1}$) and temperature (21.5 ± 1.0 °C). Since this study focused on the morphological and meristic changes from first feeding through to the completion of metamorphosis (i.e. the hatchery phase), this study examined larval fish from 0 to 16 DAH. The experiments were conducted in April/May 2016 (thyroid hormone T_3 levels) and April/May 2018 (thyroid hormone T_3 levels and all other measurements). All experiments were conducted on a single hatchery each time, and at the same facility.

2.2. Live feed

At mouth opening (2 DAH), the larvae were fed L-type rotifers of *Brachionus plicatilis* species complex. Rotifers were batch-cultured in 800 L tanks at a salinity of 36 ± 1 PSU. The rotifers were enriched with DHA Selco (INVE Aquaculture Inc., Salt Lake City, USA; 0.1 g per million rotifers) and RotiGrow *Nannochloropsis* paste (Reed Mariculture Inc., Campbell California, USA; 1 g per million rotifers). The enriched rotifers were automatically fed to the larviculture tanks at a rate of 2.62 L h^{-1} via the use of pumps (SMART Digital S DDA pump, Grundfos, Bjerringbro, Denmark) with stocks replenished twice daily (Gasset and Remoissenet, 2011). Rotifers were distributed to the larvae from mouth opening to 9 DAH at a density of 60 in. mL^{-1} . *Artemia* nauplii (INVE EG Magnetized *Artemia*, INVE Aquaculture Inc., Salt Lake City, USA) were also introduced to the larval rearing tanks at a density of 1000 *Artemia* per larva from 7 to 10 DAH and then *Artemia* A1 was added while gradually increasing feed density up to 10,000 *Artemia* per larva between 10–16 DAH (Gasset and Remoissenet, 2011). Due to the high number of larvae, the feeding protocol adopted was 24 h of continuous feeding. The larval diet was then supplemented with 300 μ m micro-pellets (GEMMA Micro 300, Skretting, Maine, USA) from 12 DAH to the end of the experiment with 1 mm pellets only introduced at 16 DAH, after which the larvae were transferred to new tanks for the “weaning phase”.

2.3. Morphological development and measurements

The orbicular batfish larvae were comprehensively examined throughout the hatchery phase to describe developmental morphology during this critical period. Samples (Fig. 1, Table 1) were obtained from $N = 80$ larvae following the methods of Leis and Trnski (1989) and Ditty et al. (1994) to the nearest 0.1 mm with a micro-ruler under a dissecting microscope (LEICA EZ4W) and analyzed using ImageJ software (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Maryland, USA). Specimens were sampled daily and measured without prior fixation in formalin or ethyl alcohol to permit observation of chromatophore and pigment development. To sample batfish larvae, fish were first euthanized in freshly prepared MS222 at 0.4 mg ml^{-1} in filtered seawater at 4 °C, and instantly placed on ice.

2.4. Measurements of growth, weight, survival and gut content

Measurements were undertaken by placing individual larvae on a glass slide and removal of as much moisture as possible. Gut contents were analyzed under the microscope using dissecting forceps to isolate

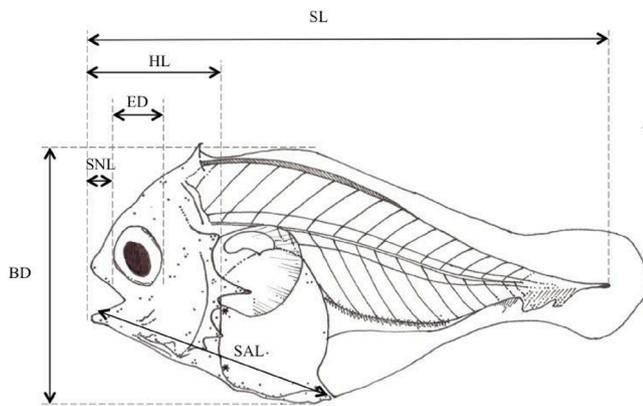


Fig. 1. Measurement parameters of orbicular batfish (*Platax orbicularis*): standard length (SL), snout-anus length (SAL), head length (HL), body depth (BD), eye diameter (ED), snout length (SNL); Adapted from Barros et al. (2015).

the abdomen and remove overlaying tissues. After puncturing the stomach pouch, its contents were observed and counted with the aid of a portable tally counter. Wet weight measurements were taken as there was no intention to return the live larvae back to the rearing tanks. Each larva was euthanized and immediately placed on a glass slide, gently patted dry with tissue paper and weighed using a precision laboratory scale (accurate to 0.001 g). Measurements were obtained daily from a randomly selected representative pool of $N = 80$ larvae (5 larvae per day) throughout the entirety of the study (1–16 days after hatching). Measurements of standard length (SL), body depth (BD), eye diameter (ED), head length (HL), snout length (SNL), snout to anus length (SAL) and flexion angle (FA) were taken daily throughout the study period. During pre-flexion stages the standard length refers to the notochord length (NL; snout to end of notochord) whereas in post-flexion larvae, SL is measured from anterior tip of jaw to the posterior border of the hypurals (Fig. 1). Body depth corresponds to the maximum dorsal-ventral length perpendicular to the SL. Fulton's condition factor (K) was calculated following Datta et al. (2013) to find out larval condition throughout ontogeny. K was calculated using the formula:

$$K = \frac{W \times 100}{SL^3}$$

Where W = weight of larvae (g), SL = standard length of larvae (mm).

Mortality is represented as a percentage of dead fish compared to the initial number of larvae. Calculations were also undertaken to determine daily mortality rate (% d⁻¹).

Table 1

Measurements of larval orbicular batfish (*Platax orbicularis*) in hatchery phase. Measurements are expressed as mm (mean \pm standard deviation) of standard length (SL), snout-anus length (SAL), head length (HL), body depth (BD), eye diameter (ED), snout length (SNL), flexion angle (FA), and egg yolk diameter (EYD).

DAH	SL	SAL	HL	BD	ED	SNL	FA	EYD
0	2.99 \pm 0.14	1.57 \pm 0.11	0.40 \pm 0.09	0.85 \pm 0.09	0.23 \pm 0.04	0.11 \pm 0.02		0.52 \pm 0.05
1	3.53 \pm 0.06	1.45 \pm 0.08	0.69 \pm 0.02	1.00 \pm 0.06	0.26 \pm 0.05	0.18 \pm 0.03		0.36 \pm 0.04
2	3.66 \pm 0.29	1.09 \pm 0.11	0.74 \pm 0.07	1.16 \pm 0.13	0.29 \pm 0.06	0.23 \pm 0.08		0.29 \pm 0.08
3	3.68 \pm 0.26	1.55 \pm 0.12	0.98 \pm 0.11	1.19 \pm 0.12	0.29 \pm 0.02	0.26 \pm 0.04	Pre-flexion	0.17 \pm 0.03
4	3.81 \pm 0.21	1.87 \pm 0.14	1.13 \pm 0.12	1.44 \pm 0.09	0.34 \pm 0.03	0.33 \pm 0.06		
5	4.63 \pm 0.18	2.28 \pm 0.21	1.32 \pm 0.10	1.67 \pm 0.37	0.45 \pm 0.02	0.33 \pm 0.05		
6	4.72 \pm 0.30	2.44 \pm 0.31	1.46 \pm 0.16	1.78 \pm 0.11	0.35 \pm 0.15	0.34 \pm 0.17		
7	5.21 \pm 0.24	2.69 \pm 0.20	1.62 \pm 0.14	2.37 \pm 0.14	0.52 \pm 0.04	0.40 \pm 0.07	16.8 \pm 3.29	
8	5.42 \pm 0.30	2.79 \pm 0.17	1.82 \pm 0.19	2.38 \pm 0.13	0.56 \pm 0.05	0.45 \pm 0.07	19.8 \pm 4.19	
9	5.98 \pm 0.09	3.56 \pm 0.34	2.53 \pm 0.28	3.12 \pm 0.15	0.62 \pm 0.12	0.49 \pm 0.05	55.28 \pm 3.21	
10	6.79 \pm 0.29	4.72 \pm 0.21	2.59 \pm 0.49	4.02 \pm 0.35	0.73 \pm 0.08	0.64 \pm 0.06	59.61 \pm 6.51	Absorbed
11	7.42 \pm 0.31	4.79 \pm 0.56	3.01 \pm 0.49	4.67 \pm 0.51	0.73 \pm 0.11	0.67 \pm 0.15		
12	7.92 \pm 0.27	5.01 \pm 0.19	3.06 \pm 0.19	4.41 \pm 0.41	0.86 \pm 0.05	0.68 \pm 0.06		
13	9.49 \pm 0.89	6.17 \pm 0.78	3.53 \pm 0.27	6.26 \pm 0.93	0.86 \pm 0.11	0.79 \pm 0.15	Flexion	
14	10.56 \pm 1.22	7.01 \pm 0.65	3.61 \pm 0.41	6.64 \pm 1.20	0.87 \pm 0.09	0.87 \pm 0.18		
15	11.05 \pm 1.26	7.09 \pm 0.81	4.14 \pm 0.35	7.69 \pm 1.31	1.07 \pm 0.17	0.99 \pm 0.12		
16	12.63 \pm 1.06	7.95 \pm 1.24	4.67 \pm 0.49	8.53 \pm 0.97	1.08 \pm 0.09	0.92 \pm 0.16		

2.5. Thyroid hormone T_3 quantification

T_3 profile were only investigated around metamorphosis, i.e. in fish between 6 and 16 DAH. Due to the small size of the larvae (and consequently, low amount of T_3), 5 individual fish were pooled per sample. Following euthanasia, each sample was weighed and stored at -20°C . Samples were subsequently defrosted and T_3 was extracted using methanol, chloroform and barbital buffer, and quantified using Roche ELICA kit on a Cobas Analyzer (detailed protocol available in Holzer et al., 2017). We performed this experiment twice (i.e. 2 technical replicates, in 2016 and 2018), for a total sample number (n) of 12–13 for each DAH, depending on larval availability and sample quality. Overall, 137 samples (N) were processed, corresponding to 685 fish collected in total.

2.6. Statistical analyses

The significance level for statistical analyses was set at $\alpha = 0.05$ and all statistical tests were performed using R software and a customized analysis code. The normality of the distribution of standard length, body depth, weight, daily mortality rate and gut content of *P. orbicularis* larvae were tested with Shapiro-Wilk Normality Tests ($W = 0.66 - 0.87$, $P = < 10^{-3}$). Differences in SL and BD, weight and mortality between DAH were analyzed by means of one-way ANOVAs followed by multiple pairwise t -test comparisons with a Bonferroni adjustment if significant differences were found. Kruskal-Wallis tests were used to compare differences between DAH and post-hoc Dunn's multiple pairwise comparison test were performed if a significant difference was found.

To analyze the dynamics of T_3 levels during larval development of *P. orbicularis*, we conducted linear mixed-effects models (lme) followed by post-hoc Tukey tests in R version 3.5.3. (R Core Team, 2019), using the nlme and multcomp packages (Hothorn et al., 2008). DAH was used as a fixed effect and the two technical replicates (corresponding to the 2016 and 2018 experiments) as a random effect. Model assumptions (normality and homoscedasticity of model residuals) were evaluated using Shapiro-Wilk tests and diagnostic plots.

3. Results

3.1. Morphological & pigmentation development

Based on morphological development parameters, our study showed that early larvae were rotund and deep-bodied with a body depth of 32 % of the standard length (SL). Egg yolk was absorbed by 3 DAH (Tables 1 and 2). At full flexion of the notochord, the BD was $> 63\%$ of SL and $> 67\%$ at 16 DAH (Table 1,2). The larvae became increasingly more deep-

bodied and laterally compressed as the culture period progressed. The heads of the larvae were large and constituted > 30 % of SL by 16 DAH (Tables 1 and 2). The head profile gradually became steeper and deeper with a terminal mouth and an upper jaw that extended to approximately the level of the middle of the eye. Spines began to develop at 3 DAH on the posterior margins of the outer shelf, initially with two visible spines, one of which was quite prominent and located at the preopercular angle (Fig. 2). Three additional smaller opercular spines developed soon after (4–5 DAH) below the dominant spine (Fig. 2). As the fish grew, the opercular spines reduced in prominence along the operculum margins from 11 to 12 DAH (Fig. 2) and as the operculum became larger the spines began to become relatively smaller in size (Table 1,2). Similarly, the supraoccipital spine, which was first observed at 5–6 DAH, became less prominent by 12–15 DAH as the larvae grew. Lastly, flexion of the notochord began at 7 DAH (mean \pm standard deviation: 16.7 ± 3.2 degrees) and complete flexion of the notochord was achieved by 11 DAH (Table 1, Table 2).

Larval eyes were round and large relative to head length and were noticeably larger at hatching (57 % of the head length (HL)) than at the end of the rearing period (only 23 % of HL at 16 DAH, i.e. about 7–9 % SL).

At hatching, the gut was a simple loop extending from the yolk to the anus, which was located around mid-body (around 52 % SL). Prior to exhaustion of the yolk, it ranged from 0.52–0.17 mm in size (0–3 DAH) (Table 1). Upon exhaustion of the yolk (4 DAH), the gut cavity became bulbous and the intestinal loop extending to the anus shortened, with the position of the anus shifting to slightly beyond mid-body by 16 DAH (> 60 % SL) (Table 2).

Pigmentation appeared within the first 24 h after hatching. In yolk sac-stage larvae, light pigmentation was observed around the head of the larvae over the mid and hindbrain and scattered over the operculum. Light patches of pigment were also concentrated above and below the notochord. Darker pigmentation was observed around the gut area where the egg yolk and oil globule were located. Larval eyes were pigmented, and the larval mouth was open by 2 DAH. By the time the yolk was absorbed at 3 DAH, which was followed shortly by the oil globule at 4 DAH, pigmentation had become more pronounced. Pigmentation was observed on the operculum and concentrated at the tips of the opercular spines, along the contours of the mouth as well as around the visceral mass immediately posterior, superior and inferior to the pectoral fin base. Pigmentation also began to concentrate along the notochord whilst the embryonic fins were yet to differentiate and remained pigment-free. Melanophores appeared on the larvae at 5 DAH (Fig. 3) and gradually dominated the anterior portion of the larval body, becoming prominent on the head of the larvae at 10 DAH. Pigmentation extended posteriorly as the larvae grew (Fig. 3). By 11 DAH, the larval body was covered in pigmentation with large melanophores across the visceral mass. Small patches of closely spaced melanophores were visible just above the gut area and extended onto the caudal and pelvic fins, with the posterior tips and caudal fin remaining pigment-free. Pigmentation terminated posteriorly around the caudal peduncle (Fig. 3). At 11–13 DAH, dark closely spaced melanophores were observed just above and below the eyes.

Table 2

Morphometrics of larval orbicular batfish (*Platax orbicularis*) in hatchery phase. Measurements are expressed as percentage (%) of standard length (SL), of body depth (BD), head length (HL), eye diameter (ED) and snout-anus length (SAL).

DAH	RATIO				
	BD/SL	HL/SL	ED/SL	ED/HL	SAL/SL
0 – 4 (First feeding)	28–38	14–30	7–8	29–57	30–52
5 - 8 (Preflexion)	38–44	29–34	7–10	24–33	49–52
9 - 12 (Metamorphosis)	52–63	34–43	9–10	24–28	59–65
13 - 16 (Metamorphosis complete)	66–68	34–37	8–9	23–26	63–66

Consolidation of these melanophores and pigments at around 14–16 DAH resulted in a band that extended inferiorly from the base of the median supra-occipital crest towards the middle of the eye and then curved posteriorly to the base of the operculum slightly behind the eye. By the end of metamorphosis (at 16 DAH), the entire larval body was scattered with melanophores with the exception of the caudal fin, indicating that this is the last body part being pigmented (Fig. 3).

3.2. Measurements of growth, weight, survival and gut content

From 0 to 4 DAH, *P. orbicularis* larvae size varied little from 2.99 ± 0.14 mm to 3.81 ± 0.21 mm in standard length (SL) with a body depth (BD) of 0.85 ± 0.09 mm to 1.44 ± 0.09 mm (Fig. 4A). From 5 to 12 DAH, moderate growth was observed with larvae growing from 4.63 ± 0.18 mm to 7.92 ± 0.27 mm in SL, with BD increasing from 1.67 ± 0.37 mm to 4.41 ± 0.41 mm (Fig. 4A). Finally, from 12 to 16 DAH, rapid growth was observed with larvae increasing from 9.49 ± 0.89 mm to 12.63 ± 1.06 mm in SL, and 6.26 ± 0.93 mm to 8.53 ± 0.97 mm in BD (SL: $\chi^2_{16} = 82.05$; $P < 10^{-3}$, BD: $\chi^2_{16} = 82.01$; $P < 10^{-3}$) (Fig. 4A). A similar trend was observed for larval weight with a significant increase commencing at 10 DAH when larvae weighed 12.33 ± 1.49 mg, and reaching 148.67 ± 6.86 mg at 16 DAH ($\chi^2_{16} = 96.68$; $P < 10^{-3}$) (Fig. 4B). The acceleration of growth and the start of weight gain at 10 DAH also appeared to be associated with a significant increase in mortality between 9 and 12 DAH, reaching a maximum of 4.73 ± 0.47 % at 11 DAH, before the cumulative mortality rate stabilized until the end of the hatchery phase ($\chi^2_{16} = 27.58$; $P = 0.024$) (Fig. 4C). The daily mortality rate (% d-1) peaked at 10 DAH at 4.75 ± 0.47 % with total mortality and at 16.52 ± 4.49 % at 16 DAH.

Apart from the transitional phase between re-absorption of yolk sac (Table 1), mouth opening and successful initiation of feeding, development of condition factors in the feeding larvae is generally linear (Fig. 5). The highest average *K*-values were observed at 3 DAH (0.08 ± 0.02) and 4 DAH (0.1 ± 0.01), whereas the lowest average *K*-values were at 9 DAH and 10 DAH ($K = 0.02 \pm 0.01$).

There were no significant differences in quantity of gut contents observed between 3 and 7 DAH, following mouth opening at 3 DAH (Fig. 4D). *P. orbicularis* was observed to be quite a voracious feeder. Prior to metamorphosis (5–7 DAH), the average rotifer counts in the gut ranged between 9.6–11.8 rotifers/individual. Gut contents drastically increased four-fold by 8 DAH (40.4 ± 13.1 rotifers/individual) and then 2.5-fold again at 9 DAH (101.0 ± 10.7 rotifers/individual). Upon the introduction of a new diet (*Artemia* at 10 DAH), the gut content steadily increased until a peak of 855.6 ± 99.4 *Artemia*/individual at 15 DAH ($\chi^2_{16} = 82.75$; $P < 10^{-3}$) (Fig. 4D).

3.3. Thyroid hormone T_3 quantification

Levels of the thyroid hormone T_3 decreased between 6 DAH and 10 DAH and then rose to a peak at 12 DAH (i.e. hormonal metamorphosis) before subsequently decreasing again ($F_{10,13} = 13.93$; $P < 10^{-3}$) (Fig. 6). This peak at 12 DAH coincides with the morphological metamorphosis of *P. orbicularis* (Figs. 2,3), further validating that coral-reef fish metamorphosis is associated with a peak in thyroid hormone levels (Holzer et al., 2017).

4. Discussion

Platax orbicularis is commercially valuable not only as a food fish but also as an ornamental fish. This species exhibits positive potential for further development in the aquaculture and the aquarium industry and can potentially provide an insight into the development of other batfish species as well as Ephippid fishes more generally. However, in aquaculture, the period of first feeding, characterized by the shift from endogenous (yolk-sac) to exogenous nutrition sources, is considered critical to the survival of marine fish (Bruno, 2016). Similarly, the larval

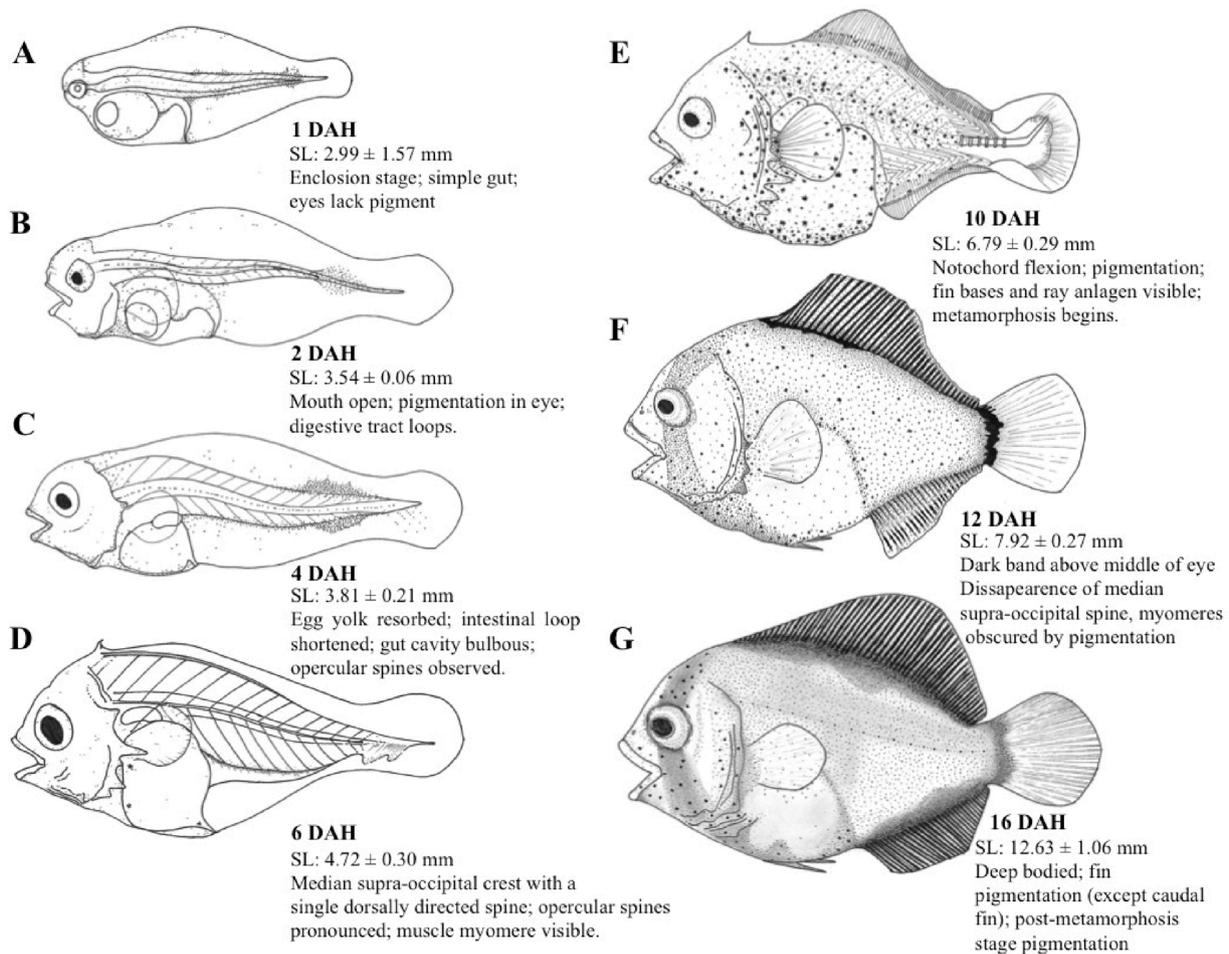


Fig. 2. Development of meristic traits in *Platax orbicularis* larvae during hatchery stages, 1 – 16 Days After Hatching (DAH). **A:** Larvae at enclosion stage, lack pigmentation; **B:** Larval mouth opens and digestive tract loops; **C:** Light pigmentation, egg yolk is resorbed and development of spines is observed on the out ridges of the operculum; **D:** Operculum spines become more pronounced and a median supra-occipital crest marked by a single dorsally directed spine is observed; **E:** Notochord flexion, ray anlagen are observed, pigmentation is more pronounced; **F:** Dark band of pigmentation are present over the eye, the single dorsally directed spine on median supra-occipital crest is no longer visible; **G:** Body is deeper with heavy post-metamorphosis stage pigmentation.

stage, typified by high mortality rates at metamorphosis, is also a substantial obstacle in fish rearing (Reverter et al., 2016; Holzer et al., 2017). Our study highlighted that metamorphosis of *P. orbicularis* is critical to their production in aquaculture. Levels of the thyroid hormone T_3 in *P. orbicularis* reached a peak at 12 DAH (i.e. hormonal metamorphosis). This peak occurred in concert with important morphological changes and increased mortality and growth between 9 and 12 DAH, clearly illustrating the sensitivity of this fish during metamorphosis. There have been recent attempts to culture *Platax teira* (Leu et al., 2018), an important food fish species in Asia, however detailed information on larviculture success is limited. Mortality rates in our study compares well with other current commercial tropical aquaculture food fish species such as snappers (30 % in *Lutjanus analis*, Benetti et al., 2007; 23–31 % in *Lutjanus guttatus*, Burbano et al., 2020) and the more difficult groupers (87–68 % in *Plectropomus leopardus*, Yoseda et al., 2003; 77 % in *Epinephelus bruneus*, Teruya and Yoseda, 2006). A notable example is flatfish larvae which undergo a particularly conspicuous and dramatic metamorphosis in which they completely re-arrange their body morphology as they transition from moving within the water column (i.e. a pelagic habitat) to along the sea floor (i.e. a benthic habitat; Laudet, 2011; McMenamin and Parichy, 2013). Metamorphosis has been a limitation to commercial success, particularly for tropical fish species, due to the fragility of larvae, mouth gape size, suitability of food, high mortality and difficulty in finding the optimal abiotic conditions for appropriate growth and development of fish at this

stage (Dabrowski, 1984). *Platax* species have been highlighted as novel and important to the aquaculture industry with some species currently being cultured in countries such as Taiwan and Thailand for human consumption (Holt et al., 2017; Le Maréchal, 2004; Yu, 2002). However, the literature concerning larval rearing protocols and associated development of Ephemeroptera larval fish is still very limited (Leu et al., 2018).

Morphological development of *P. orbicularis* was observed to be quite similar to that of other Ephemeroptera species such as *Chaetodipterus faber* (Ditty et al., 1993), with early developmental stages exhibiting elongated dorsal and anal fins which proportionally decrease in size with maturity (Kuitert and Debelius, 2001). Cavalluzzi et al. (2004) and Johnson (1984) stated that Ephemeroptera are known to have well developed head spination, which was also observed in this study. In *P. orbicularis*, the development of head spination (i.e. pre-opercular spines, supra-occipital spines) was similar to those observed in *Platax teira* and occurred at similar times (Leu et al., 2018). This type of head spination is also characteristic of other Percoid families such as Carangidae, Lobotididae, and Bramidae species (Cavalluzzi et al., 2004; Ditty et al., 1994; Johnson, 1984; Kinoshita, 2014). Body coloration, especially the band of darker pigmentation extending inferiorly from the base of the median supra-occipital crest towards the middle of the eye and then curving posteriorly to the base of the operculum slightly behind the eye (Fig. 2), is also observed in *P. teira*. However, this dark band becomes more prominent at an earlier stage in *P. orbicularis* (12 DAH) comparative to *P. teira* (17 DAH; Leu et al., 2018). At 11–13 DAH, *P. orbicularis* larvae

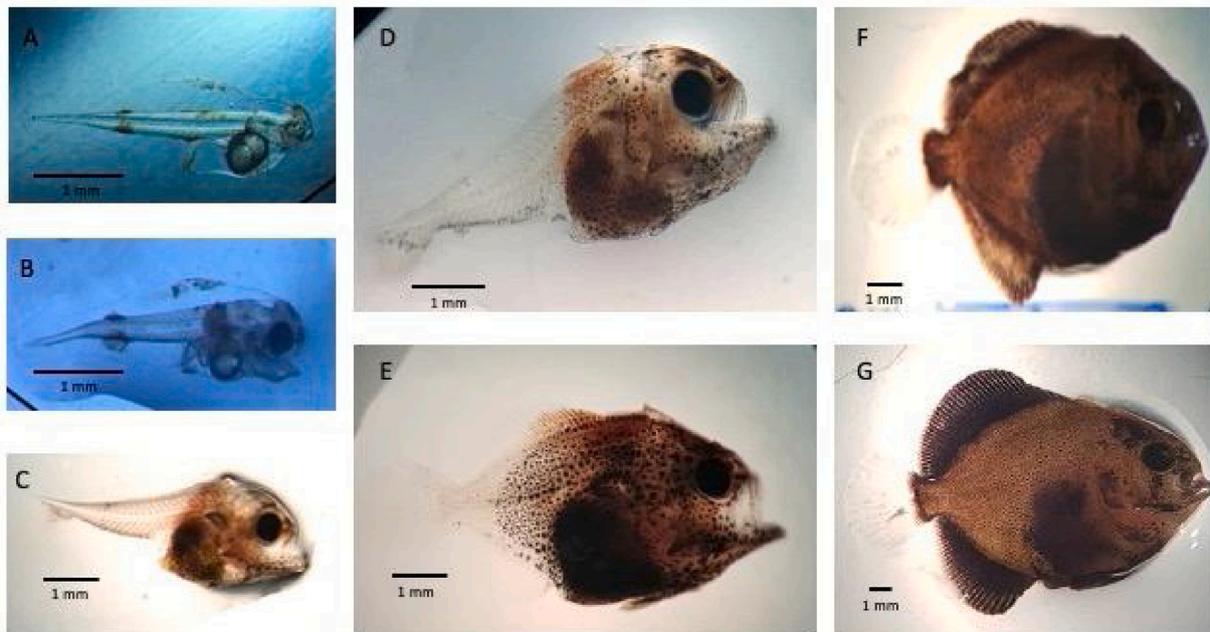


Fig. 3. Development of pigmentation in early larval development of *Platax orbicularis*. (A) Newly hatched larvae (1 DAH), lack pigmentation; (B) 3 DAH pre-flexion larvae, eye pigmented with very light scattering of pigmentation; (C) 5 DAH pre-flexion larvae, scattering of melanophores dominating the ventral side of the larval body extending anteriorly from the gut towards lower jaw; (D) 8 DAH post-flexion larvae, pigmentation extending dorsally but restricted to anterior of larval body with scattering of melanophores over operculum; (E) 10 DAH pre-metamorphosis larvae, melanophores becoming more prominent and extending posteriorly form gut, dorsal fin base and fin ray analogens begin to gain pigmentation; (F) 12 DAH larvae during metamorphosis period, melanophores and pigmentation of larval body, fins and fin rays pigmented except caudal fin which is still transparent extending posteriorly from caudal peduncle, melanophores prominent over larval body with higher concentration of melanophores located above and below eye; (G) 16 DAH post-metamorphosis larvae, pigmentation still lacking in caudal fin despite full body pigmentation, melanophores less pronounced, consolidation of pigment above and below eye creating a band extending inferiorly from the base of the median supra-occipital crest towards the middle of the eye and then curved posteriorly to the base of the operculum slightly behind the eye.

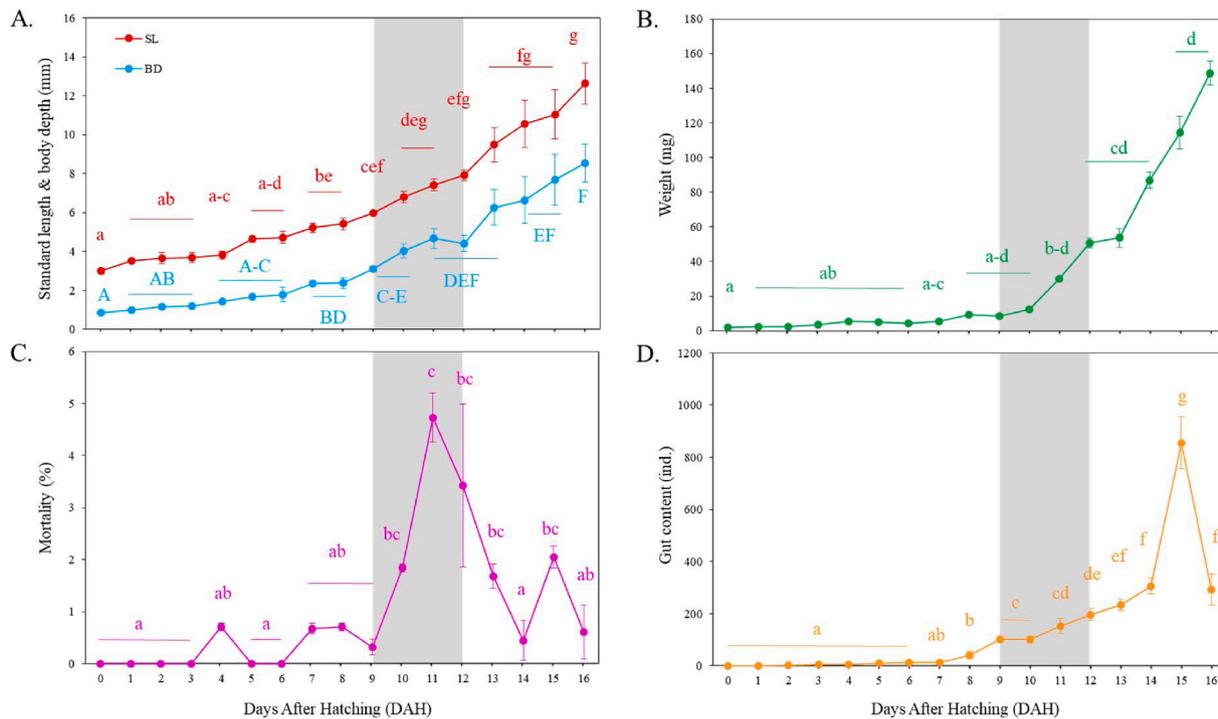


Fig. 4. (A) Standard length (SL) and body depth (BD) (B), weight, (C) daily mortality rate (%) of the remaining live population and (D) gut content counts of *P. orbicularis* larvae at 1 – 16 Days After Hatching (DAH). *Platax orbicularis* larvae were fed on L-type *Brachionus plicatilis* rotifer (1-9 DAH), *Artemia* A1 (10-16 DAH). Values are mean \pm SD. Letters indicate the results of Dunn's post-hoc tests for multiple pairwise comparisons between DAH. In A, lower case: SL; Upper case: BD. Different letters highlight significant differences at $\alpha = 0.05$, shaded areas indicate metamorphosis period.

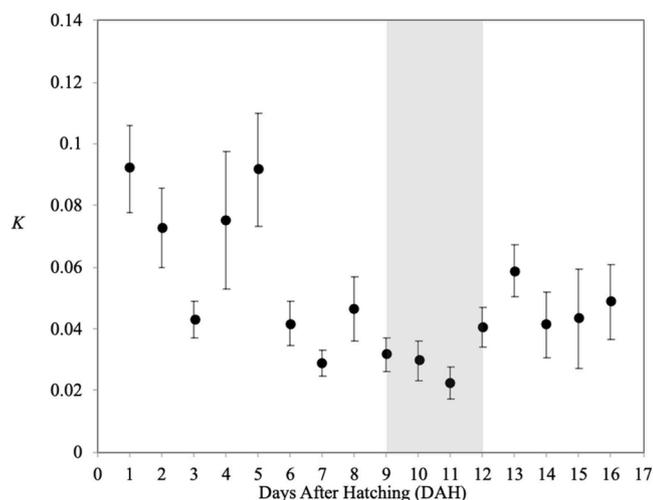


Fig. 5. Fluctuations in average condition factor (K) of *P. orbicularis* larvae sampled. Shaded area indicates metamorphosis period.

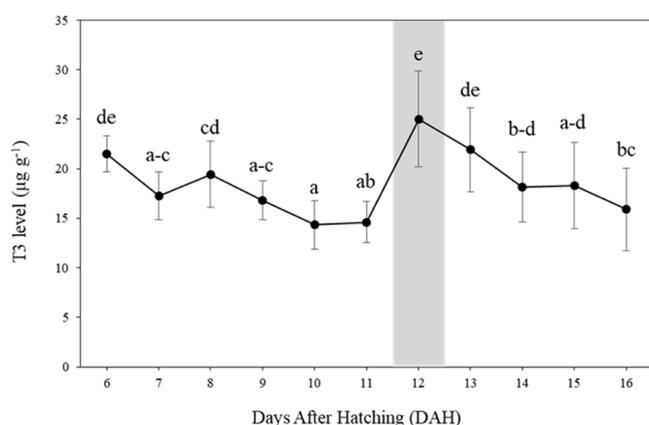


Fig. 6. Thyroid hormone T3 levels during the larval development of *Platax orbicularis*. Values are mean \pm SD ($N = 137$; $n = 12-13$ per DAH, with two technical replicates). Letters indicate the results of linear mixed-effects models followed by Tukey posthoc tests: $a < b$ (p -value = 0.0483); $b < c$ (p -value < 0.001); $c < d$ (p -value = 0.0247) and $d < e$ (p -value < 0.001), shaded area indicates metamorphosis period.

began to display leaf mimetic patterns in body shape and coloration which are also characteristic of Epphipids such as *P. teira*, *C. faber* and *Chaetodipterus zonatus* (Ditty et al., 1993; Leu et al., 2018). Similar mimetic patterns are also observed in other neotropical polycentrid fish such as *Monocirrhus polyacanthus* and *Polycentrus schonburgii*, the lobotid *Lobotes surinamensis*, and other freshwater species such as catfish of the genera *Farlowella* and *Agmus* (Randall and Randall, 1960; Liem, 1970; Lowe-McConnell, 1987; Barros et al., 2015). As is evident in many other reef fish species such as the lizardfish, *Synodus ulae*, and scorpionfish, *Scorpaena neglecta*, mimicry functions to maintain camouflage, thereby facilitating foraging success (Lowe-McConnell, 1987; Godin, 1997). However, findings by Barros et al. (2008) highlight that in *P. orbicularis*, mimicry acts more as an anti-predatory adaptation in the wild.

Larval size of *P. orbicularis* at hatching (SL: 2.99 ± 0.14 mm) was also observed to be similar to that of *P. teira* (TL: 2.57–3.16 mm) but somewhat larger when compared to the Pacific Spade fish, *C. zonatus* (NL: 1.8 mm) as reported by Leu et al. (2018). *P. orbicularis* larvae were observed to be voracious feeders, especially between 8–15 DAH. Upon examination of the gut contents, it was observed that many of the live feed were not fully digested (Fig. 7). Epphipid fish are commonly classified as being omnivorous (Kishimoto et al., 1988; Leu et al., 2018), though



Fig. 7. Undigested and partially digested live feed, *Artemia* observed from the gut content of *P. orbicularis* larvae at 14 DAH.

tending to carnivorous habits (Kuiter and Debelius, 2001; Randall, 2005), although much is still not known about their ecology in both the wild and captivity (Barros et al., 2008). Further studies on the physiological development of the early digestive system, including enzymatic activity assays, are required to better understand larval feeding behavior and digestive capabilities so that appropriate and optimized feeding strategies can be developed.

The condition of the larvae, K , was observed to peak between 3–4 DAH (Fig. 5), which coincides with reabsorption of the yolk sac (Table 1) and initiation of exogenous feeding. Blaxter and Hempel (1963) mentioned that the yolk sac impacts K -values, with increasing length and decreasing yolk sac volume, condition factors will become smaller as observed in the present study. The larvae continue to gradually decrease in condition before reaching its lowest levels at 9–10 DAH (Fig. 5). A possible reason for this decrease is that as larvae continue to grow in length, it did not increase as much in weight (Fig. 4A, 4B). This could be either due to a combination of factors such as suitability of the feed, energy expenditure, development of predatory instincts or a combination of other morphogenetic and physiological responses to metamorphosis, which affect feeding ability (Rosenthal and Hempel, 1970; Blaxter and Staines, 1971). The drastic changes that larvae undergo during metamorphosis can also cause a drop in condition in larvae and is often related to high mortality (Wang and Tzeng, 2000; Bolasina et al., 2006; Waqalevu et al., 2019). Similar behavior was observed in Japanese flounder and other flatfish species during metamorphosis (Bolasina et al., 2006; Waqalevu et al., 2019) and in anadromous salmon and catadromous eels (McCormick and Saunders, 1987; Wang and Tzeng, 2000; Björnsson et al., 2012; McMennamin and Parichy, 2013). Apart from the distinct high and low fluctuations in larval K -values throughout the study period, the development of condition factors in the larvae was generally linear (between 5 and 16 DAH). Similar condition trends have also been observed in Pacific herring, *Clupea harengus pallasi* (v Westernhagen and Rosenthal, 1981) and haddock, *Melanogrammus aeglefinus* (Laurence, 1974). Further studies on understanding the morphogenetic and physiological development of *P. orbicularis* is recommended in order to better understand its impacts on larval allometry and condition for the improvement of larviculture protocols.

Our study incorporated both observations on general morphological and meristic development of *P. orbicularis* as well as an assessment of thyroid hormone levels during the hatchery phase. Despite limited replication, our results suggested that the metamorphosis of

P. orbicularis larvae into post-metamorphic stages would be critical to the viability of their production in aquaculture (Figs. 4,5,6). The highest thyroid hormone T₃ levels coincide with the climax of metamorphosis (i.e. the period during which morphological changes occur most rapidly) (Fig. 4). This profile is similar to those observed in other coral reef fish species (Holzer et al., 2017; Besson et al., 2020), other commercial fish species (Dabrowski, 1984; Bruno, 2016) and in amphibians (Laudet, 2011). Thus, T₃ probably plays a role in the regulation of the developmental processes occurring during *P. opercularis* metamorphosis and a strong mortality occurred between 9 and 12 DAH (Fig. 4). The link between these high mortality rates and the metamorphosis climax is still unclear and further research on potentially important factors linked to thyroid hormone physiology (e.g. iodine uptake and energy expenditure) is yet to be conducted.

Thyroid Hormones (TH) are present in all vertebrates in which they are known to control very different processes: (i) in many amphibians and teleost fish they trigger and coordinate metamorphosis, a post-embryonic life history transition during which a larvae is transformed into a juvenile (Laudet, 2011; Holzer et al., 2017); (ii) in mammals, these hormones are well known regulators of basal metabolism, thermogenesis and heartbeat rate (Singh et al., 2018). It is known in mammals, including human that THs increase energy expenditure as revealed by the increase of oxygen consumption observed after TH treatment; (iii) THs are also a major effectors pathway for seasonality, the process by which species adapt their physiology and reproduction to the annual change in photoperiod (Holzer and Laudet, 2015). These apparent disparate roles are more and more integrated, for example with the observation of the metabolic control of TH in thermal regulation in zebrafish (Little et al., 2013) or on energy expenditure in stickleback (Kitano et al., 2010) but also with the realization that in amniotes too TH is regulating life history transitions (Holzer and Laudet, 2013). This explains why TH regulation is so important during fish metamorphosis. This also suggests that a better understanding of the multiple role of TH during this process will be beneficial to overcome the strong mortality often observed in this critical period. THs are key coordinators of post-embryonic development, allowing its coupling with external conditions and the adjustment of the internal physiology of the organism.

5. Conclusion

Our work is the first study to shed light on the ecological and aquacultural factors orchestrating metamorphosis in the coral reef fish, *P. orbicularis*, as well as the hormonal regulation of this major life history transition (Figs. 4,5,6), there are still many obstacles to aquaculture technologies that need to be addressed. In the present study, we highlighted that peak levels of the thyroid hormone, T₃ in *P. orbicularis* (i.e. hormonal metamorphosis) occurred in concert with important morphological changes, growth and also significant mortality, thus illustrating the sensitivity of this species during metamorphosis. Overall, our study sheds light on the metamorphosis and larval development of a novel aquaculture species, as well as how hormonal and morphological changes relate to larval growth and survival throughout a critical period of its rearing. Our findings provide foundational knowledge that may be useful to overcome current production limitations of *P. orbicularis* in aquaculture and represent major advancement for the future development of rearing technologies of other Ehippid species.

Ethics statement

All procedures followed normal aquaculture practices and the U.K. Animals Act, 1986 and associated guidelines, UE Directive 2010/63/EU for animal experiments. The fish were euthanized by anaesthetic overdose (MS222). These procedures were conducted under the supervision of the Aquaculture DRM Tahiti to ensure fish welfare.

CRedit authorship contribution statement

Viliame Waqalevu: Data curation, Formal analysis, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Marc Besson:** Data curation, Formal analysis, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Camille Gache:** Data curation, Formal analysis, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Natacha Roux:** Conceptualization, Investigation, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Lily Fogg:** Conceptualization, Investigation, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Frédéric Bertucci:** Conceptualization, Investigation, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Marc Metian:** Conceptualization, Investigation, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Marc-André Lafile:** Conceptualization, Investigation, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Moana Maamaatuaiahutapu:** Conceptualization, Supervision. **Eric Gasset:** Conceptualization, Supervision. **Denis Saulnier:** Conceptualization, Supervision. **Vincent Laudet:** Conceptualization, Supervision. **David Lecchini:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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