Downstream drifting of *Macrobrachium* (Decapoda: Palaemonidae) larvae in the Shimanto River, Japan

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**Abstract:** This study was undertaken to clarify the downstream drifting of *Macrobrachium* larvae in the Shimanto River by 24-hour sampling. Stage I zoeae accounted for most larvae collected in fresh and brackish waters. *M. formosense* and *M. japonicum* dominated in brackish and fresh waters, respectively, while *M. nipponense* occurred only in brackish waters. The amount of yolk droplets of *M. formosense* and *M. japonicum* reduced over time in freshwater. However, no larvae that had completely used up their yolks were observed. Even though *M. japonicum* larvae drifted from farther upstream than those of *M. formosense*, they had a higher residual amount of yolk droplets. Therefore, long-distance and long-time drifting in *M. japonicum* may be enabled by delaying the absorption rate of droplets.

**Key words:** developmental stage, downstream migration, *Macrobrachium* larvae, starvation tolerance, yolk droplet

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**Introduction**

While most of the subfamily Palaemoninae (Decapoda: Palaemonidae) inhabit the marine environment, the genus *Macrobrachium* is mostly adapted to diverse environments in inland water bodies (Mashiko 2011). *Macrobrachium* are often important resources in local inland waters (Bauer 2004). Fifteen *Macrobrachium* species are known to occur in Japan (Hayashi 2011), of which the important fisheries species are *Macrobrachium formosense* Bate, 1868, *Macrobrachium japonicum* (De Haan, 1849), and *Macrobrachium nipponense* (De Haan, 1849) (Hiraga 2019). These three species are amphidromous (Suzuki & Sato 1994, Suzuki & Kusamura 1997, Yoshigou 2002, Ito et al. 2003). The larvae of amphidromous shrimps require saline waters for development, hence they drift to an estuary or coastal waters after hatching (Hayashi 2000, Mashiko 2011, Bauer 2013).

Reared larvae of the three *Macrobrachium* species mentioned above metamorphose into postlarvae after nine zoal stages (Kwon & Uno 1969, Shokita 1970, Morizane & Minamizawa 1971). The survival times of stage I zoeae of the three species are the longest in water of salinity 14 (Ohno & Armada 1999). The rate of metamorphosis into postlarvae of *M. nipponense* is higher at a salinity of 10–26 (Ogasawara et al. 1979, Imai et al. 2001). On the other hand, *M. formosense* and *M. japonicum* larvae hatch and reach the estuary within about 2 hours after sunset in the Nishida River (4.5 km long), Yamaguchi Prefecture, Japan (Ideguchi et al. 2007). However, in the Shimanto River (196 km), females of both species incubating eggs about to hatch also inhabit areas 40 km or more from the river mouth (Hiraga 2020). It would seem almost impossible for the larvae of both species hatched at a 40-km or greater distance from the river mouth to reach the estuary in a short time. In general, stage I zoeae of amphidromous caridean shrimps are lecithotrophic. Therefore, they must reach saline waters where they molt to stage II and the commencement of feeding is triggered before using up nutrients (yolk droplets) (Bauer 2013). Although the larvae hatched upstream are expected to be at higher risk of starvation during downstream migration, there are no existing studies to confirm this.

Much about the ecology of the *Macrobrachium* larvae in the field remains unclear. Information on the process...
of drift downstream is useful for elucidating the early life history. In addition, catches of Japanese *Macrobrachium* prawns are steadily declining (Hiraga 2019). It is important to clarify the early life history, which is generally considered to have extremely high mortality, also from the viewpoint of conservation. Therefore, we carried out this study to clarify the downstream migration process of *Macrobrachium* larvae.

**Materials and Methods**

The survey was carried out nine times at 3-hour intervals from August 30 to 31, 2019 at D1–D3 (Fig. 1) in the Shimanto River, Kochi Prefecture, Japan. The Nakasuji River and Ushiro River, which are tributaries of the Shimanto River, join at 3 km and 5.5 km upstream from the river mouth, respectively. The sampling was started between 1–2 hours after sunset. D1 was located 4 km from the river mouth in brackish waters. The upper limit of brackish waters is at 6–7 km from the river mouth (Inouuchi et al. 2006). D2 and D3 were located in freshwater at 15 km and 34 km from the river mouth, respectively. The river width at D1, D2, and D3 was about 70, 170, and 340 m, respectively. Maximum water depth at each station was about 2 m. The larvae were collected using a conical plankton net (212-µm mesh, 30-cm diameter, 80-cm length) equipped with a flow meter (Rigosha 5571-A). Samples were collected at D1, located in brackish waters, by an oblique tow for collection from the river bottom to surface, because laminar flow conditions were expected. A float and weight were attached to the net, and the length of the rope connecting the float was adjusted according to the water depth (Fig. 2). Sampling was done once at each collection time by hauling the net from the center of the flow to the bank. The hauling distance was ca. 100 m in every sampling. At D2 and D3 located in freshwater, samples were collected by fixing the net for 3–5 minutes at the center of the riffles where turbulent flow conditions were expected (Fig. 2). Water temperature and salinity at intervals of 0.5-m depths from the surface layer at D1 were measured using a salinometer (YSI Model 30). The surface water temperatures at D2 and D3 were measured using a thermometer (Tanita TT-508N).

Collected samples were transported to the laboratory in refrigerated conditions after fixing in 5% formalin. Then, they were preserved in an incubator and maintained at about 5°C with light-shielding. The number of *Macrobrachium* larvae were counted by each species and developmental stage within 2 weeks after collection. At the same time, the yolk droplet index was defined as shown in Fig. 3 and recorded. Species identification for stage I and II zoeae was done based on the chromatophore distribution pattern described by Wada et al. (2001). We previously confirmed that chromatophores of zoeae are maintained for ca. 1 month by the above sample storage method (unpublished data). No larvae that could not be identified due

![Fig. 1. Map showing the study stations in the Shimanto River. Numerals beside each station number indicate distance (km) from the river mouth. D1: 32°57′42″N 132°58′23″E; D2: 33°00′55″N 132°53′07″E; D3: 33°05′50″N 132°48′07″E.](image-url)
to the disappearance of chromatophores occurred in this study. Stages I–III were identified as follows: Stage I, sessile eyes, uropods not free; stage II, stalked eyes, uropods not free; stage III, stalked eyes, uropods free. Stage III–IX zoeae were identified by referring to the morphological descriptions reported by Kwon & Uno (1969), Shokita (1970), Morizane & Minamizawa (1971), and Shy et al. (1987, 1990). *Palaemon* larvae are morphologically similar to *Macrobrachium* larvae and were distinguished by the elongate rostrum and a dorsal spine on the third abdominal segment as reported by Little (1969), Shokita (1977), Han & Hong (1978), and Yang (2009). Density (n·m⁻³) was calculated by dividing the number of larvae collected by the volume of filtered water. Statistical analysis was performed with Microsoft Excel or EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is
a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0) (Kanda 2013). In order to select a test method, a test of normality using EZR was conducted. The daily mean discharge (m$^3$s$^{-1}$) on the survey date at the most downstream Gudo Second gauging station was obtained from the Ministry of Land, Infrastructure, Transport and Tourism website (http://www1.river.go.jp/). Furthermore, water level data at Sanzaki gauging station in brackish waters were also obtained from the same website.

Results

The daily mean discharge (m$^3$s$^{-1}$) at Gudo Second gauging station decreased slightly from 115.38 on August 30 to 97.3 on 31. Water temperature (°C) at D3 and D2 fluctuated in the ranges of 22.9–23.6 and 23.0–23.8, respectively. On the other hand, water temperature and salinity at D1 fluctuated in the ranges of 23.4–26.9 and 0.1–32.0, respectively (Fig. 4); temperature and salinity increased from the surface to the bottom layer.

Larvae of *Macrobrachium formosense*, *Macrobrachium japonicum*, and *Macrobrachium nipponense* were collected. *Macrobrachium formosense* and *M. japonicum* occurred at all three stations, while *M. nipponense* occurred only at D1. Most of the individuals were stage I zoeae. However, one stage IV zoea, which could not be identified to species level because of significant damage, was collected at D1. In addition, three juveniles of *M. formosense* (2.0–2.1 mm carapace length) and 10 juveniles of *M. nipponense* (1.1–1.8 mm) were collected at D1. Mean densities (n·m$^{-3}$) of stage I zoeae at D3, D2, and D1 were 1.03, 5.23, and 2.83, respectively (Fig. 5). No normality was found for any of the density data by species at each station (Shapiro-Wilk test, $p<0.05$). At D2 and D3, the densities of *M. japonicum* were significantly higher than those of *M. formosense* (Welch’s $t$-test, $p<0.05$), accounting for more than 90% of collected larvae. In contrast, *M. formosense*, *M. japonicum*, and *M. nipponense* accounted for 54%, 36%, and 10% of collected larvae at D1, respectively, and no significant difference existed among the densities of three species (Kruskal-Wallis test, $p>0.05$).

Fig. 6 shows the comparison between temporal changes in density of the first zoeal larvae in the three *Macrobrachium* species at D1–D3. The densities of all three species

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**Fig. 4.** Temporal changes in vertical profiles of water temperature and salinity during 24-hour sampling at D1 in the Shimanto River. Water level (Tokyo Peil, T.P.) data at gauging station were cited from the website of Ministry of Land, Infrastructure, Transport and Tourism.

**Fig. 5.** Mean densities of the first zoeal larvae of the three *Macrobrachium* species during 24-hour sampling at D1–D3 in the Shimanto River.

**Fig. 6.** Comparison between temporal changes in density of the first zoeal larvae of the three *Macrobrachium* species during 24-hour sampling at D1–D3 in the Shimanto River.
were higher in the nighttime than in the daytime, with most peaks observed between 22:00–0:00. Thereafter, the densities declined until dawn, and remained low during the day, but rose again after sunset.

Fig. 7 shows the temporal changes in the yolk droplet index (hereinafter index) of stage I zoea by species. The indices ranged from 1 to 3 for all three species. Larvae with complete yolk droplet absorption (index 0) were not observed. Most M. formosense larvae collected at D3 indicated index 3, and no larvae indicated index 1. The proportion of index 3 at D2 decreased from 75% on 30th at 19:30 to 7% on 31th at 1:30. Thereafter, larvae at index 3 were not observed between 4:30 and 16:30, but accounted for 40% at 19:30. No clear trend was found in the temporal changes of the indices of M. formosense at D1, and the proportion of index 3 was higher at D1 than at D2. Macrobrachium japonicum larvae collected at D3 mostly indicated index 3, and did not indicate index 1. The proportion of index 3 at D3 decreased from 100% on 30th at 19:30 to 60% on 31th at 10:30. Whereas the larvae did not occur between 13:30 and 16:30, they occurred at 19:30, and all indicated index 3. The proportion of index 3 at D2 declined from 90% on 30th at 19:30 to 11% on 31th at 4:30 and 7:30. No M. japonicum indicating index 3 occurred between the 31th at 10:30 and 16:30, but most M. japonicum collected at 19:30 indicated index 3. The larvae collected at D1 mostly indicated index 3, at a higher proportion than D2, and no clear trend existed for temporal changes in the indices. Most M. nipponense collected only at D1 indicated index 2 or index 3. Temporal changes of those indices were unclear.

Discussion

Almost all the larvae collected in freshwater (D2 and D3) were Macrobrachium japonicum (Fig. 5). In contrast, most of the larvae collected in brackish waters were Macrobrachium formosense. In the Shimanto River, ovigerous females of M. japonicum inhabit freshwater, whereas those of M. formosense inhabit brackish waters as well (Hiraga 2020). Based on this, it is supposed that a considerable number of M. formosense larvae had hatched downstream from D2 (15 km from the river mouth).

The larval density during 24-hour sampling was higher at night than in the day (Fig. 6). This tendency is known in migratory shrimp larvae (March et al. 1998, Ramirez & Pringle 2001, Ideguchi et al. 2007) and aquatic insects (Flecker 1992, Ramirez & Pringle 2001). Moreover, it has been suggested that nocturnal migration is an adaptation to avoid predation by visual predators. Incidentally, in the Nishida River (4.5 km), most M. formosense and M. ja-
& Armada 1999). Among the three Macrobrachium species inhabiting the Shimanto River, M. japonicum is the one that migrates upstream the furthest (Ohno et al. 1977, Hiraga 2019), where it seems to breed and hatch (Hiraga 2020). These distribution and breeding characteristics of this species might be related to its morphology, which suggests high mobility (Kamita 1961), and the relatively high starvation tolerance of its hatching larvae described above. For example, Macrobrachium amazonicum (Heller, 1862) distributed in Brazil inhabits shallow lentic waters as well as coastal rivers and estuaries. The amount of yolk droplets in their newly hatched larvae is lower in the population of lentic waters than in the other (Anger & Hayd 2010). This is thought to be because highly productive lentic waters are not food-limited. In addition, there is genetic variability between these populations, which may lead to speciation in the future (Vergamini et al. 2011). This suggests that M. amazonicum inhabiting lentic waters might be in the process of speciation, changing their traits to adapt to less larval food-limited environments. On the contrary, M. japonicum may have acquired high starvation tolerance of larvae during the process of expanding its distribution upstream where food-limitation for the larvae is stronger. One of the reasons why M. japonicum has expanded its distribution upstream may be concerned with changes of environmental requirements with growth. Macrobrachium japonicum juveniles and adults inhabit pore spaces between the gravel of riffled bottoms during the day (Kamita 1961, Ohno et al. 1977) and they are less abundant in areas with smaller spaces (Ohno et al. 1977). The space required for habitat must increase with increasing body size. Suitable space for larger individuals is more easily found in the upstream areas where the gravel size is relatively large. In addition, the body size of M. japonicum is larger upstream (Hiraga 2019). This suggests that the expanding distribution upstream may have implications for migration to more suitable habitats according to growth.

This study revealed some ecological aspects of the larval downstream migration of three Macrobrachium species in the Shimanto River, one of the larger Japanese rivers. The information obtained in this study should contribute to the conservation of Macrobrachium prawn resources with a high market value in the Shimanto River. Future studies under relatively low flow conditions, in which larval drift times are longer, will provide more detailed information on their starvation tolerance.

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