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Burrow behaviour, structure and utilization of the amphibious mudskipper *Periophthalmus chrysospilos* Bleeker, 1853 in the Mekong Delta

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A B S T R A C T

Burrows of fishes are important to their creators, but knowledge of burrowing behaviour, structure and utilization are poorly understood. We report the burrowing behaviour of the amphibious mudskipper, *Periophthalmus chysospilos*, from estuarine and coastal sites within the Mekong Delta (Vietnam). Activities at and around the burrow were observed over 12 months (April 2020 to March 2021). Burrow casts were recovered monthly to determine burrow structure and utilization in this species. Observations revealed that males excavate burrows with their mouths during the ebb tide. Burrows were J-, Y- and U-shaped, with 1–2 openings to the surface and a bulbous egg chamber. The burrow depth (BD) and total length (BL) varied with shape and site, but not season. The BD and BL ranged from 15.1 ± 0.9 to 18.6 ± 0.80 SE cm and from 22.1 ± 1.2 to 25.7 ± 1.0 SE (standard error of mean) cm, respectively. These results provide insights into the burrowing ability and better understand the ecology of these fishes in the Mekong Delta.

Running head: Burrow behaviour, structure and utilization of *Periophthalmus chrysospilos*.

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1. Introduction

Burrows of fish species vary in design, shape, and use (Atkinson & Taylor, 1990). Some fish species inhabit burrows constructed by other organisms, such as snapping shrimps (Karplus et al., 1981; Jaafar & Hou, 2012). Others build their own burrows through excavating the substrate with their mouths, e.g., *Odontamblyopus lacepedii* (Gonzales et al., 2008) or twisting their bodies through the substrate, e.g., *Anguilla japonica* (Aoyama et al., 2005). Burrows are primary sites for shelter in many species, and are often also areas of egg deposition (Ishimatsu et al., 1998).

Burrows are commonly encountered within the littoral zone; the challenging environmental conditions here may explain the high diversity and abundance of burrows therein (Little, 2000).

Fishes living in these areas experience daily immersion and emersion following the tidal cycle. With the ebb and flow of the tide, organisms experience fluctuations in many environmental parameters such as ultraviolet radiation, salinity, temperature, and oxygen availability, amongst others (Little, 2000).

In addition to risks associated with exposure to aquatic and terrestrial systems, fishes living in littoral mudflats further contend with substrata that are not structurally complex. In the absence of readily available structures for shelter, burrows feature prominently in their ecology. Oxudercine mudskippers are a group of approximately fifty fish species well-adapted to living under such challenging conditions (Jaafar and Murdy, 2017). These fishes are amphibious and engage in foraging activities as well as territorial and courtship displays on exposed mudflats during the ebb tide (Ip et al., 1990; Clayton, 1993; Colombini et al., 1996). Following courtship, spawning and fertilization of eggs occur within designated chambers within the burrows. In this group of fishes, burrows are used as shelter areas for adults and developing eggs (Atkinson & Taylor, 1990; Martin & Ishimatsu, 2017; Polgar et al., 2017).

Mudskippers construct burrows in the mud substrate in riverine, estuarine and coastal regions (e.g., *Parapocryptes* [henceforth *Pa.*] *serperaster* (Dinh et al., 2014b), *Periopthalmodon* [henceforth

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Peer review under responsibility of King Saud University.

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Pn.] *septemradiatus* (Dinh et al., 2021b). In species such as *Pa. ser-perasters* (Dinh et al., 2014a), burrows are constructed and maintained at high tide while in *Boleophthalmus* [henceforth *B.*] *boddarti* (Clayton & Wright, 1989), *Pn. schlosseri* (Tran et al., 2020) and *Pn. septemradiatus* (Dinh et al., 2021b), these are carried out when the mudflat is exposed during low tide.

The burrows of mudskippers and other gobioid fishes are well-defined; typically I-, J-, Y-, U- and W-shaped (Atkinson & Taylor, 1990; Ishimatsu et al., 2007; Tran et al., 2020; Dinh et al., 2021b). Burrow shapes vary with habitat, e.g., *Pn. schlosseri* construct J-shaped burrows in Malaysia (Ishimatsu et al., 1998). Burrow shapes are also observed to vary between species; for example, *Pn. schlosseri* builds I- and J-shaped burrows (Ishimatsu et al., 1998; Tran et al., 2020), *Pseudapocryptes elongatus* makes Y-shaped burrows (Tran, 2008), *B. dussumieri* digs U-shaped burrows (Clayton & Wright, 1989) and *B. boddarti* makes I- and U-shaped burrows (Dinh et al., 2014a). Burrows of *Periophthalmus* species vary in shape, Y-, J-, U- and W-shaped burrows have been reported for this genus. For example, *Ps. argentilineatus* and *Ps. modestus* in Okinawa Island dig Y-shaped burrows (Kobayashi et al., 1971). In these two species, the Y-shaped burrows have two turreted openings on the substrat that meet below the surface to form a vertical tunnel then turn at a right angle for a short distance before continuing upward to form the egg chamber. Meanwhile, *Ps. waltoni* in the mudflats of Sulaibikhat Bay, Kuwait, build Y-shaped burrows with one main and one secondary opening; the main opening was the one used most often (Clayton & Snowden, 2012). J-shaped burrows are built by *Ps. minutus* in mudflats of Australia (Takeda et al., 2012). *Periophthalmus variabilis* have also been found to excavate Y-, U- and J-shaped burrows in captivity (Rupp, 2021). Burrow structures of several gobioid fish species vary with mud texture and presence of vegetation and associated roots (Brillet, 1969; Polgar et al., 2017). The density of vegetation and associated roots at mudflats influence burrow distribution (Polgar et al. (2017); for example, *B. dussumieri* excavate burrows in areas without roots, whereas *Ps. waltoni* dig burrows in areas with and without roots.

Periophthalmus chrysospilos are naturally distributed within the tropical belt, where precipitation can affect the integrity of the silt substrate. It is carnivorous fish, feeding mainly on *Acetes* spp., *Uca* spp., *Dolichoderus* sp. in the Mekong Delta (MD) (Dinh et al., 2021a), and shows temporal variation in some morphometrics and body condition factors (Le et al., 2021; Dinh et al., 2022c). This fish spawns all year round but focuses mainly on the wet season, from July to October, with a batch fecundity of 23,000 eggs (Dinh et al., 2022a), and its population parameters show a spatial change in MD (Dinh et al., 2022b). Some mudskippers in MD use burrows as a place for forging, protecting from predators, and egg releasing and hatching, but there is no data on *Ps. chrysospilos*. Moreover, in MD, precipitation shows a significant seasonal difference because there is almost no rain in the dry season but tremendous rainfall in the wet season (Le et al., 2006). The precipitation variation can affect the moisture and softness of the mud, affecting the burrow's structure and size. Therefore, this study was conducted to contribute to the burrow morphology and utilization; and verify if the burrow structure can change with season and site. The findings will help us gain a better understanding of fish ecology adaptation in the MD.

2. Materials and methods

2.1. Study sites

This study was carried out from April 2020 to March 2021 at four sites within MD in Vietnam: Duyen Hai - Tra Vinh (DHTV),

Tran De - Soc Trang (TDST), Dong Hai - Bac Lieu (DHBL) and Dam Doi - Ca Mau (DDCM) (Fig. 1). The dry season was defined from April to May 2020 and January to March 2021. Meanwhile, the wet season was determined from June to December 2020. Common plant species at these sites were *Acanthus ebracteatus* Vahl., *Avicennia marna* (Forssk.) Vierh., *Bruguiera gymnorrhiza* (L.), *Nypa fruticans* Wurmb., Savigny *Sonneratia caseolaris* (L.) A. Engl., and *Rhizophora apiculata* Blume. *Sonneratia caseolaris*, *Avicennia marina,* and *Bruguiera gymnorrhiza* were dominant at DHTV, TDST, and DHBL, respectively, whereas both *Avicennia marna* and *Bruguiera gymnorrhiza* were dominant at DDCM. The average pH (measured with a pH meter (Hanna HI98127)) and salinity (measured with a Refractometer 0–32 Brix (95000–002)) in each study area were presented in Table 1. pH and temperature were determined once a month at each sampling point at high tide.

### 2.2. Burrowing behavior observations

Periophthalmus chrysospilos were observed in the field and aquarium tanks to understand fish burrowing behavior and other activities using the method of Able et al. (1982). The burrowing behavior and other activities (grazing, foraging, and moving) were observed twice in the field, in May 2020 and October 2020. Five males were randomly chosen for observation. Fish were observed from a distance of 10 m by the camera. Each observation lasted for four continuous hours during the low tide, and if the fish entered the burrow with its head, it used the burrow as a place for predator avoidance, whereas if the fish entered the burrow with its tail, it used the burrow as a foraging place (Dinh et al., 2021b).

2.3. Burrow structure

The structure of burrows made by *Ps. chrysospilos* was recovered using polyester resin (En Chuan Chemical Industries Co., ltd, Taiwan) mixed with a hardener in the ratio (98:2) according to the method described by Atkinson & Chapman (1984). After mixing in the bottle of 500 mL, polyester was poured *in situ* into openings of burrows. This process was carried out at low tide, within the mudflats near the edge of the mangrove forest, and 5–7 burrows were casted. Fish in selected burrows are relocated to other areas. After 2–4 h, burrow casts were removed from the mudflats, washed, and transported to the laboratory. In the laboratory, the casted burrows were measured. Parameters such as burrow depth (BD) measured from the surface to the deepest point of the burrow, total burrow length (BL), was the total length measured, including all branches of the burrow, diameter of the opening, number of openings and shape were recorded for each burrow cast (Fig. 2) (Gonzales et al., 2008). The BD and BL were used to compare burrow structure between sites and between wet and dry seasons. The Robinson pipette method (Gee & Bauder, 1986) was used to analyze the mud composition (sand, silt and clay) and mud taxonomy of five burrows at each location; the substrate samples were collected at the burrow mouths. Accordingly, the bulk density was calculated as oven-dry soil weight (105 °C) of undisturbed soil samples per bulk volume. The organic matter was examined using the Walkley-Black wet oxidation method with $K_2Cr_2O_7$ and H_2SO_4 (Walkley & Black, 1934).

2.4. Data analyses

The normal distribution of substrate composition (sand, silt, and clay), BD, and BL were verified by the Kolmogorov-Smirnov test (Kim, 2015). The equality of substrate composition variances between four sites was confirmed by the Levene test. This test was also used to verify if BD and BL exhibited the equality of variances. As the substrate composition variances were equal, one-way
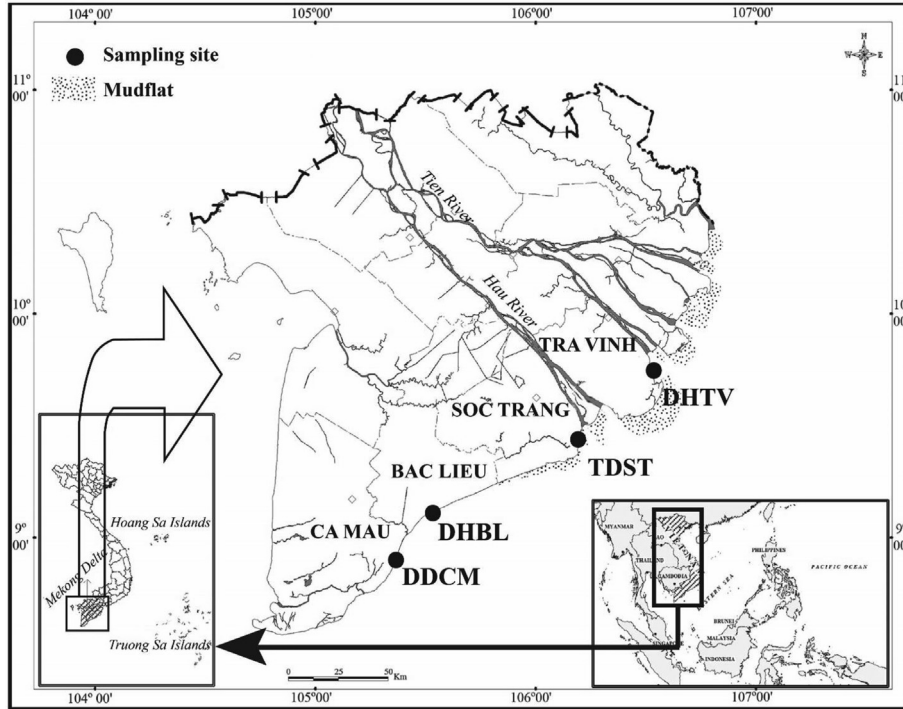
Fig. 1. The sampling map in the Mekong Delta modified from Fig. 1 of Dinh (2018) (●: Sampling area; DHTV: Duyen Hai - Tra Vinh (9°41′18.6″N 106°30′35.8″E), TDST: Tran De - Soc Trang (9°29′26.8″N 106°11′58.5″E), DHBL: Dong Hai - Bac Lieu (9°06′03.2″N 105°29′49.1″E) and DDCM: Dam Doi - Ca Mau (8°58′17.5″N 105°22′51.8″E)).

Table 1
pH and salinity at study sites.

No.	Site	pH	Salinity (‰)
1	Duyen Hai - Tra Vinh	~7.9	~15.2
2	Tran De - Soc Trang	~7.9	~16.3
3	Dong Hai - Bac Lieu	~7.8	~30.0
4	Dam Doi - Ca Mau	~7.8	~26.8

ANOVA with Tukey Post Hoc was applied to qualify the spatial variation of substrate composition, but one-way ANOVA with Tamhane's T2 was applied otherwise. If the substrate composition did not show a normal distribution, the Kruskal-Wallis test was used to verify the variation of these variables among four sites. The General Linear Model (GLM) with Tukey Post Hoc was applied to test if BD and BL change with season, burrow shape, site, and their interactions if BD and BL displayed a normal distribution and equality of variances. By contrast, as BD and BL variables did not display normal distribution, Mann-Whitney Test was involved in qualifying the variation of BD and BL between seasons, and the Kruskal-Wallis Test was performed to verify the variation of BD and BL between burrow shapes and sites. The tests were quantified by SPSS v.21 at $p < 0.05$. The Benjamini–Hochberg procedure was used to lessen the Type I error of all tests (Benjamini & Hochberg, 1995; McDonald, 2014).
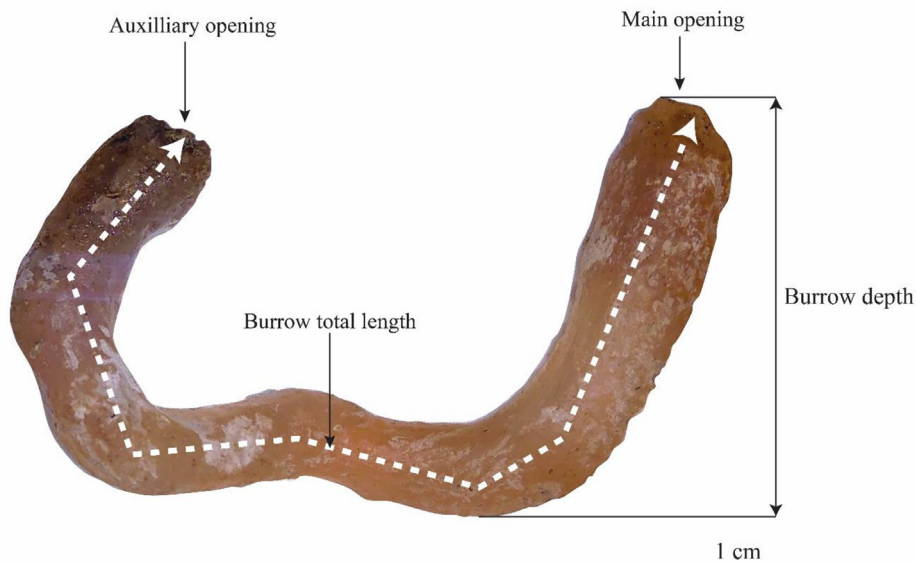
Fig. 2. Method of measuring the total length and depth of the burrow.

3. Results

3.1. Substrate composition

The normal distribution was found in the silt (Kolmogorov-Smirnov, KS = 0.12, p = 0.20), but not in sand and clay (KS_{sand} = 0.26, p_{sand} < 0.001; KS_{clay} = 0.18, p_{clay} = 0.04). The substrate composition at the four sites varied with the following [silt, clay, sand] percentage make-up [60.4, 24.6, 15.0] at DHVT, [35.0, 36.4, 28.6] at TDST, [30.8, 56.9, 12.3] at DHBL, and [33.0, 24.5, and 42.5] at DDCM. This showed that the substrate composition changed significantly according to four regions. The substrate at DHBL was composed mostly of clay (56.9 %, Kruskal-Wallis, χ^2 = 13.72, p = 0.003). At DDCM, the substrate was composed mostly of sand (42.5 %, χ^2 = 4.95, p = 0.02); and at DHTV, silt accounted for the highest percentage of total composition (60.4 %, One-way ANOVA, F = 4.23, p = 0.02). Sample substrate from TDST revealed a similar composition of silt, clay, and mud.

3.2. Burrowing behavior observations

Burrows of *Ps. chrysospilos* were observed on mud substrate at the edge of mangrove forests. It was observed that when the tide started to recede, some individuals remained at the edge of the mangrove forest without following the tide; the rest moved with the tide. A group of fish that did not proceed with the tide began to observe mudflats by moving back and forth in a specific area. Observational results showed that, after finding a suitable location with features such as porous soil or near small puddles, the fish proceeded to burrow. First, the fish used its mouth to bite into the mud to start excavating; the mud that was dug up was placed next to it. This action took place continuously for about 10 – 15 min. Next, the fish returned to the water environment to moisten the body and continue to burrow. This process was repeated until the burrow was completed. The completion time of each burrow was about 40 – 60 min.

3.3. Burrow structure

A total of 161 burrows were cast: 51 burrows at DHTV, 42 burrows at TDST, 29 burrows at DHBL, and 39 burrows at DDCM. Three burrow shapes were recovered 61 were J-shaped burrows (Fig. 3A), 29 were U-shaped burrows (Fig. 3B), and 71 were Y-shaped burrows (Fig. 3C). Burrows openings sometimes do not have turrets and pellets (Fig. 4A-E), but most of them are turreted and with pellets (Fig. 4F-G). J-shaped burrows (45.1 %) were most encountered at DHTV, followed by Y- (39.2 %) and U-shaped burrows (15.7 %). Similarly, U-shaped burrows (16.7 %) were least represented at TDST, while Y- (40.5 %) and J-shaped burrows (42.8 %) were similar in numbers. The percentage burrow distribution [Y-, J-, and U-shapes] was [51.7 %, 31.1 %, 17.2 %] at DHBL and [48.7 %, 28.2 %, 23.1 %] at DDCM. The largest opening in Y- and U-shaped burrows were often the main opening, while there were usually several smaller auxiliary openings nearby.

The BD and BL displayed a normal distribution (KS_{BD} = 0.07, p_{BD} = 0.07; KS_{BL} = 0.06, p_{BL} = 0.20) and exhibited equality of variances (Levene, F_{BD} = 1.21, p_{BD} = 0.25; F_{BL} = 1.52, p_{BL} = 0.08). Of the three burrow shapes recovered, J-shaped burrows (20.4 ± 0.6 SE cm) were the deepest, followed by Y-shaped burrows (16.4 ± 0.5 SE cm); U-shaped burrows were the shallowest (11.6 ± 0.5 SE cm, GLM, F = 38.44, p < 0.001) and widest (27.1 ± 1.5 SE cm), whereas J-shaped (23.3 ± 0.6 SE cm) and Y-shaped (23.3 ± 0.7 SE cm, F = 5.10, p = 0.007) burrows were similarly shorter (Fig. 5A).
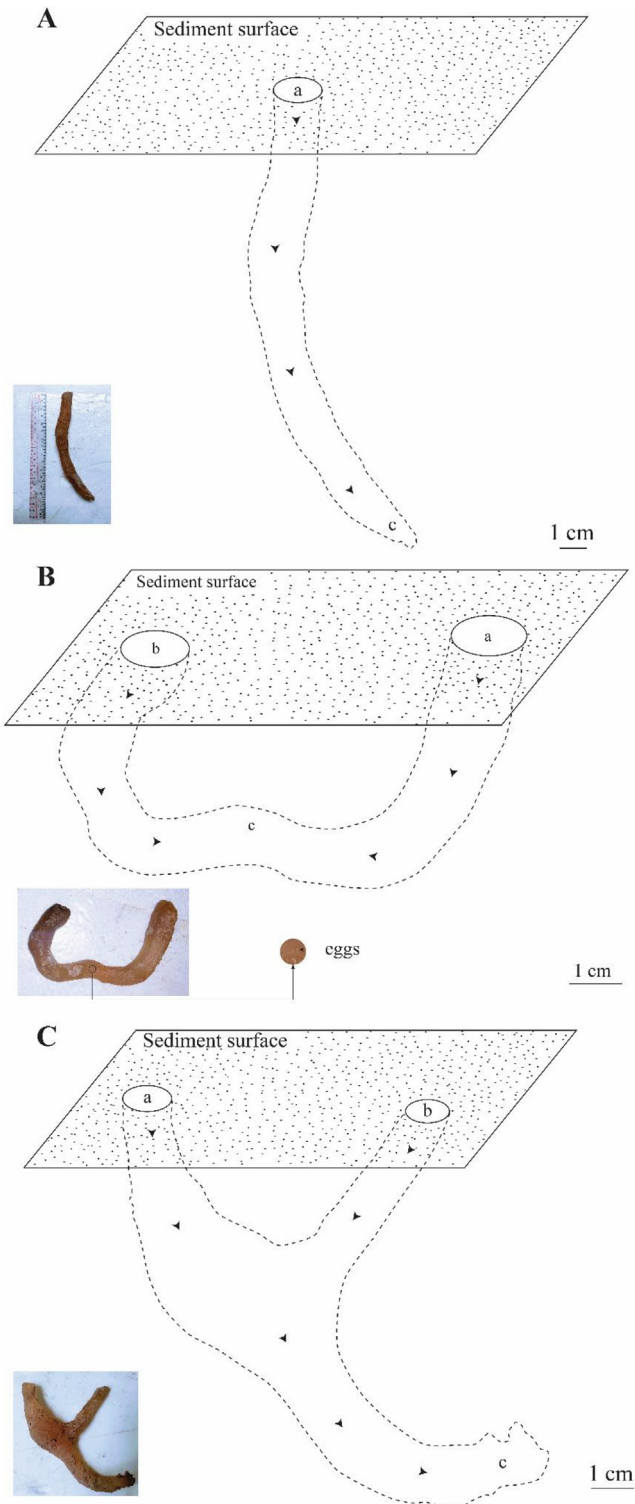
Fig. 3. The burrow drawing of *Periophthalmus chrysospilos* (A-C: J-shaped, U-shaped and Y-shaped burrows; a: opening; b: sub-opening; c: bulbous-chamber; arrowhead: tunnels).

The depth of the burrow in the dry season (17.7 ± 0.5) was not significantly different from that in the wet season (16.3 ± 0.5) (GLM, F = 2.02, p = 0.16, Fig. 5B). Similarly, BL in the dry season (24.3 ± 0.6) was not significantly different from that in the wet season (23.2 ± 0.6) (F = 1.78, p = 0.19, Fig. 5B).

The deepest burrow was found at TDST (18.6 ± 0.80 SE cm), followed by DDCM (17.2 ± 0.9 SE cm), DHTV (16.7 ± 0.6 SE cm), and
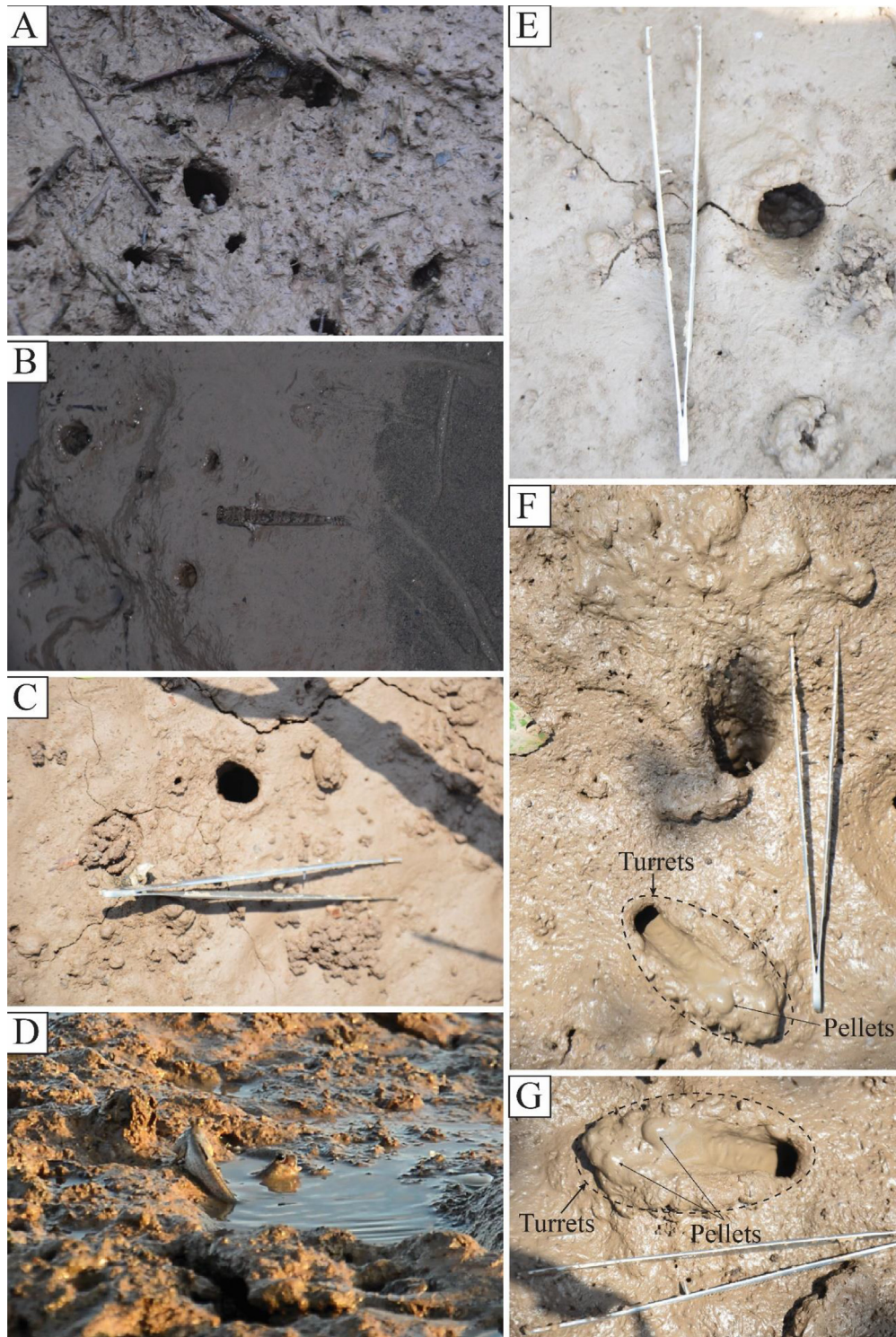
Fig. 4. Burrow openings morphology without mud pellets and turrets (A-E) and with burrow with "footprints", turrets and mud pellets (F-G) (length of forcep: 15 cm).

the shallowest at DHBL (15.1 ± 0.9 SE cm, Fig. 5C) (GLM, F = 2.34, p = 0.04). The longest burrow was found at DDCM (25.7 ± 1.0 SE cm) and TDST (25.1 ± 0.8 SE cm), the shortest at DHBL (22.1 ± 1.2 SE cm) and DHTV (22.7 ± 0.6 SE cm, F = 2.74, p = 0.04) (Fig. 5C). There appeared to be no significant effects in the interaction of season and burrow shape; season and site; shape and site; season, shape and site to BD (GLM, p > 0.05 for all cases, Table 2) and BL (p > 0.05 for all cases, Table 3).

4. Discussion

Preliminary in-situ observations revealed that *Ps. chrysospilos* built and maintained burrows during the day at low tide when the mud surface was quite soft, which was suitable for this fish to burrow. The burrowing at low tide was found in some other small-sized species, e.g., *B. dussumieri* (Clayton & Wright, 1989), whereas in larger species in MD, e.g., *Pn. schlosseri* (Tran et al.,
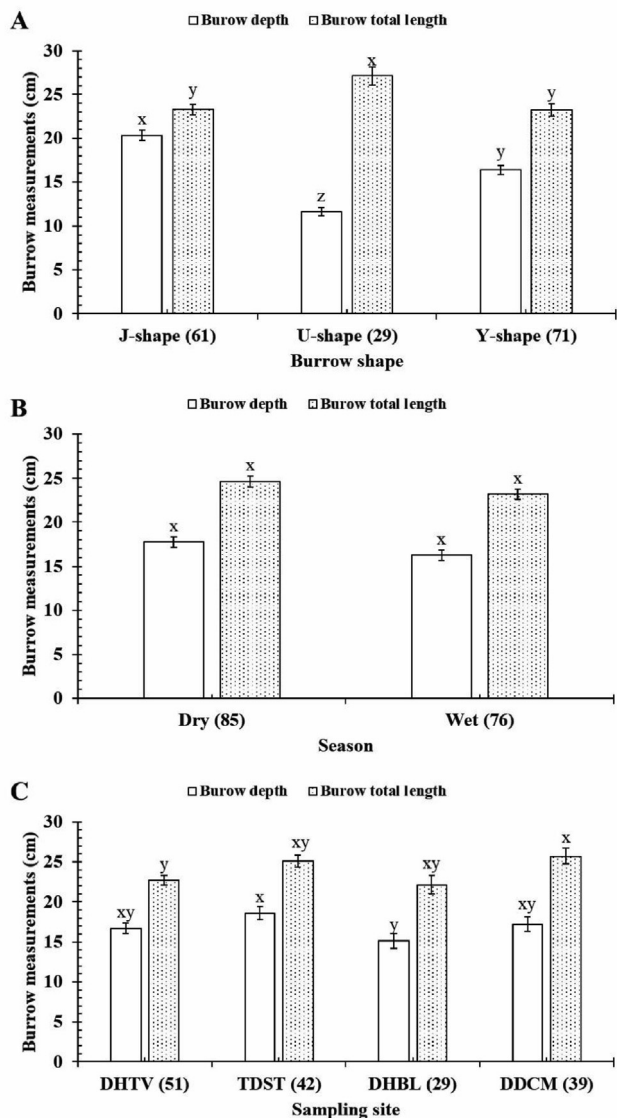
Fig. 5. Burrow depth and total length variations of *Periophthalmus chrysospilos* (A: burrow shape, B: dry-wet pattern seasons C: studied site; number in parentheses: casting burrow number; vertical line: standard error; different letter (x, y and z): significant differences).

2020) and *Pn. septemradiatus* (Dinh et al., 2021b) exhibited various times of burrow building even at high tide. The in-situ observations indicated that *Ps. chrysospilos* use their mouths to excavate

their burrows, and several mud pellets within the shelter were ejected from the fish's mouth. When studying burrow behavior, Tran et al. (2020) found that *Pn. schlosseri* in MD also created burrows by biting off the mud within the burrow and ejecting the mud pellets at the burrow's opening, but the mud pellets were larger than those of *Ps. chrysospilos*. One other congenic mudskipper in the MD, e.g., *Pn. septemradiatus* dig burrows by mouth excavating and body twisting (Dinh et al., 2021b). By contrast, *Pa. seperaster* dig burrow by turning their body into the substrate (Dinh et al., 2014b).

The burrows of *Ps. chrysospilos* in this study were found within mangrove forests and on mudflats adjacent to forested areas. The difference in vegetation (e.g., *Sonneratia caseolaris* were dominant at TDST and DHTV, while *Bruguiera gymnorrhiza* were dominant at DHBL and DDCM) and variation in mud composition between these sites could have accounted for the variation in burrow parameters. The observation and analysis results of the study areas' substrate structure show deeper burrows and more surface grooves when the substrate components are less cohesive. Mud texture and the presence of vegetation do impact burrow morphology and structure; for example, W-shaped burrows made by *Pn. septemradiatus* in Vietnam were found only in two out of five sampling sites (Dinh et al., 2021b).

Precipitation did not affect the burrow structure of *Ps. chyrsospilos* due to a similar depth and total length of burrows between dry and wet seasons. Parameters such as depth and total length in burrows excavated by *Pn. septemradiatus* (Dinh et al., 2021b) showed no seasonal variation; these parameters, however, varied seasonally of *O. lacepedii* in Japan (Gonzales et al., 2008).

Shapes of mudskipper burrows were fixed, with few variations in overall burrow shape within burrowing members of the subfamily (Polgar et al., 2017). However, there were three types of burrows (U-shaped, Y-shaped, and J-shaped) in *Ps. chrysospilos*. The analysis results of the burrow's shape and substrate structure showed that the burrow's shape depends on the structure of the substrate and the flora at the alluvial grounds. At DHTV, alluvial plains with a substrate structure with high alluvium content combined with a thick amount of roots (*Sonneratia caseolari*), there was a tendency to dig J-shaped burrows. Meanwhile, the density of roots in TDST is similar to that of DHTV, but the soil structure here was more porous but not too soft. Therefore, the common types of burrows can be J-shaped or Y-shaped. At the two sites of DHBL and DDCM, the density of roots is relatively sparse compared to DHTV and TDST. However, the clay content in DHBL was relatively high (>50 % of the substrate), leading to a rather hard alluvial deposit in this area. So the Y-shaped was a common structure of fish burrows in this area. At DDCM, there is a suitable for burrowing fish with porous substrates and low density. Thereby, more complex burrows shapes, such as Y-shaped or U-shaped, were common in

Table 2
Results of General Linear Model for burrow depth.

Source	Type III Sum of Squares	df	Mean Square	F	p
Corrected Model	1964.72[a]	23	85.42	5.19	0.00
Intercept	30745.19	1	30745.19	1868.04	0.00
Season	33.26	1	33.26	2.02	0.16
Shape	1265.43	2	632.72	38.44	0.00
Site	115.38	3	38.46	2.34	0.04
Season * Shape	10.88	2	5.44	0.33	0.72
Season * Site	68.17	3	22.72	1.38	0.25
Shape * Site	18.93	6	3.16	0.19	0.98
Season * Shape * Site	25.77	6	4.30	0.26	0.95
Error	2254.81	137	16.46		
Total	50956.17	161			
Corrected Total	4219.54	160			

a. R Squared = 0.47 (Adjusted R Squared = 0.38).

Table 3
Results of General Linear Model for burrow length.

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	1078.11[a]	23	46.87	1.67	0.04
Intercept	71010.49	1	71010.49	2527.17	0.00
Season	49.82	1	49.82	1.77	0.19
Shape	286.39	2	143.19	5.10	0.01
Site	230.77	3	76.92	2.74	0.04
Season * Shape	21.23	2	10.62	0.38	0.69
Season * Site	175.63	3	58.54	2.08	0.11
Shape * Site	18.95	6	3.16	0.11	0.99
Season * Shape * Site	91.86	6	15.31	0.54	0.77
Error	3849.53	137	28.10		
Total	97275.25	161			
Corrected Total	4927.64	160			

a. R Squared = 0.22 (Adjusted R Squared = 0.09)

the remaining areas. That showed the shape of the fish burrow was highly dependent on the substrate, demonstrating that in several previous studies. As studying the burrow morphology of *Pn. septemradiatus*, Dinh et al. (2021b) found the W-shape burrow only at two sites (An Lac Tay and Long Duc in Soc Trang province) out of a total of six sample dictionaries because the flora in these two sites was broader than in the rest of the areas. However, compared to the flora in DHTV, the plants in these two sites had deeper root systems in the lower soil, topsoil, and alluvial soils, leaving more space for fish to burrow. However, in larger fish such as *Pn. schlosseri* (Tran et al., 2020) did not find a change in burrow shape according to the study site. The difference from the results in this study may be due to *Pn. schlosseri* was significantly larger than *Ps. chrysospilos*, resulting in stronger burrowing in this fish and less dependence on substrate conditions. From these examples, it can be seen that the shape of burrows in fishes of the mudskippers group depends not only on the environmental conditions but also on the condition of the fish. The U-shaped, Y-shaped, and J-shaped burrows found in this fish were common burrows for fish in the Oxudercidae family. For example, the U-shaped burrows were also constructed by other mudskipper species, e.g., *B. boddarti* (Dinh et al., 2014a), *Pn. septemradiatus* (Dinh et al., 2021b), and *Ps. variabilis* (Rupp, 2021). Y-shaped burrows were constructed by *Pd. elongatus* (Tran, 2008), *Ps. argentilineatus*, *Ps. modestus* (Kobayashi et al., 1971), and *Ps. waltoni* (Clayton & Snowden, 2012). Similar to *Ps. chrysospilos*, *Ps. minutus* (Takeda et al., 2012), *Ps. variabilis* (Rupp, 2021) and *Pn. septemradiatus* (Dinh et al., 2021b) also excavates J-shaped burrows. In addition to these common burrow shapes, there were also some other special burrow shapes not recovered for *Ps. chrysospilos* in this study. For example, W-shaped burrowshad been reported for burrows of *Pa. serperaster* (Dinh et al., 2014b), and *Pn. septemradiatus* (Dinh et al., 2021b). In addition, there were L-shaped burrows excavated by *Ps. argentilineatus* and *Ps. modestus* (Kobayashi et al., 1971).

The number of openings depends on the behavior of each fish; indeed, the burrows of *Ps. chrysospilos* in this study had 1–2 openings that could lead to a simple burrow shape. According to our observations, this mudskipper stayed outside burrows and used burrows for egg releasing and hatching. Like many other fish species, they also mainly used burrows to protect their eggs *Ps. modestus* (Kobayashi et al., 1971), *Pd. Elongatus* (Tran, 2008), *Ps. variabilis* (Rupp, 2021). With a large number of openings as in some species, e.g., *Ps. minutus* (Takeda et al., 2012), *Pa. serperaster* (Dinh et al., 2014b), and *Pn. septemradiatus* (Dinh et al., 2021b), there were up to 4 openings, showing that the burrow was not only used as a place to lay eggs, but the burrow was also used as a hiding tool for fish to catch prey. Therefore, the burrow structure was more complex in these fishes and had more openings (Table 4).

The larger fish could create more complicated burrows; indeed, due to the small size compared to other species of the Oxudercidae

family, the burrows of *Ps. chrysospilos* were simpler than others. The results of the comparison of burrow size (BD and HL) in this fish compared with other fish of the Oxudercidae family are shown in Table 4. This table shows that the burrow size of this fish species was assessed as one of the smallest of the studied species and only more significant than the species *Ps. waltoni* (Clayton & Snowden, 2012). Comparing to other congenic species, e.g., *Ps. argentilineatus*, *Ps. modestus* (Kobayashi et al., 1971), *Ps. minutus* (Takeda et al., 2012), and *Ps. variabilis* (Rupp, 2021), showed the burrow of *Ps. chrysospilos* was 1.0 to 1.5 times smaller. Indeed, burrow openings of *Ps. chrysospilos* were fewer than those reported for *Ps. modestus* (Kobayashi et al., 1971), *Pd. elongatus* (1–3 openings) (Tran, 2008), *Ps. minutus* (2–3 openings) (Takeda et al., 2012), *Pa. serperaster* (2–4 openings) (Dinh et al., 2014b), *Ps. variabilis* (1–3 openings) (Rupp, 2021), and *Pn. septemradiatus* (1–4 openings) (Dinh et al., 2021b). Like *Ps. chrysospilos*, burrows made by *B. boddarti* had 1–2 openings (Dinh et al., 2014a). The BD of *Ps. chrysospilos* burrows was shallower than reported for *Pd. elongatus* (Tran, 2008) and *Pn. schlosseri* (Tran et al., 2020), the latter almost three times deeper. The BD of burrows constructed by *B. boddarti* (Dinh et al., 2014a) and *Pa. serperaster* (Dinh et al., 2014b) were about 1.5 times deeper than those made by *Ps. chrysospilos*. However, BD of burrows made by *Pn. septemradiatus* (Dinh et al., 2021b) was shallower than those by *Ps. chrysospilos*. Another burrow parameter, BL, was found to be four times shorter in burrows of *Ps. chrysospilos* than those of *Pd. elongatus* (Tran, 2008), *B. boddarti* (Dinh et al., 2014a), *Pa. serperaster* (Dinh et al., 2014b), and *Pn. schlosseri* (Tran et al., 2020); and 1.5 times shorter than those of *Pn. septemradiatus* (Dinh et al., 2021b). Comparisons with burrow dimensions of congeners reveal that BD of burrows of *Ps. chrysospilos* is smaller than those of *Ps. argentilineatus*, *Ps. modestus* (Kobayashi et al., 1971), *Ps. minutus* (Takeda et al., 2012), and *Ps. variabilis* (Rupp, 2021). However, this value was larger than *Ps. waltoni* (Clayton & Snowden, 2012). The variation in burrow parameters of *Ps. chrysospilos* and other burrowing gobies suggest that to an extent, burrow dimensions were species-specific.

The size of the fish burrow depends not only on the species or body size of the fish but also on the substrate structure and vegetation at each site. In this study, it was found that the burrow depth and burrow length of the burrow changed markedly across four different areas. The deepest burrows were recorded at DHTV and the shallowest in DHBL. Although the substrate structure in DHTV was not too hard, because the flora here was mainly *Sonneratia caseolari*, it leads to a relatively high root density. Space width is limited, so fish often burrow deeper than other areas. Meanwhile, in HDBL, the space on the mudflat was not as limited as in DHTV, but the soil structure was hard, so it was difficult for fish to dig deep burrows. In terms of burrow length, the highest value was recorded at DDCM and TDST and the lowest value in TVST and DHBL. The reason for this difference was that the substrate

Table 4
Burrow morphometrics of *Periophthalmus chrysospilos* and confamilial species.

No. of casts	No. of openings	Presence of Mound	Depth of burrow (cm)	Total length of burrow (cm)	Taxon	Reference
9	2–7	Yes	41.1 ± 23.7	1326.1 ± 1093.5	*Odontamblyopus lacepedii*	Gonzales et al. (2008)
10	1–2	No	23.6 ± 8.4	68.1 ± 15.9	*Boleophthalmus boddarti*	Dinh et al. (2014a)
24	2–4	No	22.3 ± 4.7	81.5 ± 30.1	*Parapocryptes serperaster*	Dinh et al. (2014b)
30	1–2	Yes	47.7 ± 2.8	88.6 ± 12.4	*Periophthalmodon schlosseri*	Tran et al. (2020)
73	1–3	Yes	10.5 ± 0.1	32.1 ± 1.5	*Periophthalmodon septemradiatus*	Dinh et al. (2021b)
–	1–2	Yes	30.0–75.0	–	*Periophthalmus argentilineatus*	Kobayashi et al. (1971)
–	1–3	Yes	25.0–30.0	–	*Periophthalmus modestus*	Kobayashi et al. (1971)
46	2	Yes	10.0	–	*Periophthalmus waltoni*	Clayton & Snowden (2012)
50	2–3	Yes	25.0–30.0	33.4 ± 6.8	*Periophthalmus minutus*	Takeda et al. (2012)
–	1–3	Yes	20.0	–	*Periophthalmus variabilis*	Rupp (2021)
161	1–2	Yes	17.0 ± 0.4	23.9 ± 0.4	*Periophthalmus chrysospilos*	This study

structure in DDCM and TDST was quite porous, which was favourable for fish burrowing. Meanwhile, DHTV was limited by the density of roots and DHBL was limited by the porosity of the substrate, so the burrow structure was shorter than the other two sites. Thereby, the suitable *Ps. chrysospilos* for burrowing in alluvial areas have a soft substrate structure and not too high root density.

Burrow openings of *Ps. chrysospilos* are oval, similar to those reported in *O. lacepedii* (Gonzales et al., 2008), *B. boddarti* (Dinh et al., 2014a), *Pn. septemradiatus* (Dinh et al., 2021b), *Ps. minutus* (Takeda et al., 2012), *Ps. variabilis* (Rupp, 2021), and *Ps. waltoni* (Clayton & Snowden, 2012). U-shaped burrows of *Ps. chrysospilos* never contained bulbous egg chambers, also reported for *Pn. septemradiatus* (Dinh et al., 2021b).

Field observations suggested that *Ps. chrysospilos* came out of burrows to forage and used burrows in avoiding predators since they entered the burrows head-first. These burrow utilizations were also observed in *Pn. septemradiatus* in MD (Dinh et al., 2021b). The use of burrows as shelters was recorded in some gobies, e.g., *O. lacepedii* living in Japan (Gonzales et al., 2008) and *B. boddarti* habiting in MD (Dinh et al., 2014a). As eggs were found in the bulbous chambers of the casting burrow, *Ps. chrysospilos* used the burrow as a place for releasing eggs. This role of burrows was in some oxudercine species living in and out MD, e.g., *Pn. schlosseri* (Ishimatsu et al., 2018) and *B. boddarti* (Dinh et al., 2014a) and *Pn. septemradiatus* (Dinh et al., 2021b).

5. Conclusion

Mudskippers, and many other closely related gobioid taxa, use burrows in complex ways. In addition to areas of shelter and respite, burrows are also sites for egg deposition and development. The common burrow shapes in *Ps. chrysospilos* was J-shape, Y-shape, and U-shape. The fish burrow shape and size varied and depended on the substrate conditions in each area. Rainfall and flora in the study areas influence the burrow structure in this fish. The burrow of *Ps. chrysospilos* has a simple structure, a small size with 1–2 openings, an average burrow length of 23.9 ± 0.4 cm, and an average burrow depth was 17.0 ± 0.4 cm. The variety in burrow shapes and disparity in burrow parameters underpins the importance of elucidating burrow structures to understand these fishes' ecology better.

Ethics approval

This research was conducted with approval from The Scientific Committee of the School of Education, Can Tho University (No. BQ2021-05/KSP) after animal welfare assessment.

Declaration of Competing Interest

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

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