Reproductive ecology of two species of the mudskippers, *Periophthalmodon septemradiatus* and *Periophthalmus modestus*

マッドスキッパー2種 *Periophthalmodon septemradiatus* と *Periophthalmus modestus* の再生産生態

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第 1 章 General introduction

Mudskippers are amphibious fish in the subfamily Oxudercinae of the family Gobiidae. Mudskippers typically live in intertidal habitats and exhibit unique adaptations to this environment that are not found in most other fishes.[1] Mudskippers are quite active when out of water, feeding and interacting with one another, for example, to defend their territories and court potential partners. Studies of mudskippers could provide important insight into the vertebrate transition from water to land, which is believed to have occurred in the Devonian period, one of the most important evolutionary events in vertebrate evolution.

第 2 章 Reproductive ecology of *Periophthalmodon septemradiatus* in the Mekong Delta

Since 2015 I have conducted field surveys along the Hau River and Tien-Co Chien River, two major tributaries of the Mekong River, from the river mouth up to the Cambodian border. Through the field survey, I found that one species of mudskippers, *Periophthalmodon septemradiatus*, inhabits over a 150 km stretch of the riparian zones along the Mekong River in Vietnam. The fish was mainly distributed in tributaries and rarely found in the mainstream of the Hau River and Tien-Co Chien River, except in the coastal areas. Adult fish are highly terrestrial and have not been observed to venture into the water during my survey. Courtship behavior was observed, and fertilized eggs were recovered from burrows in both brackish and freshwater environments. The smallest fish collected at 12, 96, and 148 km from the river mouth were juveniles shortly after starting an amphibious life. These findings suggest reproduction in both brackish and freshwater environments. In contrast, otolith Sr:Ca ratio indicates larval hatching only in brackish water. Analysis of a 940-base pair (bp) segment of the mitochondrial cytochrome c oxidase subunit II and a 934-bp segment of the mitochondrial D-loop demonstrated no genetic segregation between populations.

第 3 章 Reproductive ecology of *Periophthalmus modestus* in the Ariake Sea

Periophthalmus modestus breeds from May to August, in the Ariake Sea. Reproductive behaviors were partly reported earlier, but there are still uncertainties as to how *P. modestus* spawns and fertilizes the eggs in a burrow. This study was conducted in the Fukushoe River, Saga Prefecture, Japan in 2016. Reproductive behaviors from burrow entrance to the departure of females were recorded and analyzed on five pairs of *P. modestus*. In those cases where spawning was suspected based on a drastic reduction in the distension of the female's belly (N = 4), the female remained inside the burrows for 4-6 hours without emersion. In contrast, the male often but irregularly emerged from his burrow while the female was in his burrow. When outside his burrow, the male largely remained around burrow openings and protected the burrow from conspecifics and crabs. During burrow cohabitation of the pair, which started soon after mudflat emersion, burrow openings were often clogged by mud from inside. After the female left the

burrow, the male remained inside the burrow for variable periods (several minutes to longer than 1 hour). Then, the male guarded the burrow for about one week. Upon termination of the one-week burrow guarding, the male began excavating a new burrow near the previous one or renew the previous burrow.

第 4 章 General discussion and future direction

Reproduction in mudskippers is enigmatic because it occurs within burrows excavated in the mudflat to which it is quite difficult for a researcher to access. Our finding of *Pn. septemradiatus* inhabiting and spawing in the freshwater river banks has added further complicacy to the question.

What limits the distribution of Periophthalmodon septemradiatus? The distribution of *Pn. septemradiatus* is unique not only because of its extended range into upstream direction but also restricted occurrence along the coastal tidal flats. To gain a clue to understanding factors limiting the upstream distribution of *Pn. septemradiatus*, I measured the most basic environmental parameters within and beyond the distribution range of the species. None of the measured parameters seems to be responsible for preventing the fish from migrating beyond the observed distribution range. Some limitation that restrict successful reproduction might be involved. Larval hatching, growth and survival at various salinity conditions must be tested controlled conditions in the laboratory. The swimming ability of the larvae and juveniles must also be investigated.

Where does the fish reproduce, in freshwater, in coastal areas, or both? The courtship display was found from all observation sites from the coast to the upper limit of distribution. These results support that the fish reproduce in both freshwater and coastal areas. On the other hand, the Sr:Ca ratio showed that all the samples (coastal and freshwater samples) were spawned in high salinity conditions because Sr:Ca is much higher than 8.4 (this is presented by de Villiers, 1994). This suggests that all the fish hatched in freshwater sites were failed. More field sampling on the distribution of larvae and juveniles needs to be done together with environmental conditions.

Pn. septemradiatus is probably one of the most terrestrial fishes living today. During four years of our field survey in the Mekong Delta, I did not see *Pn. septemradiatus* ventures into the water. The high terrestriality of *Pn. septemradiatus* is further supported by the finding that ants, *Dolichoderus* sp., constituted a substantial portion of the total gut content (> 80% by biovolume analysis). This agrees with the hypothesis that fish come out of the water to find/explore new food resources. Detailed investigation into the feeding ecology is necessary to reveal resource utilization of this fish in terrestrial environment near the river.

There are many unanswered questions on the reproductive ecology of mudskippers. *P. modestus* could be a model species in this respect because of relatively easy accessibility and ample background information. Three important points during burrow resident time of *P. modestus* include;

(1) Why female fish did not come out from the burrow eggs for extended periods? One possibility is that females were not ready for spawning when they entered a burrow. An unpublished study by Prof. Takita showed that eggs were not ovulated in females foraging on the mudflat surface. Sampling of female fish is needed at various time after the fish enter the burrow to clarify this point. (2) Why they clog the burrow opening(s) during spawning? This could be to avoid the invasion of other animals living in the mudflat, including conspecifics. I observed in some cases that another non-burrow guarding male entered a burrow possibly to consume or fertilize the laid eggs, and it caused the female to leave the burrow. We need to analyze the behavior of a male and a female fish within the burrow. (3) Do male fish add air before or after spawning? After female fish left the burrow, male fish stayed inside the burrow for a while. What did male fish do during this time? It is hard to observe inside the fish burrow but we may able to observe the egg chamber by setting up an endoscope. By chance, I observed in a laboratory aquarium that a male *P. variabilis* added air only after spawning but this may be species-specific (unpublished data). To confirm this behavior we need to excavate and sample the air in the burrow chamber right after the female fish leaves the burrow or to observe with an endoscope installed in a spawning chamber with minimal disturbance to the natural behavior of the fish within the burrow.