# 東南アジア産淡水フグPao属3種の飼育下での繁殖


| | |
|---|---|
| 誌名 | 水産増殖 |
| ISSN | 03714217 |
| 著者名 | 土井,啓行<br>百田,和幸<br>小畑,洋<br>酒井,治己 |
| 発行元 | 水産増殖談話会 |
| 巻/号 | 70巻3号 |
| 巻号補足 | |
| 掲載ページ | p. 221-229 |
| 発行年月 | 2022年10月 |

*Aquacult. Sci.* **70**(3), 221–229 (2022)

# Reproduction in captivity of three Southeast Asian freshwater pufferfish species of the genus *Pao*

Hiroyuki Doi[1], Kazuyuki Momota[1], Hiroshi Obata[1] and Harumi Sakai[2,*]

**Abstract:** To advance breeding techniques for freshwater pufferfishes, *Pao abei*, *P. baileyi* and *P. suvattii* reproduction in captivity was undertaken, and compared with published reproductive characteristics of *P. cochinchinensis*, *P. palembangensis* and *P. turgidus*. All six species breed in freshwater under stable water temperature (25–27°C) and lighting (L12D12) conditions, with an acceptable spawning bed, such as an earthenware pipe or sunken driftwood with a flat surface suitable for establishing and guarding eggs in a single layer. A pair of individuals of each species spawned a number of times over two to seven month periods, a single egg batch containing hundreds of 2 or 3 mm diameter eggs. Hatching occurred in 7–10 days, larvae initially being fed with *Artemia* larvae. The data collected should contribute to successful breeding of the genus in captivity.

**Key words:** *Pao*; Freshwater pufferfish; Artificial reproduction; Aquarium fish

The pufferfish family Tetraodontidae, one of the most derived actinopterygian families, characterized by a variety of specializations, including poisonous flesh and internal organs, an inflatable body, four fused beak-like teeth, and osteological reductions, inhabits deep sea to freshwater environments (e.g., Miya et al. 2003; Yamanoue et al. 2008; Matsuura 2015; Nelson et al. 2016; Sakai 2021). The family comprises about 30 genera and 200 species (Matsuura 2015; Nelson et al. 2016), including about seven freshwater genera (40 species) (Kottelat 2013; Matsuura, 2015). Mitogenomic analyses (Yamanoue et al. 2011) revealed that the freshwater genera had independently invaded freshwater ecosystems in South America (genus *Colomesus*), Central Africa (genus *Tetraodon*), and Southeast Asia [the most speciose freshwater pufferfish region (Kottelat 2013; Matsuura, 2015)] (genera *Auriglobus*, *Carinotetraodon*, *Dichotomyctere*, *Leiodon*, and *Pao*).

A number of freshwater pufferfish species are included in the aquarium trade (Ebert 2001) or, in some cases, used for food, despite their toxicity (Japan Wildlife Research Center 2013). Although many aquarium fishes are cultured for trading purposes in Southeast Asia (Ng and Tan 1997; Raja et al. 2014), most freshwater pufferfish trade specimens are wild caught (Ebert 2001), except in rare cases, such as reported by Subamia et al. (2008). Therefore, development of culture techniques applicable to freshwater pufferfishes is essential, not only for trade purposes but also for conservation of natural biodiversity (Ng and Tan 1997; Livengood and Chapman 2007; Momota et al. 2022; Doi et al. 2022).

*Pao*, established by Kottelat (2013), is the most speciose Southeast Asian freshwater pufferfish genus, comprising 13 or 14 species (Kottelat 2013; Matsuura, 2015) (previously included in *Tetraodon*), many of which are included in the aquarium trade (Ebert 2001). A few reproduction trials for aquaculture purposes have been conducted for *P. palembangensis* (as *Tetraodon*, Subamia et al. 2008), and for the aquarium trade for *P. cochinchinensis*, *P. palembangensis*

Received 11 January 2022; Accepted 7 July 2022.
[1] Osaka Aquarium NIFREL, 2-1 Senri-banpaku-koen, Osaka 565-0826, Japan.
[2] Department of Applied Aquabiology, National Fisheries University, Shimonoseki, Yamaguchi 759-6595, Japan.
*Corresponding author: Tel, (+81) 83-286-5111 (ext. 542); Fax, (+81) 83-286-2292; E-mail, sakaih@fish-u.ac.jp (H. Sakai).

and *P. turgidus* (as *Tetraodon*, Doi et al. 2015). Recently, Doi et al. (2022) documented more detailed reproductive features of *P. palembangensis*.

Reproduction in captivity of *P. abei*, *P. baileyi* and *P. suvattii* is reported here in order to advance pufferfish breeding techniques and to clarify the reproductive characteristics of the genus comparing with other freshwater puffers including *Pao* species reported by Doi et al. (2015, 2022) and Momota et al. (2022).

## Materials and Methods

*Parental fishes and rearing*

Parental fishes, rearing conditions and spawning data are summarized in Table 1. *Pao abei* (Fig. 1a), *P. baileyi* (Fig. 1b) and *P. suvattii* (Fig. 1c), all wild-caught in Thailand, were purchased from a Japanese fish trader (Rio Co. Ltd., Tokyo) on 3 March 2015, 9 May 2018 and 14 November 2015, respectively, and one pair each of *P. abei* and *P. baileyi*, and one pair with three juvenile individuals of *P. suvattii*, maintained in closed circulation/filtering aquaria (95 *l*, 95 *l* and 327 *l* capacity, respectively), at Osaka Aquarium NIFREL. One-third of the rearing water was replaced with new water once a week. Water temperature was maintained at 25.0–27.0°C without any special pH control, with a daily light period from 07:00–19:00.

Spawning beds, comprising a concrete block with a hole (183 mm wide × 76 mm height × 178 mm depth), an earthenware pipe with a hole (70 mm wide × 40 mm height × 100 mm depth), and sunken driftwood (550 mm long × 200 mm wide), were established for *P. abei* (Fig. 1a), *P. baileyi* (Fig. 1b), and *P. suvattii* (Fig. 1c), respectively. Small pieces of sunken driftwood were also placed on the bare floor of the aquaria containing *P. abei* and *P. baileyi* to provide shelter, and on the sand-covered floor of the *P. suvattii* aquarium, together with the water plant *Aubias barteri*.

Shrimp meat (*Macrobrachium* spp. and *Lucensosergia lucens*), fish meat (*Oncorhynchus mykiss*), and commercially prepared catfish food (Kyorin Food Industries, Himeji) were provided as nutrient once a day.

*Observations*

The aquaria were checked at 8:30 AM and 5:00 PM every day. Spawning behavior of *P. suvattii* on 19 May 2016 was recorded with a

**Table 1.** Spawning data for three freshwater pufferfishes, *Pao abei*, *P. baileyi* and *P. suvattii*, in captivity

| | *P. abei* | *P. baileyi* | *P. suvattii* |
|---|---|---|---|
| Establishment date | Mar. 3, 2017 | May 9, 2018 | Nov. 14, 2015 |
| Aquarium volume (*l*) | 95 | 95 | 327 |
| Water temperature (°C) | 26 | 26 | 25-27 |
| pH | 6.7–7.5 | 7.0–7.6 | 7.2–7.5 |
| Lighting period | L12D12 | L12D12 | L12D12 |
| Shelter or spawning bed | Concrete block | Earthware pipe | Sunken driftwood |
| Size of spawning female | 133 (SL mm) | 98 (SL mm) | 143 (SL mm) |
| Size of spawning male | 106 (SL mm) | 92 (SL mm) | 133 (SL mm) |
| Spawning date | Sep. 14 ~ Oct. 19, 2017 | Oct. 25 ~ Nov. 14, 2018<br>Sep. 24 ~ Nov. 22, 2019 | Feb. 28 ~ Aug. 2, 2016 |
| Number of spawning occasions | 5 | 2 in 2018, 5 in 2019 | 11 |
| Spawning date for following observations | Oct. 3, 2017 | Nov. 14, 2018 | May 19, 2016 |
| Approximate number of eggs | 63 | 117 | 590 |
| Days until hatching | 8 | 10 | 10 |
| Egg diameter (mm) | 2.26 ± 0.04 (n=5) | 2.74 ± 0.09 (n=3) | 2.41 ± 0.41 (n=3) |
| SL of hatched larvae (mm) | 4.50 ± 0.20 (n=3) | 4.94 ± 0.07 (n=3) | 4.65 ± 0.14 (n=3) |
| TL of hatched larvae (mm) | 4.88 ± 0.11 (n=3) | 5.27 ± 0.05 (n=3) | 4.87 ± 0.18 (n=3) |

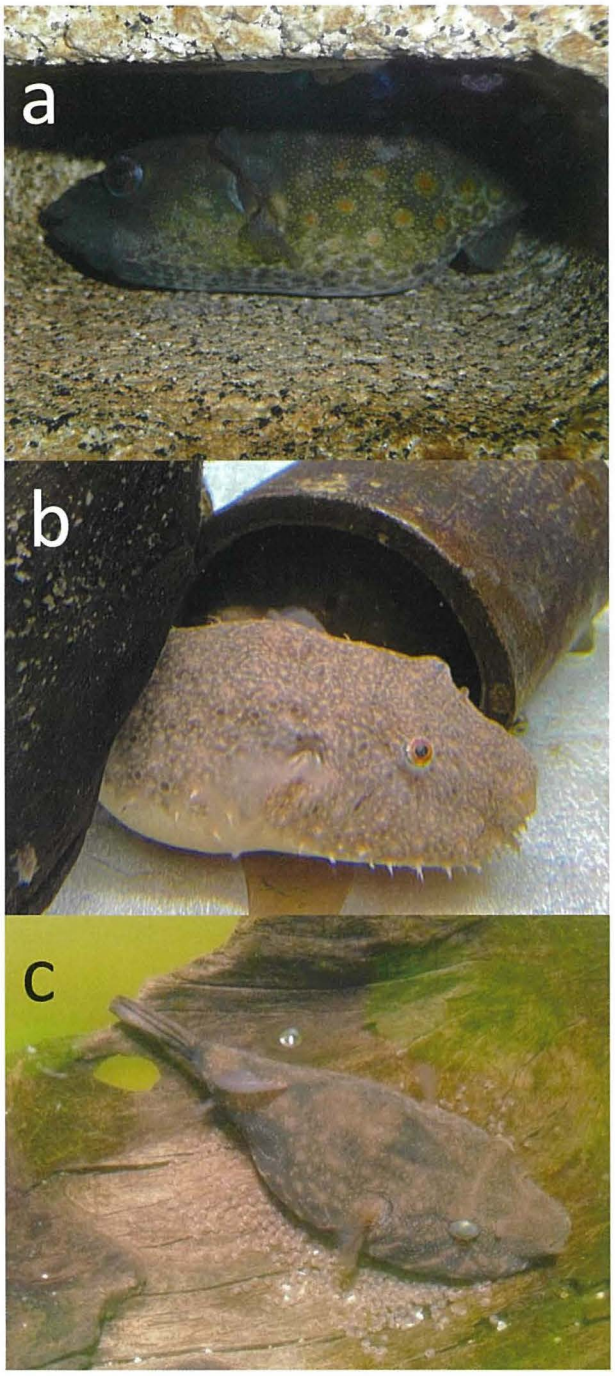


**Fig. 1.** Three freshwater pufferfish of the genus *Pao*; a, *P. abei*, male (106 mm SL) guarding egg batch deposited in a concrete block hole; b, *P. baileyi*, female (98 mm SL) in an earthenware pipe, guarded by male (92 mm SL); c, *P. suvattii*, male (133 mm SL) guarding egg batch on sunken driftwood.

digital video camera, other photos being taken with a digital camera.

Each egg batch spawned was removed from the guarding male one or two days before hatching, and the eggs collected with a glass pipette, counted and measured in a glass Petri dish.

Eggs and larvae were observed and measured using an OLYMPUS SZ61binocular stereomicroscope (equipped with measuring software Anyty Microscope with 3R-WDKMCO2, 3R System) (Olympus Co., Tokyo), following anesthetization with FA100 (DS Pharma Animal Health, Osaka). Standard length (SL, notochord length of early stage and body length of later stage larvae) and total length (TL) were measured.

*Rearing of eggs and larvae*

Antibacterial treatment with 1 ppm methylene blue solution was conducted for the eggs when removed from the guarding male. Subsequently, both collected eggs and hatched larvae were reared in 5 or 10 *l* plastic tanks set in a water bath (26℃). One-third of the tank water was changed, and excrement removed daily.

From two days after hatching, fish larvae were fed once or twice a day, initially with *Artemia* larvae, but with chironomid larvae added from 34 days (for *P. abei*), from 25 days (for *P. baileyi*), and from 33 days (*P. suvattii*). Chironomid larvae only were supplied from 49 days for *P. abei*, and from 90 days for *P. suvattii*., with commercially prepared compound feed for juvenile catfish (Kyorin Food Industries, Himeji) added from 120 days for *P. abei* and *P. suvattii*.

*Growth*

In order to compare early growth, growth regression equations between TL ($y$, in mm) and days after hatching ($x$, till about 100 days) for each species were calculated by simple regression analysis.

## Results

Spawning related data are summarized in Table 1, parental fishes on their nests shown in Fig. 1, and eggs, larvae and juveniles shown in Fig. 2. Growth till 100 days for *P. abei*, 43 days for *P. baileyi*, and 96 days for *P. suvattii* are illustrated in Fig. 3, and the growth regression equations are shown in Table 2.

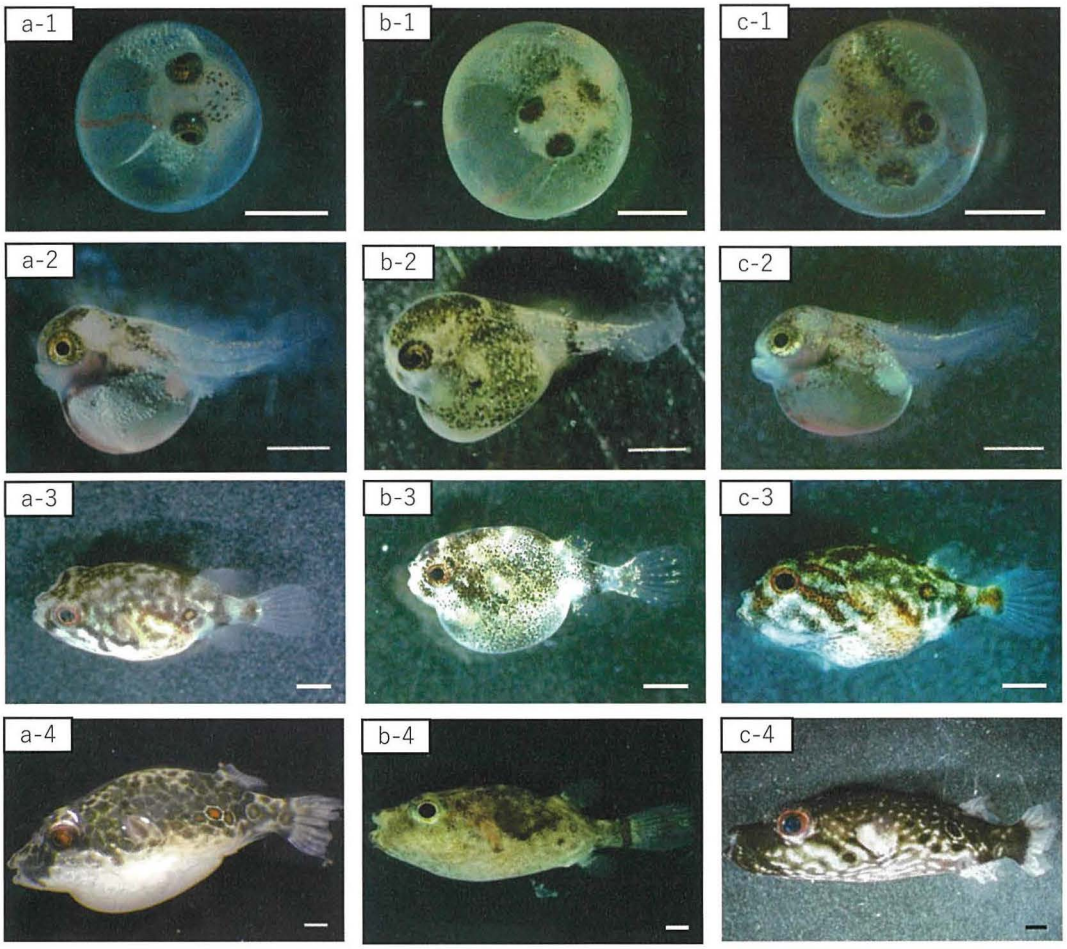


**Fig. 2.** Eggs, hatched larvae, juveniles and young of three freshwater pufferfish of the genus *Pao*: *P. abei* (a-1, egg 2.26 mm mean diameter; a-2, hatched larva 4.88 mm mean TL; a-3, juvenile 7.86 mm mean TL; a-4, young 14.8 mm mean TL), *P. baileyi* (b-1, 2.74; b-2, 5.27; b-3, 6.31; b-4,14.1). *P. suvattii* (c-1, 2.41; c-2, 4.87; c-3, 7.23; c-4, 15.5). Scale bars indicate 1 mm.

*Spawning*

*Pao abei.* The same pair spawned five times between 14th November 2016 and 19th October 2017, all in daytime. Batch sizes ranged from 49–268 eggs, excluding those eaten by the male.

Adult fish usually remained motionless next to the driftwood. Three days before spawning, red spots on the head, back and sides of the male became apparent, the remainder of the body turning darker. In contrast, the body of the female became paler. The male occupied the concrete block hole initially, subsequently being joined by the female, the two rubbing bodies with each other in the hole. Spawning behavior was not observed. However, the female departed after spawning and the male remained to protect the egg batch (Fig. 1a).

*Pao baileyi.* The same pair spawned on 25th October and 14th November 2018, and five times between 24th September and 22nd November 2019, all in daytime. Batch sizes, counted four times (once in 2018 and three times in 2019), ranged from 117–427 eggs, excluding those eaten by the male.

Adult fish often stayed between twigs on the sunken driftwood, sometimes remaining upside down under the overhanging wood, its color being similar to that of the latter. About 10 days before spawning, the pair approached each other near the earthenware pipe, their bodies swelling intermittently and becoming paler. The female then entered the hole, the male remaining near the entrance, although sometimes entering and exiting the hole. Each time the male's body became swollen,

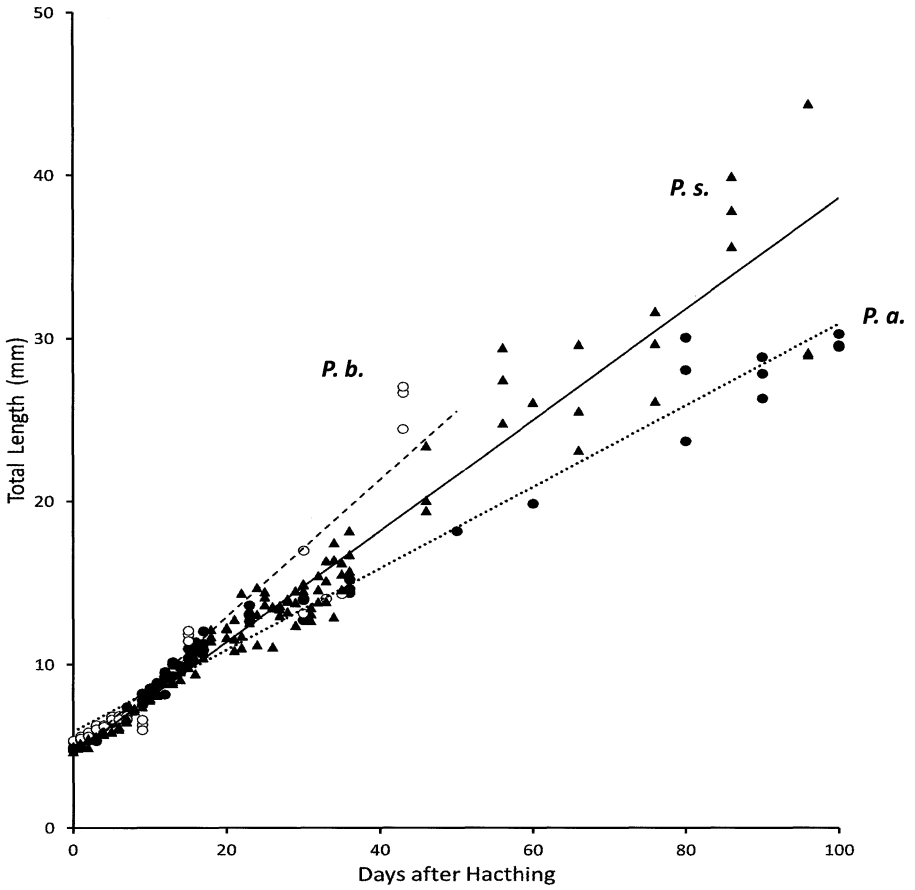


**Fig. 3.** Growth of three freshwater pufferfish of the genus *Pao* spawned in captivity: *P. abei* (*P. a.*, dotted line, solid circles), *P. baileyi* (*P. b.*, dasshed line, open circles), *P. suvattii* (*P. s.*, solid line, solid triangles). Corresponding regression equations shown in Table 2.

**Table 2.** Regression equations between total length ($y$, in mm) and days after hatching ($x$, till about 100 days) for three freshwater puffer species of the genus *Pao* spawned in captivity

| Species | Regression equation | $r$ | $p$ | $n$ |
|---|---|---|---|---|
| *P. abei* | $y = 0.250x + 5.895$ | 0.988 | <0.01 | 69 |
| *P. baileyi* | $y = 0.421x + 4.460$ | 0.953 | <0.01 | 38 |
| *P. suvattii* | $y = 0.340x + 4.572$ | 0.971 | <0.01 | 129 |

$r$, correlation coefficient; $p$, correlation test probability; $n$, number of data

the female's body also swelled accordingly (Fig. 1b). From three days before spawning, the pair stayed in the hole, although actual spawning was not observed. The female departed after spawning and the male remained to protect the egg batch.

*Pao suvattii*. The same pair spawned 11 times between 28th February and 2nd August 2016, once in the evening and the remainder in the afternoon. Batch sizes, counted four times, ranged from 113–590 eggs, excluding those eaten by the male.

Adult fish sometimes hid in the sand, only the eyes, mouth, gill opening and back remaining exposed. Three days before spawning, the pair was observed on the driftwood, the body of the male becoming darker and obscuring the usual marking pattern, in contrast to the female, in which the body became paler and the marking pattern clearer. The pair rubbed bodies with each other on the driftwood about one day before spawning, the male continuing to follow or draw close to the female as she

swam around, stretching or squirting water at the driftwood. Finally, the female stretched her body, released eggs onto the driftwood, and quickly departed. The male then ejaculated semen onto the eggs while leveling them into a single layer with his abdomen, and quivering pectoral and anal fins. Thereafter, the male guarded the egg batch (Fig. 1c).

*Development*

*Pao abei* [following observations based on egg batch spawned 3rd October 2017; with 63 eggs, excluding those eaten by male, and 63 hatched larvae (hatching rate 100%)]. Mean egg diameter was 2.26 mm (0.04 mm SD, $n = 5$), the eggs being spherical, demersal and adhesive, with a mass of small oil globules (see Fig. 2a-1, egg 5 days after spawning). Hatching occurred 8 days after spawning.

Hatched larvae (Fig. 2a-2) were 4.50 mm SL and 4.88 mm TL (means, $n = 3$), with the mouth and anus open, eyes developed, rudiments of dorsal and anal fins apparent, and small oil globules in the yolk. Melanophores occurred on the top and back of the head to the anterior lateral body region, and on the dorsal surface of the yolk. Xanthophores were scattered from head to tail, and weak erythrophores on the belly.

Notochord flexion had already started 2 days after hatching [5.24 mm SL and 5.46 mm TL (means, $n = 3$)] and was completed on day 5 [5.30 mm SL and 6.61 mm TL (means, $n = 3$)], the fish being considered as having reached the juvenile stage. By day 9, juveniles (Fig. 2a-3) [6.40 mm SL and 7.86 mm TL (means, $n = 3$)] had acquired a reticulated pattern on the back and sides, a whitish abdomen, a reddish tinge around the iris, and a red ocellus within a black circle on the flank between the dorsal and anal fin bases.

A second red ocellus appeared 15 days after hatching [8.82 mm SL and 11.0 mm TL ($n = 1$)], and a third at 22 days [10.6 mm SL and 13.0 mm TL ($n = 1$)], just encircling the first ocellus. Thirteen days after hatching, juveniles [11.9 mm SL and 13.6 mm TL (means, $n = 3$)] started to bite each other's caudal fin. Thirty-six days after hatching, juveniles (Fig. 2a-4) [13.4 mm SL and 14.8 mm TL (means, $n = 3$)] became darker on the back and sides, with a whitish reticulated pattern, white belly, and some weak to bright red ocelli with black and white circles on the flanks.

By sixty days after hatching [16.7 mm SL and 17.7 mm TL (means, $n = 3$)], the white belly of juveniles had gradually become darker and patterned. Red ocelli became encircled with white, some turning brownish after 120 days [30.7 mm SL and 34.0 mm TL (means, $n = 3$)] and 180 days [43.4 mm SL and 49.5 mm TL (means, $n = 3$)]. By three hundred days after hatching [73.8 mm SL and 82.8 mm TL (means, $n = 3$)], white circles around the ocelli had become obscure, similar to the adult condition. Individuals grew to 75.4 mm SL and 89.3 mm TL (means, $n = 3$) by day 337, and to 76.0 mm SL and 88.7 mm TL (means, $n = 3$) by day 360.

*Pao baileyi* [following observations based on egg batch spawned 14th November 2018; 117 eggs, excluding those eaten by male, and 66 hatched larvae (hatching rate 56.4%)]. Mean egg diameter was 2.74 mm (0.09 mm SD, $n = 3$), the eggs being spherical, demersal and adhesive, with a mass of small oil globules (see Fig. 2b-1, 8 days after spawning). Hatching occurred 10 days after spawning.

Hatched larvae (Fig. 2b-2) were 4.94 mm SL and 5.27 mm TL (means, $n = 3$), with the mouth and anus open, eyes developed, rudiments of the dorsal and anal fins apparent, and small oil globules in the yolk. Melanophores were widely distributed on the top and back of the head to the anterior lateral body region, dorsally on the yolk, and around the tail base. Xanthophores were scattered from the head to tail, and on the base of the dorsal fin rudiment. Weak erythrophores occurred on the abdomen.

Notochord flexion had already started 3 days after hatching [5.03 mm SL and 6.22 mm TL (means, $n = 3$)] and was completed on day 5 [5.37 mm SL and 6.76 mm TL (means, $n = 3$)], the fish being considered as having reached the juvenile stage. By day 7, juveniles (Fig. 2b-3) [5.48 mm SL and 6.75 mm TL (means, $n = 3$)]

had acquired melanophores on top of the head and nape, xanthophores on the side of the body, and a black band around the tail base. Xanthophores were also scattered on the dorsal, anal and caudal fins, and on the caudal peduncle. A reddish tinge occurred around the iris, but no ocellus had formed on the body.

The back had become darkened and the abdomen whitish with vague markings by 15 days after hatching [9.37 mm SL and 11.8 mm TL (means, $n = 3$)]. Xanthophores persisted on the dorsal, anal and caudal fin rays. Undefined patterns on a darker body were apparent 33 days after hatching (Fig. 2b-4) [11.5 mm SL and 14.1 mm TL ($n = 1$)]. Forty-three days after hatching [20.9 mm SL and 26.1 mm TL (means, $n = 3$)], individuals had a dark body and pale yellowish abdomen, and had developed whitish to yellowish skin flaps arising from the snout, above the eyes, and from the cheeks to above the anal fin. At no time did an ocellus develop. Individuals often nipped one another, and consequently died from bacterial infections.

*Pao suvattii* [following observations based on egg batch spawned 19th May 2016; 590 eggs, excluding those eaten by male, and 400 hatched larvae (hatching rate 67.8%)]. Mean egg diameter was 2.41 mm (0.41 mm SD, $n = 3$), the eggs being spherical, demersal and adhesive, with a mass of small oil globules (see Fig. 2c-1, 9 days after spawning). Hatching occurred 10 days after spawning.

Hatched larvae (Fig. 2c-2) were 4.65 mm SL and 4.87 mm TL (means, $n = 3$), with the mouth and anus open, eyes developed, rudiments of dorsal and anal fins apparent, and small oil globules in the yolk. Melanophores were present on top of the head and anterolaterally on the body, with xanthophores scattered on the head and body, and weak erythrophores on the belly.

Notochord flexion had already started by 3 days after hatching [5.31 mm SL and 5.54 mm TL (means, $n = 3$)], and was completed on day 7 [5.44 mm SL and 6.61 mm TL (means, $n = 3$)], the fish being considered as having reached the juvenile stage. By day 8, juveniles (Fig. 2c-3) [5.94 mm SL and 7.23 mm TL (means, $n = 3$)] had a pattern of red with black bands radiating backward from the eyes, a whitish abdomen, reddish tinge around the iris, and a small red ocellus with a black circle on the flank between the dorsal and anal fin bases.

A black "V" shaped pattern, diagnostic of the species, appeared 14 days after hatching [7.96 mm SL and 9.44 mm TL (means, $n = 3$)]. The dorsum had darkened with whitish spots, and the whitish abdomen acquired a dark reticulated pattern by 35 days [14.5 mm SL and 15.5 mm TL (means, $n = 3$)] (Fig. 2c-4); the ocellus had turned brownish. Individuals sometimes bit the tails of others. Sixty days after hatching [24.7 mm SL and 26.1 mm TL (means, $n = 3$)], the dorsum remained darkened with small whitish spots, and abdomen whitish with a brown pattern. The ocellus remained brown.

After 120 days [42.5 mm SL and 47.6 mm TL (means, $n = 3$)], the snout had become more pointed, but biting behavior had lessened. Individuals attained 84.4 mm SL and 94.8 mm TL (means, $n = 3$) by 180 days, 82.3 mm SL and 100.6 mm TL (means, $n = 3$) by 213 days, and 93.6 mm SL and 110.0 mm TL (means, $n = 3$) by 330 days.

Three hundred and eleven days after hatching, an offspring pair (both ca. 106.0 mm SL) spawned for the first time on 20th February 2017, with repeated spawning on a further 6 occasions to October of the same year. In 2018, the same pair spawned 35 times from February (663 days after hatching: female 113 mm SL, male 129 mm SL) through July.

*Growth*

Growth of *Pao abei* (*P. a.*, dotted line, solid circles), *P. baileyi* (*P. b.*, dashed line, open circles), and *P. suvattii* (*P. s.*, solid line, solid triangles) are illustrated in Fig. 3, and growth regression equations shown in Table 2. The growth of *P. baileyi* was fastest [growth coefficient (GC) = 0.421], next *P. suvattii* (GC = 0.340), and slowest *P. abei* (GC = 0.250).

## Discussion

According to the spawning data of *Pao abei*, *P. baileyi* and *P. suvattii* (Table 1) and those of *P. cochinchinensis*, *P. palembangensis* and *P. turgidus* (documented by Doi et al. 2015, 2022), all the species could breed in freshwater in captivity under stable water temperature (25–27°C) and lighting conditions (L12D12), with a suitable spawning bed, such as an earthenware pipe, concrete block with a hole, and sunken driftwood, all with a flat surface suitable for setting eggs in a single layered batch, followed by guarding by the male. The same pair in each species spawned a number of times through two to seven months within a single year. Single egg batches included hundreds of eggs, of 2 to 3 mm diameter. Hatching occurred after 7–10 days. These spawning characteristics are similar to those of the ocellated puffer *Leiodon cutcutia*, which spawns all year round (Doi et al. 2015, as *Tetraodon cutcutia*; Momota et al. 2022).

On the other hand, dwarf pufferfish of the genus *Carinotetraodon*, including *C. irrubesco*, *C. lorteti* and *C. travancoricus*, scatter smaller eggs (about 1 mm diameter) on water plants or other surfaces, without subsequent guarding (although Doi et al. 2015 stated that *C. travancoricus* protected its eggs, observations by HD and HS have shown such not to be the case), and hatched larvae are less developed than those of *Pao* species (Doi et al. 2015). The eyespot pufferfish *Dichotomictere ocellatus* breeds in brackish water, scattering smaller eggs (less than 1 mm diameter), again without subsequent guarding (Doi et al. 2015 as *Tetraodon biocellatus*).

Based on growth, six *Pao* species, three in the present study (Fig. 3, Table 2) and other three shown by Doi et al. (2015, 2022), are divided into two groups, faster (GC more than 0.3; *P. baileyi*, *P. suvattii*, *P. turgidus*) and slower groups (GC less than 0.3; *P. abei*, *P. cochinchinensis*, *P. palembangensis*). However, even the two groups grow faster than *Carinotetraodon* species and *Dichtomictere ocellatus*, GC being about 0.1. The growth of *Leiodon cutcutia* is similar to those of the faster group, GC being about 0.3 (Doi et al. 2015; Momota et al. 2022).

Larval care of *Pao* and *Leiodon* species was straightforward, *Artemia* larvae being supplied for initial nutrition, whereas *Carinotetraodon* and *Dichotomictere* species needed smaller food, such as fresh and brackish water *Brachionus* (Doi et al. 2015, 2022; Momota et al. 2022), probably reflecting the size differences of eggs and hatched larvae between the former and latter genera. Growth of *Pao* and *Leiodon* species was faster (growth coefficient GC > 0.2) than in the latter genera (GC ca. 0.1) as discussed above, perhaps suggesting more optimal growth conditions for the former. An artificially reared offspring pair of *P. suvattii* bred within two years.

The above data should provide a useful aid for future aquarium-based breeding trials of freshwater *Pao* species. However, the present study failed to successfully grow *P. baileyi* much beyond 43 days due to incessant nipping between individuals and resulting bacterial infection. Additional measures, such as rearing in larger tanks, providing more shelter, feeding more frequently, or utilizing antibiotics should improve the level of breeding success in that species.

## Acknowledgements

We are indebted to Dr. Hiroko Aoki, Chiba University and Dr. Shun Satoh, Osaka City University for literature, and Miss Yukiko Sugiyama, Shimonoseki Maine Science Museum for information on *Pao* spawning. We also thank Mrs. Hideto Nakagawa, Satoru Miyagawa and staff of Osaka Aquarium NIFREL, and Miss Mami Tanada, Ten-noji Zoo for technical assistance, and Mr. Nen Miwa and Dr. Kiyonori Nishida, Osaka Aquarium Kaiyukan for kind support. English corrections were made by Dr. Graham Hardy (Ngunguru, New Zealand).

## References


Doi, H., T. Akita and H. Sakai (2022) Reproduction and development in captivity of the Southeast Asian freshwater pufferfish *Pao palembangensis*. *Aquacul. Sci.*, **70**, 197-199.

Doi, H., H. Sakai, Y. Yamanoue, T. Sonoyama and T. Ishibashi (2015) Spawning of eight Asian brackish and freshwater puffers of the genera *Tetraodon* and *Carinotetraodon*. *Fish. Sci.*, **81**, 291-299.

Ebert, K. (2001) *The Puffers of Fresh and Brackish Waters*. Aqualog Verlag GmbH, Mörfelden-Walldorf, 96 pp.

Japan Wildlife Research Center (JWRC) (2013) *Gift of Waterfront (Mizube no Sachi) - Illustrated Handbook of Edible Plants and Animals Indigenous to the Mekong River Basin*. Nagao Natural Environment Foundation, Tokyo, 441 pp.

Kottelat, M. (2013) The fishes of the inland waters of Southeast Asia: a catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries. *Raffles Bull. Zool., Suppl.*, **27**, 1-663.

Livengood, E. J. and F. A. Chapman (2007) The ornamental fish trade: an introduction with perspectives for responsible aquarium fish ownership. *IFAS Extent. Univ. Florida, FA124: https://edis.ifas.ufl.edu/publication/FA124*.

Matsuura, K. (2015) Taxonomy and systematics of tetraodontiform fishes: a review focusing primarily on progress in the period from 1980 to 2014. *Ichthyol. Res.*, **62**, 72-113.

Miya, M., H. Takahashi, H. Endo, N. B. Ishiguro, J. G. Inoue, T. Mukai, T. P. Satoh, M. Yamaguchi, A. Kawaguchi, K. Mabuchi, S. M. Shirai and M. Nishida (2003) Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Mol. Phylogenet. Evol.*, **26**, 121-138.

Momota, K., H. Doi, H. Obata and H. Sakai (2022) Spawning and development of ocellated pufferfish *Laiodon cutcutia* in captivity. *Aquacul. Sci.*, **70**, 9-15.

Nelson, J. S., T. C. Grande and M. V. H. Wilson (2016) *Fishes of the World, 5th ed.* John Wiley & Sons, Hoboken, 707 pp.

Ng, P. K. L. and H. H. Tan (1997) Freshwater fishes of Southeast Asia: potential for the aquarium fish trade and conservation issues. *Aquar. Aci. Conserv.*, **1**, 79-90.

Raja, S., T. D. Babu, P. Nammalwar, C. T. Jacob and K. P. B. Dinesh (2014) Potential of ornamental fish culture and marketing strategies for future prospects in India. *Internat. J. Biosci. Nanosci.*, **1**, 119-125.

Sakai, H. (ed) (2021) *Science about Fugu Eating Culture*. Seibutsu Kenkyusha, Tokyo, 199 pp. (in Japanese).

Subamia, I. W., N. Meilisza, Sudarto and S. Sugito (2008) Domestication of freshwater puffer fish or buntal. *Indones. Aquacul. J.*, **3**, 133-138.

Yamanoue, Y., M. Miya, M. Doi, K. Mabuchi, H. Sakai and M. Nishida (2011) Multiple invasions into freshwater by pufferfishes (Teleostei: Tetraodontiformes): a mitogenomic perspective. *PLoS ONE*, **6**, e17410.

Yamanoue, Y., M. Miya, K. Matsuura, M. Kato, H. Sakai and M. Nishida (2008) A new perspective on phylogeny and evolution on tetraodontiform fishes (Pisces: Acanthopterygii) based on whole mitochondrial genome sequences: basal ecological diversification? *BMC Evol. Biol.*, **8**, 212.


# 東南アジア産淡水フグ *Pao* 属3種の飼育下での繁殖

土井啓行・百田和幸・小畑　洋・酒井治己

東南アジア産淡水フグ Pao 属3種 *P. abei*, *P. baileyi* および *P. suvattii* の飼育下での繁殖を試み，既報の *P. cochinchinensis*, *P. palembangensis* および *P. turgidus* と比較した。これら6種はすべて一定の水温（25-27℃）及び日長条件（L12D12）下の淡水で，平らな床のある土管や沈水性流木を産卵床とすることにより繁殖した。雄は数百個の卵径 2-3 mm の卵からなる一層の卵塊を保護した。それぞれの種で1ペアが1年のうちの2-7ヶ月の間に複数回産卵した。卵は7-10日でふ化し，*Artemia* 幼生を初期餌料として育てることができた。これらの知見は飼育下における淡水フグ類の繁殖技術向上に貢献するものと思われる。