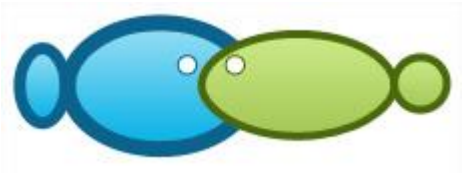

Study on environmental condition at a spawning area and morphological characteristics of mudskipper (*Periophthalmodon septemradiatus* Hamilton, 1822) at the embryonic and larval stages

[1]Vo T. Toan, [1]Mai V. Hieu, [1]Huynh T. Toi

[1] College of Aquaculture & Fisheries, Campus II, 3/2 Street, Ninh Kieu District Can Tho University, Viet Nam. Corresponding author: H. T. Toi, httoi@ctu.edu.vn

Abstract. Mudskipper *Periophthalmodon septemradiatus* is a euryhaline and an amphibian species, both aquatic and terrestrial habitats**.** It is widely distributed in the mangrove swamps and muddy areas in the Asian regions. This study was conducted to provide basic information on the morphology of embryonic and larvae and describe the environmental parameters of this species in the lowland area of the Mekong River Basin for artificial reproduction, management, and conservation implication. The study was conducted from November to December 2020. Eggs of *P. septemradiatus* were collected from the natural habitat, a burrow in the small tributaries of the Mekong River in Binh Thuy District, Can Tho City, located in the Mekong Delta, and incubated under hatchery conditions. The results showed that the fertilized eggs have an elliptical shape with one adhesive side. The fish larvae are more active inside the eggshell before hatching. The eggshell is broken, and hatching occurs. The newly hatched larva has a mouth and eyes, but they are not completely developed yet. The eyes develop completely between 24 and 30 hours after hatching, and the mouth becomes fully developed between 5 and 7 days after hatching (DAH), at which time oil globules in the yolk sac are very small or disappear. After approximately 7–8 DAH, the fish larvae use up the yolk sac. The results of this study indicated that eggs and larvae of *P. septemradiatus* can develop in freshwater. This information can be useful in studies on artificial reproduction.
Keywords: Mudskipper*, Periophthalmodon septemradiatus*, fry, morphology.

Introduction. Mudskipper fish belong to the family Gobiidae and are classified in the suborder Gobioidei of the order Perciformes (Tran et al 2013). The species *Periophthalmodon septemradiatus* is an amphibian species, both aquatic and terrestrial, that occurs in marine, brackish, and freshwater; this species has potential as an ornamental fish in the Mekong Delta (Dang & Nguyen 2009, Dinh et al 2018b). According to Polgar et al. (2010), species *P. septemradiatus* has a partial life cycle on land and is an indicator organism in the assessment of biology and toxicity in the aquatic environment; this is also a carnivorous fish (Dinh et al 2018a; Dinh et al 2020). *P. septemradiatus* has a total length from 5.3 to 10.9 cm (Murdy 1989, Dinh et al 2018b), and it has a gray to a brown color similar to the color of mud. According to Mai et al. (2019), when attracting female fish, the male fish changes body color from light brown to iridescent dark green; it may show blue-violet spots on its body and light red and light green spots on the snout and gills (Dinh et al 2018c). Pelvic and dorsal fins are purple in males. Color is likely to vary through distribution and may also be genetic (Takita et al 1999). The female maintains a light brown color that does not change throughout its life. Male and female fish mate in the burrow, the eggs are laid on the wall of the spawning chamber in the burrow, and the periods end at high tide. The mudskipper can spawn all year-round (Dinh et al 2018b). *P. septemradiatus* favors freshwater and is considered an endangered species, extinct in Malaysia (Polgar 2008). It is in danger of extinction elsewhere, which would cause the loss of a link in the natural food chain (Dang & Nguyen 2009), urbanization, environmental pollution and climate change (Dinh et al 2018b). Its

population has been subjected to overfishing in some regions in the Mekong delta (Tran & Lam 2020). Besides egg-tending chambers, burrows are used for feeding, as it was estimated from its otolith size (Dinh et al 2021a). However, there is no study on its morphology at fry stage. The present study has two objectives. The first is to collect basic information on spawning areas and the environmental parameters of fish spawning. The second is to describe the morphology of embryonic and larvae. Thus, the study aims to contribute to the knowledge on mudskippers in the lowland area of the Mekong River Basin that can be used for its artificial reproduction study, management, and conservation.

Material and Method

Wild eggs collection. Samples of mudskipper were collected four times between November and December 2020 at tributaries of the Mekong River in Long Tuyen Ward, Binh Thuy District, Can Tho City, Viet Nam. The location of the receiving points was determined by the global positioning system (GPS) at the following coordinates on November 2, 2020 (sample 1; A1 in Figure1B): 10°2'47"N 105°43'31"E; November 6, 2020 (sample 2; A2 in Figure1B): 10°2'51"N 105°43'27"E; December 3, 2020 (sample 3; A3 in Figure1B): 10°2'41"N 105°43'43"E; December 16, 2020 (sample 4; A4 in Figure 1B): 10°2'33"N 105°43'44"E.

1A 1B

Figure 1. Map of Long Tuyen District, Can Tho City (Figure 1B) in the Mekong delta (Figure 1A), showing the collection site (Red balloon) for mudskipper eggs.
Source: Google Earth Pro 2020

Fish eggs were collected directly from fish burrows in the wild. The number of egg nests burrows^{-1} in 4 samples was—sample 1: 2 egg nests 7 burrows^{-1}; sample 2: 1 egg nest 7 burrows^{-1}; sample 3: 1 egg nest 8 burrows^{-1}; sample 4: 1 egg nest 10 burrows^{-1}. The collected eggs were put in a 3 L plastic containing water at the site of the collected eggs, and aeration was continuously supplied and brought to a hatchery of the College of Aquaculture & Fisheries, Can Tho University, for incubation. The collected eggs were then transferred into a 1.5 L aquarium containing 1 L of filtered freshwater lightly aerated, collected at the egg nests, and were incubated at room temperature (28±2 °C). The collected water was filtered through a Whatman membrane filter (0.45 µm) before its use for egg incubation. Around 30% of water in the incubation vessel was replaced daily with filtered water collected from the sampled site. When the eggs hatched, fries of fish were sampled to observe and analyze the morphological indicators.

Data collection and analysis. Indicators of pH were measured using a pH meter, the temperature was measured using a thermometer, water clarity was measured with Secchi disc, and salinity was measured using a refractometer at the spawning area at

high tide (one sampling for each parameter). Ten eggs and larvae were sampled to observe and analyze the morphology by stereomicroscopes (Nikon SMZ745T, China) and they were photographed using a digital camera (Nikon 25 Megapixel, Japan). Accordingly, the eyepiece micrometer was used to measure the total length of fry under 10× magnification. Data were recorded and calculated using Microsoft Excel software (2016). Photoshop CS6 and Clip Studio software were used to process images describing morphological parameters related to the development of mudskippers in the larval stage.

Results and discussion

Water parameters at the sampling site. Fish eggs were found in a freshwater environment, and there was no change in salinity under the influence of tides. The slight change in water environment parameters between sampling periods was recorded. pH in the second sampling was lower compared to other days because that sampling day was partly cloudy. At the third sampling, due to the influence of erratic rain, the water clarity is slightly higher than the other days (Table 1).

Table 1

Water parameters at the sampling site

Sampling	*Time*	*Weather condition*	*pH*	*Temperature (°C)*	*Water clarity (cm)*
1	9:00	Sunny	8.7	28.7	22.6
2	13:00	Partly cloudy	7.8	27.6	21.8
3	9:00	Shower	8.4	28.4	23.3
4	10:00	Sunny	8.3	28.2	23.0

The results show that *P. septemradiatus* is a species that lives in a less polluted environment, so it is very rarely found in densely populated areas with high levels of domestic wastewater. However, mature males and females are abundant 5 km away from residential areas. Eggs are laid on the wall of the spawning chamber in the fish burrow, and they are evenly spaced next to each other on the same layer, with no overlapping eggs, the cave is full of water, but the spawning chamber is not flooded because it contains a sufficient amount of air for the eggs to develop, the fish burrow has two mouths, including a large mouth (where fish move in and out) and a small mouth (which can be camouflaged by fish or covered with a thin layer of mud to protect themselves and escape) (Figure 2). These findings are similar to the study reported by Mai et al. (2019). *P. septemradiatus* is euryhaline, its fecundity increases from estuary to upstream area (Dinh et al 2018b) and Mai et al. (2019) reported that *P. septemradiatus* spawn in both brackish and freshwater environments. The findings of previous studies and present study confirmed that the eggs of *P. septemradiatus* are laid in freshwater areas.

Figure 2. The burrow of *P. septemradiatus* in mudflat (site cross).
Source: authors' personal archive (photo taken on November 2, 2020)

Morphological characteristics of P. septemradiatus at embryonic and larval stage. The eggs of *P. septemradiatus* were collected from four samples in the burrows, where there is a mudflat (Figure 2). The fish eggs were treated with freshwater, and the fry was arranged in the aquarium. The results show that the eggs have an elliptical shape with one adhesive side and were in the stage of cell division in which the one-cell animal

pole subdivides. The middle part is the yolk containing all the nutrients; when the eggs hatch into fry, they fully utilize this yolk nutrition (Figure 3A). After 4 hours, the cells subdivide into the embryo, which lies around the yolk (Figure 3B). When the eggs are about to hatch, the embryo fish begin to work, moving around inside the egg; the degree of movement of the fish embryo increases gradually until the eggshell is broken, and hatching occurs (Figure 3C). Studies showed that mudskipper *P. septemradiatus* spawned throughout a year-round in the Mekong delta, that it digs I-, J- and Y-shapes burrows (Dinh et al 2018b), and that the egg has a similar shape to mudskipper *Scatellaos gigas,* but that it does not have a melanophore (Kim et al 2011). However, the mouth of *P. septemradiatus* embryo is not opened as it happens in the case of *P. schlosseri* (Tsukaho et al 2003).

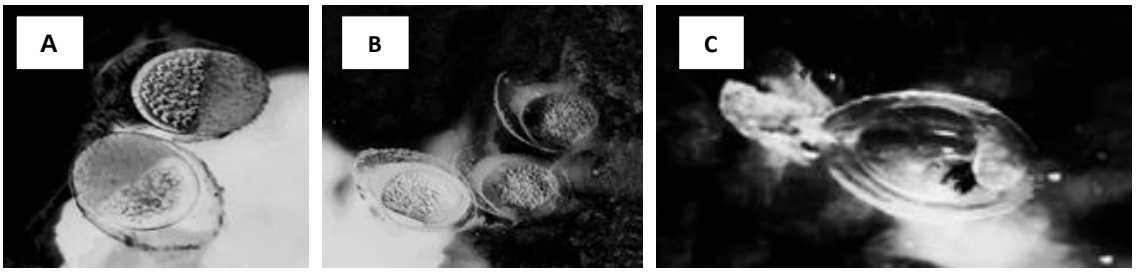

Figure 3. Egg cleavage, germ development, and embryonic phase of *P. septemradiatus* (Magnification 10X).
Source: authors' personal archive (photo taken on November 2, 2020

Observation results show that the maximum hatching rate of eggs is 70%, and the rest are mainly unfertilized eggs or weak embryos. According to Ishimatsu et al. (2007), eggs immersed in water have a high hatching rate (80%), whereas eggs remaining in the air had reduced hatching competence. After hatching (Figure 4A), the fish has not fully developed organs, and the body tends to curve slightly around the yolk sac, with inflated air bubbles with large oil globules on the head. Two hours after hatching (Figure 4B), the fish body develops straight and elongated. The body length is in the range of 1.72–1.95 mm. Its size is smaller than the newly hatching fry (2.09–2.60 mm) of *Periophthalmus schlosseri* (Tsuhako et al 2003). The total length of mature *P. septemradiatus* is nearly two times smaller than mature *P. scholosseri* (Mazlan & Rohaya 2008; Dinh et al 2018b), leading to a smaller larval size. At this time, the fry has not fully developed eyes and lacks an open mouth. Other organ functions are not fully developed. The backbone has been formed, and melanophore on the skin at the fry stage was not detected.

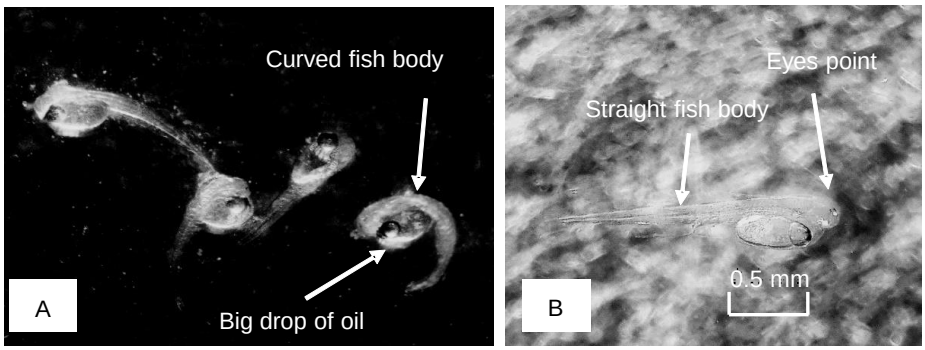

Figure 4. The free-swimming larval stage (Magnification 10X).
Source: authors' personal archive (photo taken on November 4, 2020)

Total length od fish larva's (TL) is from 1.76 to 2.04 mm, 24 hours after hatching. At this time, the completed eyes appear but are not yet fully developed, and no eye movements are present. The blood vessels in the anus work, start to grow and move faster, and the fish's mouth has not yet developed (Figure 5A). The difference in the TL of hatched fish is recorded in some previous studies, the TL of *B. pectinirostris* is 3.0-3.4 mm (Ryu et al

1995) and 3.1-3.3 mm (Okiyama, 1988), and *S. gigas* larva is 2.58-3.24 mm (Kim et al 2011).

On day two after hatching (DAH), the size of the fish is bigger, its total length is 2.38–2.48 mm, the eyes are completely developed and have started to move, and the development of the intestines and blood vessels has not yet been detected. Moreover, the oil globules inside the fish are smaller, indicating that this is the source of nutrients used by the fish to develop organs during the fry stage (Figure 5B). The fry at 3 DAH has a TL of 2.57–2.62 mm (Figure 5C), the oil globules inside are gradually smaller, the organ functions continue to develop, the mouth and pulse appear, and blood vessels increasingly appear in the body. There are oil globules in the egg development and newly hatched mudskippers. The size of oil globules changes during fish development. There are many small oil globules in egg development of *B. pectinirostris* and two oil globules in the embryo of *S. gigas* just prior to hatching (Ryu et al 1995). Howerer, a single oil globule is found in *S. gigas* larvae, in *B. pectinirostris* larvae (Ryu et al 1995; Kim et al 2011) and in *P. septemradiatus*, according to the present study. According to Tsuhako et al. (2003), the oil globules are completely absorbed when the fry of *P. schlosseri* reaches 2.09–2.61 mm. Although the results of the current study show that the sizes of *P. schlosseri* and *P. septemradiatus* at the fry stage are similar, the growth of other organs of *P. septemradiatus* is slower, and the fish is still in the stage of utilizing the yolk sac.

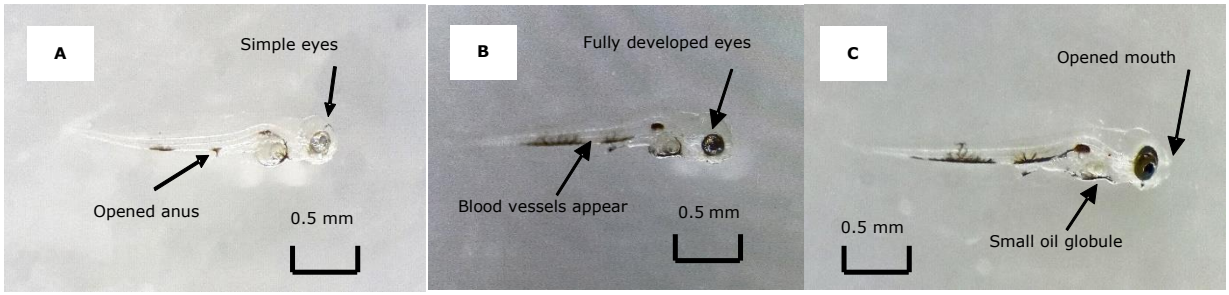

Figure 5. Fry at 1 day after hatching or DAH (A), 2 DAH (B), and 3 DAH (C) (Magnification 10X).
Source: authors' personal archive (photo taken on November 5-7,2020)

The fry from 4 to 5 DAH (Figure 6A and 6B) reaches a length of 2.57–2.62 mm; during these two days the growth in length of the fish slows down, and it starts to grow other organs. The oil globule inside gets smaller, and the melanophores pigment spots appear on the fish body. By 6 and 7 DAH (Figures 6C and 6D), the length reaches 2.72–2.81 mm. The growth in length in these two days is not significantly different, but it is greater than that of day 5 and day 6. The fully developed fish mouth starts to open, the digestive organs develop, the oil globules are completely absorbed by now, and the fish begin to eat outside by filtering. Nutrition is entirely reliant on outside food sources. The melanophore is an important characteristic for the identification of species during the larval stage (Kendall et al 1984). The appearance of the melanophore is different between species. It is present on day 4 and day 5 in *P. septemradiatus*; on the ventral contour of the mid-tail in *S. gigas* from hatching until it reached 3.26 mm (Kim et al 2011); and no melanophore in *B. pectinirostris* at 3.1-3.3 mm (Okiyama, 1988).

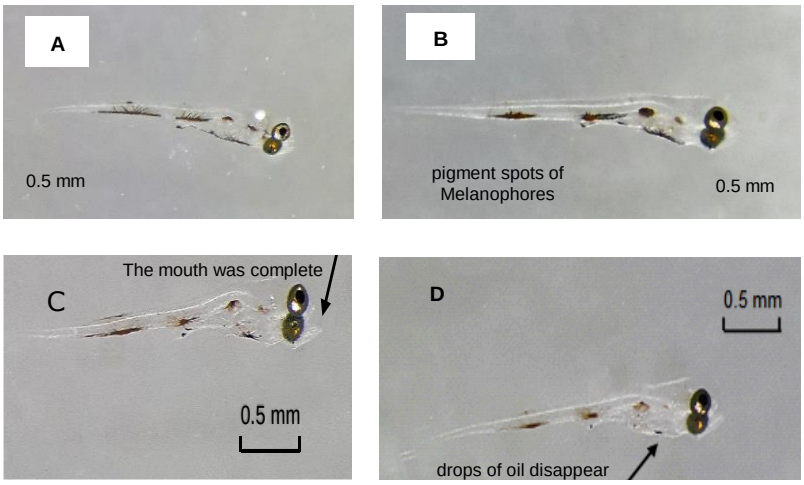

Figure 6. Fry at 4 DAH (A), 5 DAH (B), 6 DAH (C), and 7 DAH (D) (Magnification 10X).
Source: authors' personal archive (photo taken on November 8-11, 2020)

Conclusions. Mudskipper *P. septemradiatus* lives in freshwater at zero salinity, with pH from 7.8–8.7, temperature from 27.6–28.7°C, and water clarity from 21.8–23.3 cm. The fertilized eggs have an elliptical shape, and eggs stick to one end. After hatching, the yolk sac is used up 7–8 DAH. The fish's eyes are fully developed from 24–30 hours after hatching, and its mouth is fully developed at 5–7 DAH. The results indicated that eggs and larvae of *P. septemradiatus* can develop in freshwater. This information can be helpful in studies on the artificial reproduction of *P. septemradiatus*.

Acknowledgements. This study is funded by the scientific research and technology transfer budget of Can Tho University.

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Recieved: 4 August 2021. Accepted: 27 September 2021. Published online: 01 October 2021.
Auhors:
Vo Thanh Toan, Department of Fisheries Management and Economics, College of Aquaculture and Fisheries, Cantho University, Campus II, 3/2 Street, Nink Kieu District, Can Tho City, Viet Nam, e-mail: vttoan@ctu.edu.vn
Mai Van Hieu, Department of Fisheries Management and Economics, College of Aquaculture and Fisheries, Cantho University, Campus II, 3/2 Street, Nink Kieu District, Can Tho City, Viet Nam, e-mail: mvhieu@ctu.edu.vn
Huynnh Thanh Toi, Department of Applied Hydrobiology, College of Aquaculture and Fisheries, Cantho University, Campus II, 3/2 Street, Nink Kieu District, Can Tho City, Viet Nam, e-mail: httoi@ctu.edu.vn

How to cite this article:
Toan V. T., Hieu M. V., Toi H. T., 2021 Study on environmental condition at a spawning area and morphological characteristics of mudskipper (*Periophthalmodon septemradiatus* Hamilton, 1822) at the embryonic and larval stages. AACL Bioflux 14(5):2772-2778.