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Captive breeding and seed production of lyretail anthias *Pseudanthias squamipinnis* (Peters, 1885) in a recirculating aquaculture system (RAS)

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Abstract

Lyretail anthias, *Pseudanthias squamipinnis* (Peters, 1885), belongs to the subfamily Anthiinae (Family:Serranidae), is a highly priced (US$ 30) coral reef fish distributed in the Indo-West Pacific region. The present study investigates and describes the captive maturation, volitional spawning, larval rearing, and seed production of the *P. squamipinnis,* for the first time globally. Broodstock maintained in 2 t recirculating aquaculture system (RAS) spawned naturally without any hormonal induction. Fertilised eggs were planktonic, spherical, non-adhesive, transparent, and measured between 596 to 615 µm in size and had a single oil globule (152.14±4.24 µm). A green water medium consisting of a combination of microalgae was used for larval rearing. Eggs were maintained at 35 ppt salinity and 28±1°C temperature, which hatched out within 15-16 h after spawning. Newly hatched larvae were planktonic and measured 1.43±0.09 mm in total length (TL). The yolk sac was completely absorbed by 3 days post-hatch (dph). The preflexion stage was observed till 14 dph, and flexion began by 15 dph, which was completed by 20 dph. Between 30-40 dph, the larvae metamorphosed into juveniles with adult orange-yellow colour. Larvae were reared using copepod nauplii (*Parvocalanus crassirostris*) as the first feed, and in later stages, they were fed with copepods, Artemia and micro-encapsulated feed. The present study provides an improved RAS-based broodstock development protocol that enables the simultaneous rearing of several pairs of the same species or different species. It also provides an improved larval rearing and feeding schedule, resulting in improved survival rates compared with previously reported works.

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Introduction

The annual global trade value of live tropical marine ornamental fishes is approximately 1.5 billion US$, and the trade includes around 4000 coral reef fish species. The world's most significant marine ornamental fish exports are from tropical and subtropical countries. The trade has negatively impacted ecosystems and coral reef systems (Olivotto *et al*., 2003) as around 90% of the world's commonly traded marine reef ornamental fishes are wild-caught (Wabnitz *et al*., 2003; Olivotto *et al*., 2011; Palmtag, 2017). Hatchery-produced fishes are available in the market only for 30-35 species (Biondo, 2017). Aquaculture can prevent over-harvesting, save critically endangered species, and produce new varieties of domesticated fish well adapted to captive conditions (Teletchea, 2016). Captive broodstock development, breeding, rearing, and seed production of commonly traded marine ornamental fishes are essential for their conservation and sustainable utilisation. These measures help to protect the species from further depletion and to reduce pressure on wild populations (Majories *et al*., 2018). However, only a few reports are available that document the breeding and culture of commonly traded aquarium reef fish (Olivotto *et al*., 2005; Moorhead and Zeng, 2010; Anil *et al*., 2012; Madhu and Madhu, 2014; Madhu *et al*., 2016; Anil *et al*., 2018; Rohini Krishna *et al.,* 2018, 2019; Anzeer *et al.,* 2019; Raheem *et al*., 2021).

Fish belonging to the genus *Pseudanthias* are small plankton-feeding reef fishes that inhabit rocky coral reef habitats and are characterised by schooling behaviour, sexual dichromatism and protogynous hermaphroditism (Randall and Pyle, 2001; Parenti and Randall, 2020). They are known for their haremic reproductive behaviour and a colonial system consisting of distinctively coloured single large territorial males and females of adults and sub-adults (Bray, 2020). The dominant male defends its territory and associated females. Nineteen species of the genus *Pseudanthias* have been described from the south-west coast of India, including *P. squamipinnis* (Heemstra and Akhilesh, 2012).

P. squamipinnis commands a high value in the ornamental fish trade, fetching around 20 US$ per individual in the Indian market and up to 30 US$ in the international market. *P. squamipinnis* is one of the most traded ornamental fishes in the Red Sea (Froukh, 2007) and one of the top ten ornamental species imported by the European Union (Wabnitz *et al*., 2003). They are pelagic spawners found along the tropical and subtropical Indo-west Pacific region (Allsop and West, 2003; Heemstra and Akhilesh, 2012). Like other *Pseudanthias* fishes, the *P. squamipinnis* is also a sexually dichromatic/dimorphic protogynous hermaphrodite. The maximum size of the male and female reported is 15 cm and 7 cm, respectively, in Australian waters (Bray, 2020). Attempts on breeding and larval rearing of anthias fishes are very few, except the publication on successful broodstock production, breeding and larval rearing of Marcia anthias, *Pseudanthias marcia* (Anil *et al.,* 2018), and embryology and larval development studies of the same species (Gomathi *et al*., 2020). This study describes the broodstock maturation, spawning behaviour, embryonic development, larval rearing and seed production of the high valued *P. squamipinnis* for the first time.

Materials and methods

Broodstock development and spawning

Broodstock was developed using 11 fishes comprising 2 males (total length 7.55 ±0.77cm; size range 7 - 8.1 cm): and 9 females (total length 6.72±0.50 cm; size range of 6.3 to 7.7 cm) of *P. squamipinnis* procured from an aquarium shop. After a quarantine period of two weeks, the active, healthy, and disease-free juvenile fishes were transferred to a 2 t FRP tank connected to a recirculating aquaculture system (RAS). This broodstock RAS tank is part of a multi-tank system provided with a common filtration designed for broodstock development of ornamental fishes (Fig. 1).

The system consists of five 2 t tanks, a settling tank, a biological filter, a reservoir tank, a pump, a protein skimmer, and a UV filter. Each broodstock tank is a dual-drain RAS; each having a central drain and an overflow conduit or side drain. The overflow drain is provided with a device for egg collection, which is designed to collect the pelagic eggs flowing out of the overflow drain in filter cloth (kept immersed in water) while the excess water flows out. The water which is pumped after the filtration goes into each tank and gets circulated. That is, the amount of water coming in goes out either through the central drain or the side drain. The central drain valve regulates the percentage of water going out through the central drain and the side drain. From the central drain, water goes to the settling tank, where the waste gets settled. The water overflowing from the settling tank will then pass through the biological filter unit and then to the reservoir. Water which goes out through the side drain goes directly to the reservoir tank. The one-hp pump sucks water from

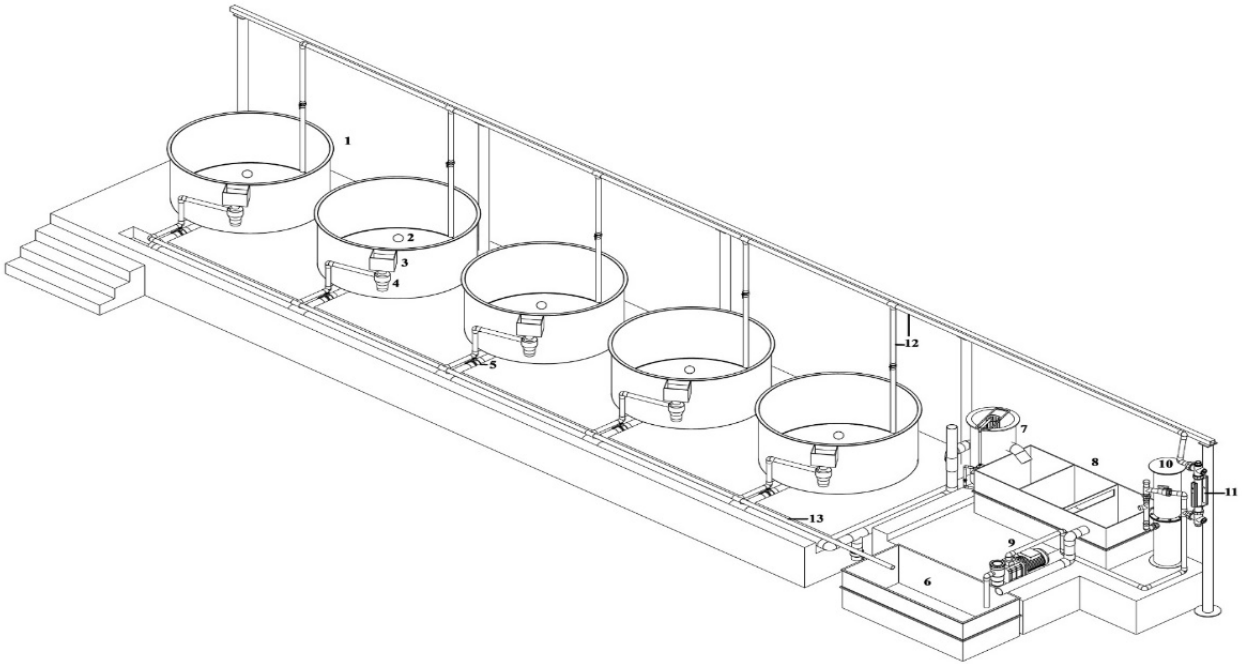

Fig. 1. Multi tank single filtration RAS: 1: Tank; 2: Central drain; 3: Side drain; 4: Egg collector; 5: Valve; 6: Reservoir; 7: Settling tank; 8: Biological filter; 9: Pump' 10: Foam fractionation unit; 11: UV unit; 12: Water distribution line; 13: Drain pipe

the reservoir, and part of the water goes to the protein skimmer; which goes back to the reservoir after the fractionation process. The other part of the delivery from the pump gets distributed to all five broodstock tanks after passing through a UV filter. Water quality parameters of the broodstock system, such as water temperature, salinity, pH, ammonia and nitrate, were checked at regular intervals and recorded. Natural photoperiod of 12 h L: 12 h D was maintained throughout the culture period.

Fishes were fed *ad libitum* with chopped squid meat and compounded semi-moist feed (protein-40%, lipid - 9.5%, fibre - 2% and moisture - 31%) at 10.00 and 15.00 hrs, respectively. 1-2 % of the water is changed daily, along with any waste settled at the bottom. Further, 30-40% water was exchanged once in a week to once in a month depending on the algal development on the sides of the tank after scrubbing. Fishes were monitored daily to record behavioural changes in both males and females. Once fish started spawning, regular monitoring was carried out to record the spawning time, fecundity, spawning behaviour, and frequency of spawning.

Sex change in captivity

A separate experiment was conducted to study the sex change of *P. squamipinnis* in captivity. Eight females with a total weight of 2.5 to 2.8 g and a total length of 5.7 to 7 cm were reared in a tank, without males, after taking proper quarantine measures. The fish were fed twice daily, as mentioned above. Fishes were monitored regularly to record the differences in morphology, changes in colour patterns and behaviour.

Live feed culture

P. squamipinnis larval rearing was carried out using copepod and artemia nauplii as larval live feeds. Mass culture of copepod species such as *Parvocalanus crassirostris, Pseudodiaptomus serricaudatus* were carried out separately using microalgal species *Nannochloropsis salina* and *Isochrysis galbana* as feed in FRP tanks of 300 l capacity with seawater of salinity 32-35 ppt and temperature 29±1° C, pH-7.6-8.0. Cleaning and washing of copepod culture tanks of each species were done once a week to maintain the culture and they were filtered, washed, and added to the larval rearing tanks (LRT).

Freshly hatched Artemia nauplii, within 8 h post-hatch (hph) (OSI brand, USA), were fed to the larvae from day 15 onwards. Eight hours of post- hatch (hph) Artemia was enriched for about 6-8 h using Ori-green (Skretting, France) enrichment medium and was used for feeding advanced stages (from 25th dph).

Spawning, egg collection and stocking

Fertilised eggs at optic vesicle stage of embryonic development were collected from the water coming out of the overflow conduit of the broodstock tank by keeping 200 µm mesh below the drainpipe. Collected eggs were washed and counted volumetrically to assess the percentage of fertilised eggs. The fertilised eggs were floating and transparent. After counting, eggs were treated with iodophor (10 ppm for about 5 min) and stocked (@10 eggs l^{-1}) in pre-set, yellow-coloured larval rearing tanks of 500 l capacity filled with chlorinated, filtered and UV-treated seawater of 35 ppt and provided with mild aeration.

Larval rearing

Larval rearing trials were continued in 500 l FRP tanks in triplicates with pre-treated seawater of 35 ppt salinity. Newly hatched larvae were cultured in green water medium. The water quality parameters of the larval rearing tanks (LRTs) were maintained at optimum level and monitored on alternate days till metamorphosis. To estimate the hatching rate, 500 numbers of floating eggs were stocked in 1 l of seawater with mild aeration till hatching. The next day, larvae were counted to calculate the percentage of hatching. The hatching % was obtained by the following formula:

Hatching % = (No. of larvae/No. of fertilised eggs stocked) x 100

Embryonic development

To study embryonic development, 300-350 fertilised eggs were collected directly from the RAS tank immediately after the spawning and incubated in a 1000 ml glass beaker filled with filtered (2 µm), UV-treated seawater of 35 ppt salinity and maintained at 28±1°C. Subsamples (10-15 nos.) of eggs were observed under a Leica S8APO stereo zoom-microscope at 5 min intervals to record the time taken to reach different developmental stages, *i.e.* from fertilised egg to cleavage period (2, 4, 8, 16, 32, 64, 128 cell stages), dome, gastrulation (25% epiboly, 50% epiboly, 75% epiboly, 100% epiboly), optic vesicle stage, heart formation, motility and hatching.

Larval development and growth

To study the larval development, growth, and critical stages, larvae were randomly collected from LRT on 1, 3, 5, 10, 15, 20, 25, 35, and 40 dph, photographed and measured using Zeiss Primostar Axiocam 105 colour (till 3 dph), followed by the measurement of larval length till 25 dph using Leica S8APO stereo zoom microscope. Larvae were anaesthetised using MS222 before taking photographs. Metamorphosed larvae and juveniles were photographed using a digital camera (Canon EOS 5D Mark IV). All the data were represented in mean±standard deviation (SD).

Results

Broodstock management and breeding

In *P. squamipinnis,* males were larger than females (Fig. 2 and 3). Among the two males in the broodstock, the larger male with bright purple colour acted as the dominant one and showed courtship behaviour such as swimming around the females and exhibited darting movements and this was observed after 6 months of rearing. Thus, in the present study, one dominant male controlled 9 females in the adult and sub-adult stages.

Water quality parameters of the broodstock system during the breeding period were as follows; temperature: 29±1°C, salinity: 33–35 ppt, pH: 7.6–8.0, total ammonia: 0.35–0.51 mg l^{-1}, dissolved oxygen (DO): 4.7–5.6 mg l^{-1}, alkalinity: 100–130 mg l^{-1} CaCO3, turbidity: 0.16–0.39 NTU, nitrate (NO3): 0.3–0.7 mg l^{-1}, nitrite (NO2): 0.01–0.03 mg l^{-1} and photoperiod: 12 h L: 12 h D.

Fig. 2. *Pseudanthias squamipinnis* male

Fig.3. *Pseudanthias squamipinnis* female

Sex change in captivity

Sex change was noticed after 50 to 60 days of rearing when the tank was stocked exclusively with females. From the stocked females, the largest one (the one which had 7.2 cm total length at the time of stocking) started showing male characteristics such as an elongated third dorsal fin and a flexible pectoral fin with a red spot at the tip (Fig. 4). Complete sex reversal was observed after 3-4 months of rearing.

Volitional spawning and stocking

In the RAS broodstock tank, the fish were allowed to spawn naturally without any hormonal induction. Natural spawning without any hormonal intervention was observed in the evening hours between 18.00 to 20.00 hrs. The total number of eggs spawned per day ranged between 200 to 5000 (2512.11±1730.7), and the rate of fertilisation was 50-60%. Fertilised eggs were pelagic, spherical, non-adhesive, and transparent, measuring 596-615 µm in diameter (605.08±8.03 µm) and had a single oil globule (152.14±4.24 µm). Eleven hours post fertilisation (hpf), the eggs attained the optic vesicle stage of embryonic development and were collected from the broodstock tank and transferred to larval rearing tanks (LRT) for further rearing (10 eggs l^{-1}). Hatching was observed after 15-16 hpf at 28±1ºC. A hatching rate of 78 to 85% was observed in different rearing trials of *P. squamipinnis*.

Embryonic development

The sequence of embryonic development of *P. squamipinnis* from fertilised egg to till hatching is described in Table 1 and Fig. 5. The cleavage started 15 min after fertilisation (2 cell stage) and reached blastula stage/128 cell stage by 1 hr 22 min. Gastrulation began 3 h 52 min of hpf (25% epiboly) and reached 100% epiboly after 7 h10 min. Optic vesicle stage, heartbeat and hatching were observed at 11h 20 min, 13 h 50 min and 15 h 20 min hpf, respectively.

Larval rearing

P. squamipinnis larvae were raised using a green water medium with a combination of microalgae (*N. salina* and *I. galbana* with a cell density of 2.0- 2.26 × 10^6 ml^{-1}), and it continued till 25 dph. For larval rearing, mild aeration with a static water system of rearing was followed. On 0 dph along with eggs, the tanks were stocked

Fig. 4. *P. squamipinnis* sex reversal in progress

Table. 1. Sequence of embryonic development of *P. squamipinnis* from fertilised eggs till hatching

Developmental stages	Time from post-fertilisation	Features
Fertilised egg	0	Planktonic, spherical, non-adhesive and transparent eggs with single oil globule
2 cells	15 m	First cleavage resulted in forming 2 blastomeres of equal size
4 cells	20 m	Second cleavage occurred perpendicular to the first axis and form 4 blastomere
8 cells	28 m	Third cleavage resulted in 8 blastomere formation
16 cells	35 m	Forth cleavage resulted in formation of 16 blastomeres.
32 cells	45 m	Size of blastomeres reduced
64 cells	1 h	Formation of morula stage with ball-shaped blastodisc
128 cells/ blastula	1 h 22 m	Blastula stage with flowery appearance of balstomeres.
Dome	3 h 35 m	Transitional stage from blastula to gastrula
25% epiboly	3 h 52 m	Begins the gastrulation. 25% yolk enveloped with blastomeres
50% epiboly	5 h 5 m	50% yolk enveloped with blastomeres
75% epiboly	6 h	75% yolk enveloped with blastomeres
100% epiboly	7 h 10 m	100% yolk enveloped with blastomeres.
Somite	8 h 55 m	Somites formation begins from the middle portion of the embryo
Optic vesicle	11 h 20 m	Pair of optic vesicle formation at the cephalic end
Heartbeat	13 h 50 m	Rudimentary heart-formed, and heartbeat observed. Pair of placoid otic vesicle formed
Motility	14 h 40 m	Twitching and jerking movements were observed. Vigorous jerking movement in the later stages made the embryo detached from the egg case.
Hatching	15 h 20 m	Hatchling is transparent, with a heavy yolk.

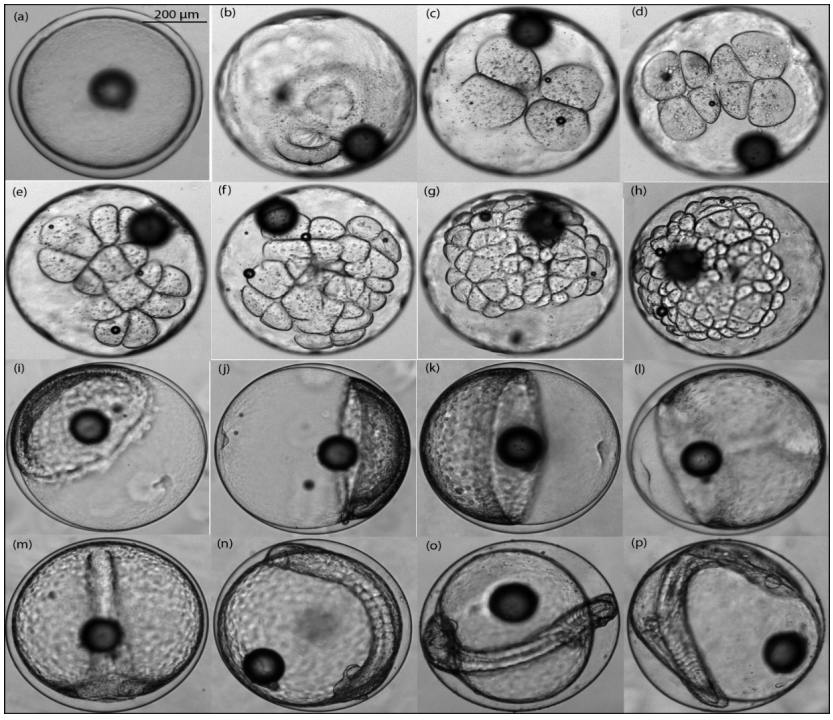

Fig. 5. Embryonic development of *P. squamipinnis*: (a) Zygote; (b) 2 cell; (c) 4 cell; (d) 8 cell; (e) 16 cell; (f) 32 cell; (g) 64 cell; (h) 128 cell (blastula); (i) dome; (j) 25% epiboly; (k) 50% epiboly; (l) 75% epiboly; (m) 100% epiboly; (n) somite stage; (o) optic vesicle stage; (p) motility stage

with the calanoid copepod, *P. crassirostris* adults at the rate of 0.3 to 0.7 nos. ml^{-1} to ensure availability of nauplii when the larvae start feeding. The nauplii size of this species is smaller (55-65 µm in length and 35-45 µm in width). From 7 dph onwards, larvae were supplemented with another species of copepod *Pseudodiatomus serricaudatus* (0.4-0.5 nos. ml^{-1} which has a larger nauplii size, 115 µm in length and 90 µm in width.

Larvae were fed with copepods till 25 dph. Meanwhile from 15 dph onwards larvae were fed with newly hatched artemia nauplii along with copepods. Co-feeding (copepod and artemia) was continued till 25 dph. From 15 to 40 dph, larvae were fed with artemia nauplii and enriched artemia. Weaning of juvenile with microencapsulated feed (skretting) began on 40 dph with 300 µm size and subsequently with 500 µm on 50 dph. Details of larval feeding and water management are given in Table 2.

Table 2. Details of larval feeding and water management

Days post hatch (dph)	0	1	2	3	4	5	6	7	8	9	10	12	15	20	25	30	35	40	45	50
N. salina & *I. galbana* (1:1) (average total cell density: 2-2.26x10^6)																				
Parvocalanus crassirostris (0.3 to 0.7 nos. ml^{-1})																				
Parvocalanus srricaudatus (0.4 to 0.5 nos. ml^{-1})																				
Artemia nauplii (1-2 nos. ml^{-1})																				
Enriched artemia (1-2 nos. ml^{-1})																				
Artificial feed (300-500 µ)																				
Water management																				
Water exchange 20-30%																				
Water exchange-40-50%																				

Larval development and growth

Eggs of *P. squamipinnis* hatched out within 15-16 h after spawning at a water temperature of 28±1°C. Newly hatched larvae were planktonic, measuring 1.43±0.09 mm in TL, with yolk-sac (1027.64±6.28 µm) and single oil globule (152.14±4.24 µm). Yolk sac length and oil globule diameter of the larvae from 0 to 3 dph is given in Table 3.

Table 3. Yolk sac length and oil globule diameter of *P. squamipinnis* larvae

Days of post hatch (dph)	Yolk sac length (µ)	Oil globule diameter (µm)
Hatchling	1027.64±6.28	152.14±4.24
1st dph	432.38±14.52	72.4±8.50
2nd dph	141.96±1.53	55.91±2.48
3rd dph	Exhausted	Exhausted

Mouth and gut of the newly hatched larvae were not developed, and eye pigmentation was not evident. On 1 dph, larvae measured 1.99±0.036 mm and had non-pigmented eyes and primordial fin fold extending the larvae's whole trunk. On 2 dph, the larvae measured 2.01±0.052 mm in length and developed mild eye pigmentation and had anus and pectoral fin bud. By 3 dph, yolk sac absorption was completed, and the mouth (size 85-105 µm) opened and had a heavily pigmented retina and well-developed pectoral fin. The first feeding began by 3-4 dph. There was a greenish tinge indicating the presence of algae in the gut on 3rd dph.

The shell of copepod nauplii was found in the gut of sampled larvae on 4 dph. On 5dph, larvae measured 2.65±0.22 mm and had a well-developed colourful pectoral fin and extended anus with the formation of melanophore at the post-anal fin fold. On 10 dph, larvae (3.21±0.18 mm) had the typical serranid shape with a big head and opercular spination. Additionally, the larvae also had an elongated filamentous third spine. By 15 dph, the larvae measured 5.11±0.28 mm and had fin folds changing into spiny and soft dorsal, anal, and pelvic fins.

In *P. squamipinnis,* notochord flexion began at 13 dph with caudal fins ray formation and was completed by 25 dph. The larval metamorphosis began by 25 dph with the development of intense greenish-yellow colour mottled with black spots on the head region and orange-red in the opercular region. However, the larval body was transparent. Pelvic fins were elongated and filamentous. Resorption of the opercular spine and dorsal fin elongation was completed by 25 dph, but larvae were still planktonic. Later during the advanced stages, they developed a deep and transparent body, well-formed fins, a mottled head region with black spots and the opercular region was orange- red.

By 30 dph, the larval body changed from transparent to orange in red colour, squamation was completed, and the larvae measured 17.63±1.52 mm. On 40 dph, all larvae completed metamorphosis with the development of the adult colour of orange red. Between 30 and 40 dph, along with the metamorphosis, the larva began to move from surface to bottom frequently. Hideouts (cut and cleaned PVC pipes - 3 to 4 nos.) were kept in the tank by 35 dph, and the larvae were observed to hide inside these pipes most of the time, except during feeding time. The larvae were fed with enriched artemia till metamorphosis and the larvae attained a size of 23.10±2.81 mm.

Bottom waste was siphoned from 12 to 35 dph at 4-5 days intervals, and 20-30% of the water was exchanged. After 35 dph it was increased to 50-60 %. Larval development of *P. squamipinnis* from hatchling to 30 dph and 40 dph juvenile is given in the Fig 6 and 7. Summary of the growth of larvae from hatchling to 50 dph is graphically represented in Fig. 8. The final survival obtained at 50 dph was around 18.3±1.5.

Discussion

Serranid fishes are highly valued as food (which includes several species of groupers) and ornamental fish, with several reports on their spawning in captivity. Tucker (1994) reported the volitional spawning of 23 serranid fish species and induced spawning of at least 31 species in captivity; most of them being groupers. Some serranid fishes bred under captivity without hormonal induction are *Epinephelus akaara* (Ukawa *et al*., 1966; Chen *et al*., 2003); *E. tauvina* (Hussain and Higuchi, 1980); *E. fuscoguttatus* (Lim *et al*., 1993); *E. polyphekadion* (James *et al.,* 1997), and Marcia's anthias *P. marcia* (Anil *et al*., 2018).

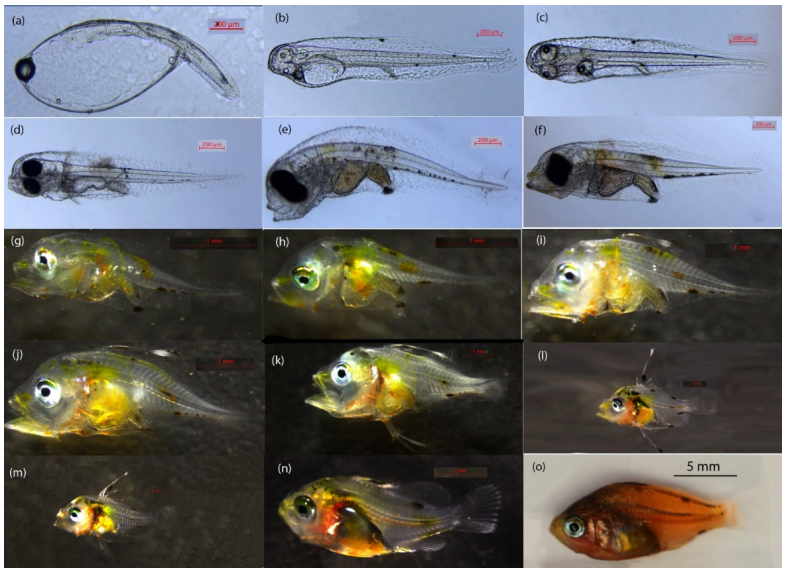

Fig. 6. Larval development of *P. squamipinnis* from hatchling to 30 dph; (a) Hatchling; (b) 1 dph larva; (c) 2 dph larva; (d) 3 dph larva; (e) 4 dph larva; (f) 5 dph larva; (g) 6 dph larva; (h) 10 dph larva; (i) 11 dph larva; (j) 13 dph larva; (k) 15 dph larve; (l) 17 dph larva; (m) 20 dph larva; (n) 25 dph larva; (o) 30 dph larva

Fig. 7. 40 dph juvenile of *P. squamipinnis*

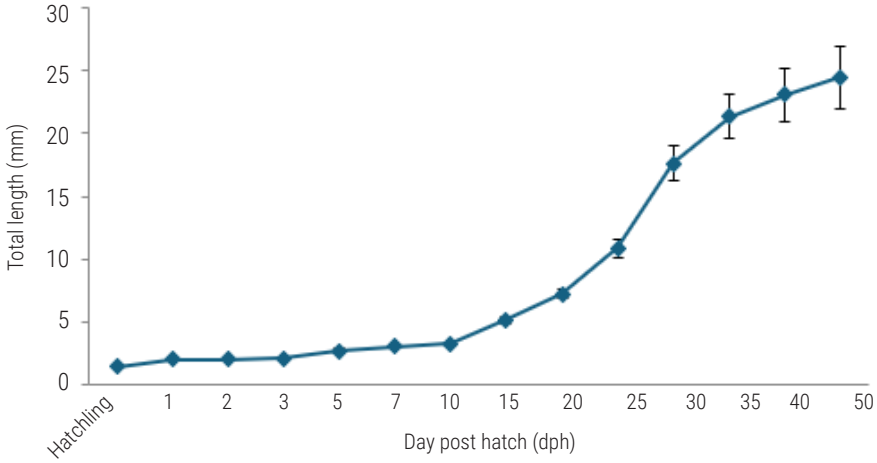

Fig. 8. Larval growths from hatchling to 50 dph

Fishes kept under proper husbandry practices, and appropriate environmental conditions like ambient temperature, natural light and uncrowded conditions undergo gonadal maturation and spawn naturally in captivity (Olivatto *et al*., 2003). Tucker (1999) reported that the groupers spawn voluntarily in captivity when they are well-fed and kept uncrowded during the natural spawning season under conditions of ambient temperature and partial or total natural light. He also reported that at least 27 serranid species had spawned voluntarily in captivity. Maintenance of brooders with good quality feed and optimum environmental conditions in RAS enhanced the gonadal maturation and led to volitional spawning of food fish and ornamental fish such as *P. marcia (*Anil *et al*., 2018)*, Lethrinus lentjan* (Anil *et al.,* 2019) and *L. nebulosus* (Anil *et al.,* 2021). Similarly, *P. squamipinnis* spawned volitionally without any hormonal application during the present study by providing suitable conditions.

Sex reversal in protogynous fish involves a change in colouration, gonadal function, and behaviour in females (Shapiro, 1981). Heemstra and Akhilesh (2012) in their review of the anthiinae-fish genus *Pseudanthias* reported that the sex reversal (female to functional male) could be accomplished in a week or two, but that might be influenced by the number and behaviour of other females in the colony. In captivity, sex change in *P. squamipinnis* was associated with the elongation of the third flexible dorsal fin and the development of a red spot at the tip of the pectoral fin in females reared for 50-60 days without males. Complete male characters were observed after 3-4 months of rearing.

Fishes from the genus *Pseudanthias* have the basic colour differences between sexes and males often show enhanced variations of their colour patterns associated with courtship and spawning: and the largest male fish defends its territory and its females (Heemstra and Akhilesh, 2012). Similarly, in the present study also, during the spawning season, largest territorial male had developed a bright intense pinkish red colour and controlled 5-10 adult and sub-adult females. It is opined that the members of the family Serranidae have smaller eggs compared to other fishes (Tucker Jr., 1991; Watanabe *et al.*, 1995). The egg of camouflage grouper, *E. polyphekadion* has an average size of 757.3 µm (James *et al*., 1997); *E. microdon* has an egg size range of 769–832 µm (Tamaru *et al*.,1996). While studying the embryonic and larval development of *P. marcia,* Gomathi *et al*. (2020) reported an average egg size of 617.9±14.9 µm and opined that it could be the smallest egg among serranids. But the present study revealed that the egg size of *P. squamipinnis* is 605.08±8.03 µm, which is smaller than that of *P. marcia*.

The sequence of embryonic developmental stages from fertilised egg to cleavage period (2, 4, 8, 16, 32, 64, 128 cell stages), dome, gastrulation (25% epiboly, 50% epiboly, 75% epiboly, 100% epiboly), optic vesicle stage, heart formation, motility and hatching were similar to the basic developmental events reported in most of the teleost fishes. The duration of stages was similar to that of *P. marcia,* and the hatching took 15 h 20 min at 28±1ºC in *P. squamipinnis,* whereas in *P. marcia* it was 14 h 30 min at 29º C (Anil *et al.,* 2018).

The hatchling size of *P. squamipinnis* was 1.428±0.094 mm in TL but was found to be smaller than many groupers species: gold blotch grouper *E. costae*, 1.76±0.048 mm (Glamuzina *et al.,* 2000); dusky grouper, *E. marginatus*, 1.52±0.07 mm (Glamuzina *et al*., 1998); Malabar grouper, *E. malabaricus* 1.71±0.16 to 1.84±0.10 (Yoseda *et al.,* 2006); brown spotted grouper, *E. tauvina*, 2.25 mm (Nazar and Hussain, 1980). In *P. marcia* notochord flexion stage was completed on 25 dph. The larval metamorphosis began by 25 dph and was completed around 50 dph with adult colouration in *P. squamipinnis*.

Doi *et al.* (1997) reported that marine fish larvae are characterised by small mouth size. Tucker (1999) also reported that the larvae of most grouper species are small and fragile and have small mouths at the time of first feeding. Similarly, larva of *P. squamipinnis* was also tiny and fragile and had a mouth size of 85-105 µm. Species with small mouths need small zooplankton such as small rotifers, trochophores (oyster or clam larvae) or copepods at first feeding (Tucker, 1999). Tucker (1999) opined that growth and survival tend to be better in grouper larvae, if copepods or mixed zooplankton are included in the diet. Similarly in the present study also, two species of calanoid copepod, *P. crassirostris* which has smaller naupliar size was used as the first feed and in later stage (after 7 dph) *P. serricaudatus* with larger nauplii size was used.

Development of an elongated second dorsal and pelvic spine on 14 dph is a characteristic feature of grouper larvae as reported in the case of *E. septemfasciatus* (Kitajima *et al*., 1991) and *E. lanceolatus* (Garcia-Ortega *et al*., 2012). Similar observation was made in *P. squamipinnis* between 11 to 20 dph, and the larvae acquired its final fin shape after 20 dph with a transparent body. Larval metamorphosis of *P. squamipinnis* began at 25 dph with the intense colour formation on the head and was completed by 50 dph with adult colouration. In giant grouper, *E. lanceolatus* metamorphosis began by 25 dph and was completed by 38 dph (García Ortega *et al*., 2012).

As reported in the case of Marcia's anthias*, P. squamipinnis* also had three critical periods during their larviculture *i.e*. during the first feeding stage (3 to 5 dph), between 15 to 20 dph, and finally between 20 to 30 dph. In the first critical period, larvae had about 60% survival. A survival rate between 38-48% was observed in the second critical period, while in the final critical stage, the survival was about 16 to 18%. The first critical period is during the first feeding phase of the larvae, where the factors like live feed size, mouth size, algal density, and water movement can directly influence larval survival. The second critical period was observed during the flexion stage, opercular and head spination. The third critical period coincided with the resorption of spination and initiation of metamorphosis. Moorhead and Zeng (2010) and Olivotto *et al.* (2017) are of the view that there is a possibility of having several critical stages before the fish reaches commercial production. Utmost care is to be taken regarding the water quality, dissolved oxygen, ammonia content, and live feed size and density according to the larval stages to maximise survival. The excess live feed can lead to the accumulation of ammonia and waste products, whereas low live feed concentration can lead to poor nutrition and reduced growth, in addition to more energy requirements to search for food. RAS or flow-through with a mild flow rate might improve the water quality and, therefore, survival.

The reported survival rate of *P. marcia* (larvae to juvenile) was 5.2±1.07% only, whereas in the present study, we could achieve a better survival rate of 18.3±1.5% for *P. squamipinnis*. This could be attributed to the successful first feeding of larvae by ensuring the sufficient number of copepods nauplii available when they began to

feed. In order to ensure a sufficient number of nauplii, on 0 dph itself, the tank was stocked with a sufficient number of copepod adults *i. e.* 0.3 to 0.7 nos ml^{-1} and ensured good algal density in the tank to keep the copepod adults well nourished. Stomach content analysis also revealed the presence of copepod naupliar appendages in the gut. Tucker (1999) opined that the larval period of groupers is long (35 to 70 days), and it requires live food longer than most marine fish that have been reared. In *P. squamipinnis* also, live feeds were provided up to 40 dph with the right amount of copepod and artemia.

The results of the present study indicate that *P. squamipinnis* can be taken as a candidate species for commercial ornamental aquaculture, thereby increasing the number of species bred from the sub-family Anthiinae of the family Serranidae which has more than 52 species described with ornamental importance (Randall and Pyle, 2001). Further, it could also help in preventing the overexploitation of coral reef fish communities. The author's previous study on Marcia's anthias (Anil *et al.*, 2018) reported 100-120 days for the fish to reach marketable size, whereas, in the present study, *P. squamipinnis* reached marketable size within 70-80 days, thereby substantially reducing the cost of production. However, further investigations of the critical stages could lead to a better understanding of the reasons for mortality which can be used to improve larval survival. Developing hatchery technologies would help reduce the pressure on the natural population in addition to helping to create additional income for the coastal community.

The present study demonstrated the successful captive spawning and larval rearing of the anthias species *P. squamipinnis* with improved survival rate compared to the previous studies. Spawning was achieved in RAS without hormonal induction. Copepod nauplii of *P. crassirostris* were used as the first feed, and in later stages, larvae were weaned directly to Artemia without use of rotifer. The larvae had three critical stages that significantly affected the survival rates. Further studies using RAS and manipulation of feeding schedules improved the survival rate, leading to the mass scale production of anthias species.

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References

Allsop, D. J. and West, S. A. 2003. Constant relative age and size at sex change for sequentially hermaphroditic fish. *J. Evol. Biol.*, 16(5): 921-929. https://doi.org/10.1046/j.1420-9101.2003.00590.x.

Anil, M. K., Gomathi, P., Ambarish, P. G., Surya, S., Siju, R. and Raju, B. 2021. Successful breeding of green snapper *Lethrinus nebulosus*. *CMFRI Newsletter*, 171: 16-17.

Anil, M. K., Gomathi, P., Raheem, P. K., Raju, B., Philipose, K. K. and Gopalakrishnan, A. 2018. Captive broodstock development, breeding and seed production of Anthid fish (family: Serranidae), Marcia's anthias, *Pseudanthias marcia* in recirculation aquaculture system (RAS). *Aquaculture*, 492: 265-272. https://doi.org/10.1016/j.aquaculture.2018.03.043.

Anil, M. K., Gomathi, P., Sugi, V. V., Raheem, P. K., Raju, B., Ambarish, P. G. and Gopalakrishnan, A. 2019. Captive maturation, breeding and seed production of pink ear emperor, *Lethrinus lentjan* (Lacepede, 1802) (Family: Lethrinidae) in recirculating aquaculture system (RAS). *Aquaculture*, 503: 207-216. https://doi.org/10.1016/j.aquaculture .2018.12.084.

Anil, M. K., Santhosh, B., Prasad, B. O. and George, R. M. 2012. Broodstock development and breeding of black-finned anemone fish *Amphiprion nigripes* Regan, 1908 under captive conditions. *Indian J. Fish.*, 59(1): 77-82.

Anzeer, F. M., Aneesh, K. S., Abraham, M. V., Darsana, S., Santhosh, B., Anil, M. K. and Yoyak, G. 2019. Breeding, early development and larval rearing of cloudy damsel, *Dascyllus carneus* Fischer, 1885. *Aquaculture*, 505: 374-385. https://doi.org/10.1016/j.aquaculture.2019.03.001.

Biondo, M. V. 2017. Quantifying the trade in marine ornamental fishes into Switzerland and estimation of imports from the European Union. *Glob. Ecol. Conserv.*, 11: 95-105. https://doi.org/10.1016/j.gecco.2017.05.006.

Bray, D. J. 2020. *Pseudanthias squamipinnis in fishes of Australia* (Accessed 15 Nov 2021).

Chen, Z. S. L. and Ye, X. S. H. 2003. Establishment of a pluripotent embryonic cell line from sea perch (*Lateolabrax japonicus*) embryos. *Aquaculture.*, 218(1-4): 141-151. https://doi.org/10. 1016/S0044-8486(02)00570-7.

Doi, M., Ohno, A., Taki, Y., Singhagraiwan, T. and Kohno, H. 1997. Nauplii of the calanoid copepod, *Acartia sinjiensis* as an initial food organism for larval red snapper, *Lutjanus argentimaculatus*. *Aquac. Sci.*, 45(1): 31-40. https://doi.org/10.11233/aquaculturesci 1953.45.31.

Froukh, T. 2007. *Genetic population structure, gene flow, and evolutionary history of selected ornamental fish in the Red Sea*. Ph. D. Thesis, Universität Bremen, Germany.

García-Ortega, A., Daw, A. and Hopkins, K. 2012. Feeding hatchery-produced larvae of the giant grouper *Epinephelus lanceolatus*. In: *Proceedings of the 40th US–Japan Aquaculture Panel Symposium: Hatchery technology for high quality juvenile production*, US Department of Commerce, Washington, DC, USA, pp. 37-44.

Glamuzina, B., Glavic, N., Tutman, P., Kozul, V. and Skaramuca, B. 2000. Egg and early larval development of laboratory reared gold blotch grouper, *Epinephelus costae* (Steindachner, 1878) (Pisces, Serranidae). *Sci. Mar.*, 64: 341-345. https://doi.org/10.3389/scimar. 2000.64n3341.

Glamuzina, B., Skaramuca, B., Glavic, N. and Kozul, V. 1998. Preliminary studies on reproduction and early life stages in rearing trials with dusky grouper, *Epinephelus marginatus* (Lowe, 1834). *Aquac. Res.*, 29: 769-771. https://doi.org/10.1046/j.1365-2109.1998.29100769.x.

Gomathi, P., Anil, M. K., Raheem, P. K., Raj, P. N., Krishna, M. V. R., Ambarish, P. G. and Surya, S. 2020. Egg and larval development of serranid fish Marcia's anthias, *Pseudanthias marcia* (Subfamily: Anthiinae) spawned and reared under captive condition. *Thalassas*, 36(1): 23-31. https://doi.org/10.1007/s41208-020-00193-0.

Heemstra, P. C. and Akhilesh, K. V. 2012. Review of the anthiine fish genus *Pseudanthias* (Perciformes: Serranidae) of the western Indian Ocean, with description of a new species and a key to the species. *Aqua Int. J. Ichthyol.*, 18(3): 121-164.

Hussain, N. A. and Higuchi, M. 1980. Larval rearing and development of the brown-spotted grouper, *Epinephelus tauvina* (Forskal). *Aquaculture*, 19: 339-350. https://doi.org/10.1016 /0044-8486(80)90082-4.

James, C. M., Al-Thobaiti, S. A., Raseem, B. M. and Carlos, M. H. 1997. Breeding and larval rearing of the camouflage grouper *Epinephelus polyphekadion* (Bleeker), in the hypersaline waters of the Red Sea coast of Saudi Arabia. *Aquac. Res.*, 28: 671-681. https://doi.org/10.1046/j.1365-2109.1997.00908.x.

Kitajima, C., Takaya, M., Tsukashima, Y. and Arakawa, T. 1991. Development of eggs, larvae and juveniles of the grouper, *Epinephelus septemfasciatus*,

reared in the laboratory. *Jpn. J. Ichthyol.*, 38(1): 47-55. https://doi.org/10.11369/jji1950.38.47.

Lim, L. C. 1993. Larviculture of the greasy grouper *Epinephelus tauvina* and brown-marbled grouper *E. fuscoguttatus* in Singapore. *J. World Aquac. Soc.*, 24: 262-274. https://doi.org/ 10.1111/j.1749-7345.1993.tb00062.x.

Madhu, K. and Madhu, R. 2014. Captive spawning and embryonic development of marine ornamental purple firefish *Nemateleotris decora* (Randall and Allen, 1973). *Aquaculture*, 424: 1-9. https://doi.org/10.1016/j.aquaculture.2013.12.027.

Madhu, K., Madhu, R. and Retheesh, T. 2016. Spawning, embryonic development and larval culture of redhead dottyback *Pseudochromis dilectus* Lubbock, 1976 under captivity. *Aquaculture*, 459: 73-83. https://doi.org/10.1016/j.aquaculture.2016.03.017.

Majoris, J. E., Francisco, F. A., Atema, J. and Buston, P. M. 2018. Reproduction, early development and larval rearing strategies for two sponge-dwelling neon gobies, *Elacatinus lori* and *E. colini*. *Aquaculture*, 483: 286–295. https://doi.org/10.1016/j.aquaculture. 2017.10.028.

Moorhead, J. A. and Zeng, C. 2010. Development of captive breeding techniques for marine ornamental fish: A review. *Rev. Fish. Sci.*, 18(4): 315–343. https://doi.org/10.1080/ 10641262.2010.516035.

Nazar, A. and Hussain, H. 1980. Larval rearing and development of the brown spotted grouper, *Epinephelus tauvina* (Forskal). *Maric. Fish. Dept. Kuwait Inst. Sci. Res., Aquaculture*, 19: 339–350. https://doi.org/10.1016/0044-8486(80)90082-4.

Olivotto, I., Cardinali, M., Barbaresi, L., Maradonna, F. and Carnevali, O. 2003. Coral reef fish breeding: The secrets of each species. *Aquaculture*, 224(1–4): 69–78. https://doi.org/10. 1016/S0044-8486(03)00207-2.

Olivotto, I., Leu, M. Y. and Blázquez, M. 2017. Life cycles in marine ornamental species - Fishes as a case study. In: Calado, R., Olivotto, I., Oliver, M. P. and Holt, G. J. (Eds.), *Marine ornamental species aquaculture*. Wiley, pp. 23-49. https://doi.org/10.1002/ 9781119169147.ch3.

Olivotto, I., Planas, M., Simões, N., Holt, G. J., Avella, M. A. and Calado, R. 2011. Advances in breeding and rearing marine ornamentals. *J. World Aquac. Soc.*, 42(2): 135-166. https://doi.org/10.1111/j.1749-7345.2011.00453.x.

Olivotto, I., Zenobi, A., Rollo, A., Migliarini, B., Avella, M. and Carnevali, O. 2005. Breeding, rearing and feeding studies in the cleaner goby *Gobiosoma evelynae*. *Aquaculture*, 250(1-2): 175-182. https://doi.org/10.1016/j.aquaculture.2005.02.057.

Palmtag, M. R. 2017. The marine ornamental species trade. In: Calado, R., Olivotto, I., Oliver, M. P. and Holt, G. J. (Eds.), *Marine ornamental species aquaculture*. Wiley, pp. 3-14. https://doi.org/10.1002/9781119169147.ch1.

Parenti, P. and Randall, J. E. 2020. An annotated checklist of the fishes of the family Serranidae of the world with description of two new related families of fishes. *FishTaxa*, 15: 1-170.

Raheem, P. K., Rohini Krishna, M. V., Surya, S., Gomathi, P., Ambarish, P. G., Santhosh, B., Raju, B. and Anil, M. K. 2021. Breeding, larval rearing and growth of black *Amphiprion ocellaris* (Cuvier, 1830) under captivity. *Indian J. Fish.*, 68(2): 60-69. .

Randall, J. E. and Pyle, R. L. 2001. Four new serranid fishes of the anthiine genus *Pseudanthias* from the South Pacific. *Raffles Bull. Zool.*, 49(1): 19-34.

Rohini Krishna, M. V., Anil, M. K. and Neethu Raj, P. 2018. Larval development and growth of red saddleback anemonefish, *Amphiprion ephippium* (Bloch, 1790) under captive conditions. *Indian J. Mar. Sci.*, 47: 2421-2428.

Rohini Krishna, M. V., Anil, M. K., Gomathi, P., Raheem, P. K. and Pushparajan, N. R. 2019. Studies on the broodstock production and larval rearing of coral demoiselle *Neopomacentrus nemurus* (Bleeker, 1857). *Aquac. Res.*, 50(2): 412-422. https://doi.org/10.1111/are.13898.

Shapiro, D. Y. 1981. Sequence of coloration changes during sex reversal in the tropical marine fish *Anthias squamipinnis* (Peters). *Bull. Mar. Sci.*, 31(2): 383-398.

Tamaru, C. S., Carlstromtrick, C., Fitzgerald, W. J. and Ako, H. 1996. Induced final maturation and spawning of the marbled grouper, *Epinephelus microdon* captured from spawning aggregations in the Republic of Palau, Micronesia. *J. World Aquac. Soc.*, 27(4): 363-372.

Teletchea, F. 2016. Domestication level of the most popular aquarium fish species: is the aquarium trade dependent on wild populations? *Cybium*, 40(1): 21-29.

Tucker, J. W. Jr. 1994. Spawning by captive serranid fishes: A review. *J. World Aquac. Soc.*, 25(3): 345-359. https://doi.org/10.1111/j.1749-7345.1994.tb00218.

Tucker, J. W. Jr., Parsons, J. E., Ebanks, G. C. and Bush, P. G. 1991. Induced spawning of Nassau grouper *Epinephelus striatus*. *J. World Aquac. Soc.*, 22: 187-191.

Tucker, J. W. Jr. 1999. Species profile grouper aquaculture. *SRAC Publication* No. 721.

Ukawa, M., Higuchi, M. and Mito, S. 1966. Spawning habits and early life history of a serranid fish, *Epinephelus akaara* (Temminck and Schlegel). *Jpn. J. Ichthyol.*, 13: 156-161. https://doi.org/10.11369/jji1950.13.156.

Wabnitz, C., Taylor, M., Green, E. and Razak, T. 2003. *From ocean to aquarium*. UNEP–WCMC, Cambridge, UK.

Watanabe, W. O., Ellis, S. C., Ellis, E. P., Head, W. D., Kelley, C. D., Moriwake, A., Lee, C. S. and Bianfang, P. K. 1995. Progress in controlled breeding of Nassau grouper (*Epinephelus striatus*) broodstock by hormone induction. *Aquaculture*, 138: 205-219. https://doi.org/ 10.1016/0044-8486(95)01057-2.

Yoseda, K., Dan, S., Sugaya, T., Yokogi, K., Tanaka, M. and Tawada, S. 2006. Effects of temperature and delayed initial feeding on the growth of Malabar grouper (*Epinephelus malabaricus*) larvae. *Aquaculture*, 256: 192-200. https://doi.org/10.1016/ j.aquaculture.2006.01.031.