Settlement patterns of two sesarmid megalopae in the Sai River Estuary, Ishikawa Prefecture, Japan

Takehiro Matsumoto1,†,*; Hiroaki Arakawa2; Takaya Murakami3 & Seiji Yanai1

1Department of Environmental Science, Ishikawa Prefectural University, Nonoichi, Ishikawa 921–8836, Japan
2Division of Sciences for Bioproduction and Environment, Ishikawa Prefectural University, Nonoichi, Ishikawa 921–8836, Japan
3Division of Environmental Science, Ishikawa Prefectural University, Nonoichi, Ishikawa 921–8836, Japan

†Present Address: Graduate School of Human Development and Environment, Kobe University, 3–11 Tsurukabuto, Nada-ku, Kobe, Hyogo 657–8501, Japan

Received 13 April 2020; Accepted 7 September 2020 Responsible Editor: Shinji Shimoda
doi: 10.3800/pbr.15.306

Abstract: Two semi-terrestrial crab species, Chiromantes haematocheir and Chiromantes dehaani, are closely related and have a sympatric distribution. The ecological characteristics of these species, including habitat and behavior during the adult stage, have been investigated. However, the larval stage remains poorly understood because it is difficult to identify larvae morphologically. Our study objectives are to clarify the settlement patterns involved in recruitment of the two species of megalopae using genetic analysis and the effects of ecological factors on settlement, including 1) seasonal changes, 2) spatial variations (dominated by conspecific or heterospecific adults), and 3) tidal effects. The Chiromantes megalopae were collected in the lower reach of the Sai River during spring and neap tides from August to November 2017. Megalopa larvae were collected from three areas: one dominated by C. haematocheir adults, one dominated by C. dehaani adults, and a revetment area. Both species of megalopae showed seasonal and spatial variations in settlement, which also differed with tidal fluctuations. Chiromantes dehaani megalopae settled from mid-August to early October and C. haematocheir megalopae settled from early September to early November. Chiromantes haematocheir megalopae preferentially settled in the area dominated by conspecific adults over the area dominated by C. dehaani and the revetment area, while no difference in the settlement of C. dehaani megalopae was observed among areas. Chiromantes haematocheir megalopae returned on the spring tide; however, C. dehaani megalopae showed no tidal pattern.

Key words: brackish water area, megalopa larvae, recruitment processes, semi-terrestrial crab

Introduction

The semi-terrestrial crabs, Chiromantes haematocheir (De Haan, 1833) and Chiromantes dehaani (H. Milne Edwards, 1853) are closely related species of Sesarmidae. In Japan, these crabs are distributed in vegetated land along estuaries (Hashiguchi & Miyake 1967b, Kobayashi 2000). They can be found sympatrically in river systems. However, the adult habitats of the two species differ slightly: C. haematocheir inhabits mainly inland forest areas, while C. dehaani occurs mainly in areas near water, such as wetlands (Matsuda & Kamihogi 2004, Ito et al. 2011). The adults of both species migrate to riverbanks or coasts from July to September, where they release zoea larvae (Hashiguchi & Miyake 1967b). The zoea larvae are transported to coastal waters and then return to brackish areas during growth to the megalopa larval stage (Kobayashi 2000). Megalopae settle on riversides and shift from a free-floating to benthic lifestyle; after transformation into juveniles, they move from water to land habitats (Pawlik 1992, Anger 2006). The megalopa settlement process involves selecting a promising habitat and then metamorphosing into a juvenile crab (Rodriguez et al. 1993). After metamorphosis, most juvenile crabs grow into adults in the area where they first settled, although some move to more suitable habitats after growth (Wada 1983, Kobayashi 2006). The natural recruitment process, especially settlement behavior, which
is the beginning of a benthic lifestyle in a suitable environment, is a major factor affecting the population persistence and distribution of Decapoda species with a dispersive larval stage (Castejón et al. 2019). Settlement patterns are known to differ among species (Epifanio et al. 1988, Tankersley et al. 1995, Hasek & Rabalais 2001), of which the  

 frags. megalopae depend on salinity in estuaries (Epifanio et al. 1988). Megalopa larvae prevent competition with other species that occupy similar ecological niches by occupying species-specific settlement environments.

Chiromantes haematocheir and C. dehaani utilize the ecotone between water and land for spawning and settlement during their life cycle. Recently, the population of these crabs has been decreasing because of loss of this ecotone due to habitat destruction, including concrete re-  

 vetment work and fragmentation due to the construction of facilities and roads (Matsuda & Kamihogi 2004, Washitani & Kamihogi 2010, Yamanishi 2013). Megalopa larvae prefer complex substrates for settlement, such as vegetated areas, to avoid predation and obtain food efficiently (Perrins-Visser et al. 1996, Moksnes et al. 1998). Therefore, loss of emergent plant communities along the riverbank due to river restoration projects has the potential to affect the settlement behavior of megalopa larvae. For the con-  

 servation of both species, corridors between their land and water habitats and animal-friendly concrete revetments were created based on the migration of adult crabs (Sakurai et al. 2001, Okano et al. 2016). However, few conservation trails focused on their megalopa larvae have been developed. Megalopa larvae have a high rate of mortality related to delayed metamorphosis, biological or physical disturbances, hydrodynamics, physiological stress, predation, and competition (Hunt & Scheibling 1997), which influences the abundance of the overall population (Gosselin & Qian 1997). In addition, the larval recruitment processes of benthic invertebrates remain unclear (Hasek & Rabalais 2001, Pineda et al. 2010). Since the larvae of sympatric Decapoda species, such as C. haematocheir and C. dehaani are difficult to identify morphologically and are treated as congeneric species, interspecific differences in their larval recruitment processes are not well understood (Baba & Miyata 1971, Epifanio et al. 1988, Oh et al. 2007, Anger 2006, Cannicci et al. 2019). Recent studies have shown the effectiveness of genetic analyses for identifying morphologically similar crustacean species (Radulovicic et al. 2009, Raupach et al. 2015). This method can also be applied to identifying megalopa larvae to species, with the potential to clarify the ecology of the larval stages of crus-  

tacean species.

Our study objectives were to reveal the settlement patterns of C. haematocheir and C. dehaani megalopa larvae in the lower reach of the Sai River in Ishikawa Prefecture, central Japan, where these species occur sympatrically. We also investigated the effects of various factors on the density of settled megalopa, including 1) seasonal changes, 2) spatial variations (dominated by conspecific or heterospe-  

 cific adults), and 3) tidal effects. Megalopa samples collected from the river were identified based on DNA sequencing. Sesarmid crab megalopae have been reported to return to areas near their adult habitats (Paula et al. 2003, Cannicci et al. 2019). Therefore, we also investigated the distribution of the adults of each species within the land area adjacent to the river during their active period, based on the hypothesis that megalopa larvae will return to an environment dominated by adults of the same species.

Materials and Methods

Study area

This study was conducted along the Sai River, which is located in central Japan and flows into the Sea of Japan. The river is 35 km long, and the basin area is 256 km². The tidal reach of this river extends to approximately 3.7 km upstream of the river mouth (Fig. 1). In this study, sampling was conducted in the lower reach, between 0.6 and 1.0 km from the river mouth. Forests composed of mainly Japanese black pine (Pinus thunbergii Parlatore), black locust (Robinia pseudoacacia Linnæus), and Chinese hackberry (Celtis sinensis Persoon) flourished on the left side

---

**Fig. 1.** Location of the study area in the lower reach of the Sai River: Zone A (dry forested area dominated by Chiromantes haematocheir), Zone B (Chiromantes dehaani dominant area, which contains a swamp with a spring in the flood channel), Zone C (forest area behind Zone A), and Zone D (river revetment area). The broken lines indicate the location where the distribution survey of adult crabs was conducted and the filled circles indicate the site of the sample collection of megalopae.
of the river. In this forest, an artificial stream flows from pumped groundwater into a pond approximately 600 m in diameter. The river width and depth are approximately 3 and 0.15 m, respectively. The riverbed is composed of boulders and cobbles. The right bank of the river is covered with concrete and inhabited by herbaceous plants such as members of the Gramineae and emergent plants including common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) (Fig. 1, Zone D).

### Distribution of adult crabs

To survey the distribution patterns and abundances of adults of the two crab species, a line census survey was conducted once every 2 weeks from May to October 2017. Adults of both *Chiromantes dehaani* and *Chiromantes haematocheir* inhabit downstream and upstream areas for reproduction and feeding, respectively (Murakami unpublished data). The line census survey of adult crabs was conducted in three areas along the left bank (Fig. 1, Zones A–C). In Zone A, grasses and forests comprised of Japanese walnut (*Juglans mandshurica* var. sachalinensis) and *R. pseudoacacia* are dominant. Zone B is a wetland with a spring in the flood channel. Zone C is located in the inland area near Zone A, with *C. sinensis* forest alongside an artificial stream and pond. One survey line was set in each zone. The survey lines were 350, 280, and 320 m long in Zones A, B and C, respectively and 4 m wide in all zones. Two investigators counted the numbers of *C. haematocheir* and *C. dehaani* adults visually while walking the survey lines in each zone (Fig. 1) from 06:00 AM to 09:00 AM since these crabs were active in the morning (Yoshida 1961). Individuals smaller than 15 mm in width could not be identified to species visually or morphologically. These individuals do not participate in reproductive activity along the riverside (Hashiguchi & Miyake 1967b). Therefore, we counted only individuals larger than 15 mm to estimate the effect of the presence of conspecific or congeneric mature crabs on attracting megalopae. In addition, temperature and relative humidity were measured using a temperature/humidity data logger (Hygrochron, KN Laboratories) from June 7 to October 18. The measurement interval was set to 1 h, and the daily mean temperature and relative humidity were calculated by averaging the observed values over 24 hours.

### Sample collection of megalopae

To collect settled megalopae samples from the river for genetic analysis, concrete blocks (20.0 × 20.0 × 2.0 cm³) were used. The concrete blocks were covered with polyethylene turf (referred to as artificial substrate hereafter) that has leaf-like projections approximately 1.0 cm in height and many small gaps between the projections. Three artificial substrates were placed at the edge of the water in each area, including Zone A, Zone B, and the revetment on the right bank, referred to as Zone D (Fig. 1). The tidal fluctuations are smaller along the Sea of Japan than along the Pacific Ocean coast. At the observation station in Kanazawa City near the study river, the tidal range was 29–42 cm during the spring tide and 29–40 cm during the neap tide from August 1 to November 3, 2017 (Japan Meteorological Agency 2017). The artificial substrates were placed so that they were not exposed to the water surface during the tidal fluctuation. No sampling was conducted in Zone C because only freshwater flowed through this zone, and larvae cannot survive in freshwater (Saigusa 1981). The collection period was from August 1 to November 3, 2017, and sampling was set to coincide with the spring and neap tides. The artificial substrates were submerged overnight. The next day, the larvae and invertebrates that had settled on the artificial substrates were carefully lifted out of the water. Then, the polyethylene turf containing megalopae was removed, placed into coolers, and transported to the laboratory. The polyethylene turf was washed with a brush, and the megalopae were collected using filter paper (Tea Pack, Aeon Co., Ltd.) made of PET/PE composite fiber. All megalopae samples were preserved in 70% ethanol until genetic analysis.

### Genetic analysis

Of the 257 megalopae samples collected in the survey, 191 were used for the genetic analysis. Because the greatest number of megalopae were collected on August 16 (2–46 versus 0–11 on other days), we randomly selected 10 subsamples from six artificial substrates, onto which more than 10 megalopae had collected on August 16 for genetic analysis. DNA extraction from megalopae samples was carried out using a modification of the method reported by Marco-Herrero et al. (2014). Each megalopae sample was placed in a 1.5-mL microtube and homogenized using a pestle to facilitate DNA extraction. Protein was degraded via incubation for 1 h in 100 µL lysis buffer supplemented with 1 µL protease K (Lysis Buffer for PCR, Takara Bio) at 60°C. To inactivate protease K, the solution in the 1.5-mL microtube was transferred to a 0.2-mL tube and incubated for 2 min at 98°C using a PCR thermal cycler (TaKaRa PCR Thermal Cycler Dice Gradient TP600, Takara Bio). After incubation, the solution was returned to the 1.5-mL microtube. Protein was precipitated by adding 25 µL 7.5 M ammonium acetate and centrifuging for 10 min at 18°C and 14,000 rpm. The supernatant was transferred to a new 1.5-mL microtube. DNA precipitation was initiated by adding 3 µL glycogen (20 mg/mL glycogen solution from Oyster, nuclease tested, Nacalai Tesque) and 75 µL isopropanol. Then, the mixture was stirred for 3 min using a microtube mixer (MT-360, Tomy Digital Biology), incubated for 10 min at room temperature and then centrifuged for 10 min at 18°C and 20,000 G. To remove residual salts, the pellet was washed with 20 µL 70% ethanol and dried. Finally, the pellet was resuspended in 20 µL TE Buffer and stored at −20°C.
We used universal primers for crustaceans, including 16S ar-L (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16S br-H (5′-CCG TGC TGA ACT CAG ATC ACG T-3′), which amplify a fragment of the mitochondrial 16S large ribosomal subunit (Palumbi et al. 1991). DNA amplification was performed following standard PCR protocols recommended for the GoTaq® Master Mix (Promega Corporation) in 20-µL volumes consisting of 10 µL Go Taq Green Master Mix, 1 µL each of the primers, 7 µL distilled water, and 1 µL DNA solution. The thermal cycling procedure for DNA amplification was as follows: 10 s at 25°C, 2 min at 95°C, 30 cycles of 30 s at 95°C, 30 s at 50.2°C, 1 min at 72°C, and 4 min at 72°C using a TaKaRa PCR thermal cycler (Dice Gradient TP600, Takara Bio). DNA amplification was confirmed by electrophoresis using 1% agarose gels. The successfully amplified PCR products were then purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific) and sent to Eurofins Genomics Japan for sequencing. Species identification was conducted by searching for the species with the highest homology in the National Center for Biotechnology Information database. Finally, the estimated number of settled megalopae for each species was calculated from the total number collected and the ratio of megalopae for each species was calculated from the total number collected and the ratio of Chiromantes haematocheir and Chiromantes dehaani samples identified by DNA sequencing. The estimated number was used for the statistical analysis and other results.

**Statistical analysis**

A generalized linear model (GLM) was used to examine the effect of climate elements and the spatial habitat difference on the abundance of adult; Chiromantes haematocheir and Chiromantes dehaani. The dependent variable was the counted number of each crab species in the line census areas. First, we assumed that our GLM models followed a Poisson distribution; however, our count data in the Poisson model indicated over-dispersion. Therefore, a negative binomial regression model was used for datasets exhibiting over-dispersion. The independent variables were zone (A to C), daily mean temperature and daily mean relative humidity in each zone. An offset based on the line census area (m²) was included in the model to account for the unequal areas. To examine the effects of various factors on the settlement pattern of C. haematocheir and C. dehaani megalopae, a generalized linear mixed model (GLMM) was used. The dependent variable was the mean number of megalopae on each artificial substrate. The number of C. haematocheir megalopae fit the Poisson distribution without over-dispersion. For C. dehaani megalopae, it was initially assumed that our GLMM models followed the Poisson distribution; however our count data in the Poisson model indicated over-dispersion. Therefore, a negative binomial regression model was used for datasets exhibiting over-dispersion. Fixed effects were the date (elapsed days), to represent seasonal changes, zone (A, B, D), as an indicator of spatial changes, and tide (spring tide, neap tide). This study is based on the hypothesis that megalopae larvae are willing to settle in an area where conspecific adults are dominant. Therefore, regarding the zone in the fixed effect, the analysis was based on Zone A for C. haematocheir megalopae and Zone B for C. dehaani megalopae. The random effect was the three artificial substrates placed in each zone. All statistical analyses were performed using the statistical package R (ver. 3.5.3.)

**Results**

**Adult stages of two crabs**

The first observation of Chiromantes haematocheir adults was in late May in Zone C, which was further inland compared with the other study areas (Fig. 2a). The number of individuals in Zone C increased until June and then decreased slightly in July. In Zone A, which is dry forested area dominated by C. haematocheir, the abundance of crabs began to increase on July 5 and peaked on August 10. The number of individuals observed in Zone A decreased on August 16 and decreased further after September 1. In contrast, the crab density in Zone C increased after September 1; although the crabs remained less abundant than those in Zone A from mid-July to August, they peaked in September and were not observed on October 18. In Zone B, which is a swamp dominated by Chiromantes dehaani, adults were distributed at a low density throughout the study period. Chiromantes dehaani adults were observed in Zone C for the first time on May 24 (Fig. 2b).

![Graph](image-url)
In Zone B, the three highest peaks in the density of this crab in any of the study areas were observed between July and September. In Zone C, the density remained low and constant throughout the study period. In Zone A, crab density was lowest among the study areas, and the density dynamics were similar to those in Zone B. No individuals were observed in any of the study areas on October 18.

Throughout the survey period, *C. haematocheir* adults were observed in Zone A more dominantly than Zone B (GLM, \(z = -4.154, p < 0.001\), Table 2a) and less dominantly than Zone C (GLM, \(z = 2.886, p < 0.01\)). *Chiromantes dehaani* adults were observed in Zone B more dominantly than Zone A (GLM, \(z = -5.115, p < 0.001\)) and there was no significant difference between Zone B and C (GLM, \(z = -1.428, p = 0.153\)). The daily mean temperature and the daily mean relative humidity were positively correlated with abundance of both adult crabs (GLM, *C. haematocheir*: temperature, \(z = 5.541, p < 0.001\), humidity, \(z = 2.999, p < 0.01\), *C. dehaani*: temperature, \(z = 4.465, p < 0.001\), humidity, \(z = 3.687, p < 0.001\), Table 2a). Zone A, where *C. haematocheir* adults were dominant, had warm mean temperatures and high mean relative humidity from July 5 to August 16 (mean ± SD: 24.3 ± 1.7°C and 91.9 ± 6.5%, respectively, Table 1). In Zone C, the mean relative humidity was higher (93.7 ± 5.1%) than in other areas. The mean temperature in Zone C from June 21 to September 27, when the adults of both species were active, was 21.7 ± 2.1°C. The mean temperature and relative humidity in Zone B, where

| Day     | Daily mean temperature (°C) | Daily mean relative humidity (%) |
|---------|-------------------------------|----------------------------------|
|         | Zone A | Zone B | Zone C | Zone A | Zone B | Zone C |
| May 24  | ---    | 18.6   | 18.4   | 17.2   | 83.1   | 86.2   | 93.5   |
| Jun. 7  | 19.0   | 22.1   | 23.3   | 21.8   | 93.2   | 95.2   | 97.2   |
| Jun. 21 | 25.6   | 26.2   | 26.0   | 24.0   | 87.6   | 86.3   | 94.6   |
| Jul. 5  | 22.1   | 24.2   | 24.4   | 22.9   | 92.8   | 91.1   | 98.9   |
| Jul. 19 | 25.6   | 22.6   | 22.8   | 21.3   | 91.1   | 90.2   | 97.1   |
| Aug. 3  | 26.2   | 23.5   | 23.6   | 22.9   | 98.6   | 86.0   | 90.0   | 99.1   |
| Aug. 10 | 24.2   | 22.7   | 22.5   | 20.9   | 73.9   | 75.7   | 86.6   |
| Aug. 16 | 23.5   | 22.6   | 23.3   | 21.4   | 14.8   | 14.1   | 83.1   |
| Sep. 1  | 22.7   | 20.1   | 20.5   | 19.7   | 85.6   | 90.0   | 99.1   |
| Sep. 13 | 22.6   | 19.8   | 19.9   | 18.7   | 73.4   | 75.9   | 86.0   |
| Sep. 27 | 20.3   | 19.8   | 19.9   | 18.7   | 15.1   | 14.8   | 14.1   |
| Oct. 18 | 15.1   | 14.8   | 14.1   | 14.1   | 14.1   | 14.1   | 14.1   |

### Table 1. Changes in the daily mean temperature and relative humidity in Zones A (*Chiromantes haematocheir* dominant area), B (*Chiromantes dehaani* dominant area), and C (forest area behind Zone A).

### Table 2. Summary of GLM results comparing the effects of zone, daily mean temperature, and daily mean relative humidity on the abundance of adult crabs of (a) *Chiromantes haematocheir* and (b) *Chiromantes dehaani*. This study is based on the previous survey that adults of both *C. haematocheir* and *C. dehaani* were dominant species in Zone A and Zone B, respectively. Therefore, regarding the zone in the fixed effect, the analysis was based on (a) Zone A for *C. haematocheir* and (b) Zone B for *C. dehaani*.

| (a) *C. haematocheir* adults | Source | Estimate | Std. Error | z value | Pr (>|z|) |
|------------------------------|--------|----------|------------|---------|----------|
| Zone                         | C      | 1.133    | 0.393      | 2.886   | <0.01*** |
| Temperature                  | 0.301  | 0.054    | 5.541      | <0.01*** |
| Humidity                     | 0.071  | 0.023    | 2.999      | <0.01**  |

| (b) *C. dehaani* adults      | Source | Estimate | Std. Error | z value | Pr (>|z|) |
|------------------------------|--------|----------|------------|---------|----------|
| Zone                         | A      | -1.814   | 0.355      | -5.115  | <0.01*** |
| Temperature                  | -0.525 | 0.368    | -1.428     | 0.153   |          |
| Humidity                     | 0.224  | 0.050    | 4.465      | <0.01*** |
|                             | 0.082  | 0.022    | 3.687      | <0.01*** |
adult *C. dehaani* were dominant when this crab showed high activity, were 23.4±2.0°C and 89.3±7.8%, respectively, from July 5 to September 27. The relative humidity in Zone B was 99.2, 98.6, and 90.2% on July 5, August 16, and September 13, respectively, which were the highest peaks in the density of *C. dehaani*. Conversely, on August 3, September 1, and September 27, when crab density was low, the relative humidity was 83.2%, 75.7%, and 90.0%, respectively.

**Settlement period of megalopæ of the two crab species**

A total of 257 *Chiromantes* spp. megalopæ were collected from all study areas throughout the study period. Of the all individuals collected, 240 were identified as *Chiromantes haematocheir* (*n*=33) or *Chiromantes dehaani* (*n*=207), and sequencing failed for 17 megalopæ. As a general trend, settlement began in mid-August and extended to early November.

Elapsed days, to represent seasonal changes, had significant effects on the settlement patterns of both species (GLMM, *C. haematocheir*: *z*=3.321, *p*<0.001, Table 3a; GLMM, *C. dehaani*: *z*=-5.261, *p*<0.001, Table 3b). From mid-August to late August, settlement of only *C. dehaani* megalopæ was observed, with the highest peak on August 16 (Fig. 3b). From early September to early October, both species of megalopæ settled (Fig. 3a, b). From October to early November, *C. haematocheir* megalopæ settled mainly (Fig. 3a). Tidal effects on the settlement were observed only for *C. haematocheir* megalopæ (GLMM, *C. haematocheir*: *z*=2.629, *p*<0.01, Table 3a). *Chiromantes haematocheir* megalopæ tended to return on spring tide days: October 4, 19, and November 3 (Fig. 3a). For *C. dehaani*, a greater number of megalopæ returned on August 16 with the neap tide than on other days; however, there was no notable difference between spring and neap tides during the study period, aside from August 16 (Table 3b, Fig. 3b).

*Chiromantes haematocheir* megalopæ were most abundant in Zone A and significantly more so than in Zone B or Zone D (GLMM, Zone B: *z*=-1.989, *p*<0.05, Zone D: *z*=-2.587, *p*<0.01; Table 3a, Fig. 4a). By contrast, spatial change was not a significant variable affecting the number of settled *C. dehaani* megalopæ (Table 3b, Fig. 4b).

### Table 3. Summary of GLMM results comparing the effects of date (elapsed days), zone and tide on the settlement of (a) *Chiromantes haematocheir* and (b) *Chiromantes dehaani* megalopæ: Zone A (*C. haematocheir* dominant area), Zone B (*C. dehaani* dominant area), and Zone D (river revetment area). This study is based on the hypothesis that megalopa larvae are willing to settle in an area where conspecific adults are dominant. Therefore, regarding the zone in the fixed effect, the analysis was based on (a) Zone A for *C. haematocheir* megalopæ and (b) Zone B for *C. dehaani* megalopæ.

#### (a) *C. haematocheir* megalopæ

| Source     | Estimate | Std. Error | z value | Pr (>|z|)  |
|------------|----------|------------|---------|-----------|
| Elapsed days |         |            |         |           |
| Zone       | B        | -0.799     | 0.401   | -1.989    | <0.05*    |
|            | D        | -1.204     | 0.465   | -2.587    | <0.01**   |
| Tide       | Spring tide | 1.176     | 0.447   | 2.629     | <0.01**   |

#### (b) *C. dehaani* megalopæ

| Source     | Estimate | Std. Error | z value | Pr (>|z|)  |
|------------|----------|------------|---------|-----------|
| Elapsed days |         |            |         |           |
| Zone       | A        | -0.675     | 0.639   | -1.055    | 0.291     |
|            | D        | -0.687     | 0.628   | -1.093    | 0.274     |
| Tide       | Spring tide | -0.896    | 0.619   | -1.447    | 0.148     |
Discussion

Migration and distribution patterns of adult crabs

In this study area, the active migration of *Chiromantes* spp. adults on the ground was observed in late May, when the mean daily temperatures in all areas were 18.3–19.0°C. Several studies have reported that *Chiromantes* spp. become active when the mean temperature is over 20°C (Hashiguchi & Miyake 1967a, Suzuki 1981). In addition, the mean temperature in May 2017 at the Kanazawa Local Meteorological Office, located near the study area, was 18.9°C. This temperature is consistent with the temperature at which the crabs become active. The temperature in late September was 18.7–20.3°C, and the abundance of *Chiromantes dehaani* tended to decrease on days with lower humidity. Hashiguchi & Miyake (1967a) reported that humidity was also a factor influencing the activity of *Chiromantes* spp. According to Yoshida (1961), sesarmid crabs are not active if the humidity is below 80%. In our study, the humidity was below 80% on 1 day only, and the number of active *Chiromantes haematocheir* individuals decreased on that day. Therefore, the threshold humidity for *C. haematocheir* activity is approximately 80%.

Meanwhile, the activity of *C. dehaani* decreased when the humidity was lower than approximately 90%. This result suggests that *C. dehaani* has a higher threshold for humidity than does *C. haematocheir*.

The number of *C. haematocheir* adults showed seasonal variations in both areas. The majority of *C. haematocheir* first appeared in the inland forest area and moved to the riverbank during the breeding season. Before hibernation, they returned to the inland forest area. Although several species of land crab live mainly in inland habitats such as forests, they move to shore or riverbank areas to release larvae. The pattern of this movement differs among species. The gecarcinid crab *Discoplax longipes* reproduces in inland adult habitats, and only females travel to the shore for hatching (Ng & Guinot 2001). Meanwhile, both males and females of the gecarcinid crab *Gecarcoidea natalis* travel to the sea, where they mate (Hicks 1985). In addition, *Johngarthia lagostoma*, also belonging to Gecarcinidae, mates not only in its adult habitat but also during and after migration to the coast (Hartnoll et al. 2010). There are no reports of the migration pattern of *C. haematocheir*. However, during the survey, mating individuals were observed in both the inland area and riverbank. This result suggests that *C. haematocheir* adults mate not only in the inland area used for their overwintering habitat but also in the riverbank area. A constant population of *C. haematocheir* was found in the inland area in July, during the breeding season. Males that successfully mated before migration to the riverbank and immature individuals were assumed to remain in the inland area.

*Chiromantes dehaani* did not show a clear migration pattern, in contrast to *C. haematocheir*, and its population density was higher in the wetland area along the riverbank throughout the study period. Among Decapoda, land crabs are highly adaptable to land areas due to their ability to obtain oxygen from air (Adamczewska & Morris 2000). It is assumed that *C. dehaani* depends more on water than does *C. haematocheir*, as the number of active individuals decreased when the humidity was lower. Thus, *C. dehaani* may remain in areas near water, such as wetlands, during their life cycle.
Spatial settlement patterns of megalopae of the two species

Chiromantes haematocheir megalopae tended to settle preferentially in areas dominated by conspecific adults. Megalopa larvae actively select settlement areas that will increase their survival rates based on protection from predation and availability of post-recruitment food resources (Perkins-Visser et al. 1996, Moksnes et al. 1998). Selection of settlement areas by megalopae requires chemical and physical recognition systems (Forward et al. 2001, Anger 2006). The factors recognized by crustacean species include various habitat-derived substances such as substratum, biofilms, and humic acids, as well as specific biological substances such as odors from conspecific adults, closely related species, predators, and potential prey (Forward et al. 2001, de Noronha Campos et al. 2016). In particular, as chemicals derived from conspecific adults reduce the time to metamorphosis in many brachyurans, they are effective factors attracting megalopa larvae and promoting appropriate habitat usage by conspecific individuals (Epifanio & Cohen 2016). Chiromantes haematocheir megalopae might selectively settle in response to substances released by conspecific adults. However, our adult distribution survey showed that the majority of C. haematocheir adults migrated from the riverside to the inland area for overwintering before the megalopae settled on the riverside. Therefore, C. haematocheir megalopae may settle in areas where conspecific adults were dominant during the breeding season, as these areas generally contain suitable habitats.

There was no difference in the number of settled Chiromantes dehaani megalopae among the areas. Chemical cues from congeneric adults also attract megalopae and enhance settlement in habitats dominated by closely related species (Gebauer et al. 2002, Anger 2006). Based on breeding experiments using the sesarmid crab Sesarma curacaoense, the odor derived from conspecific adults and those of the closely related species Sesarma rectum shortened the time to metamorphosis from megalopa to juvenile crab (Gebauer et al. 2002). Similarly, based on experiments involving C. dehaani megalopae, leachate from both conspecific adults and adults of the closely related species C. haematocheir reduced the time to metamorphosis (Murakami unpublished data). Therefore, C. dehaani megalopae show a strong preference toward settlement areas dominated by not only conspecific adults but also C. haematocheir adults. In contrast to C. haematocheir megalopae, which settled only at sites dominated by conspecific adults, C. dehaani megalopae also settled on the revetment area along the opposite bank (Zone D). Zone D contained a levee covered with vegetation consisting mainly of herbaceous plants such as Gramineae and emergent plants such as Phragmites australis. The area inland of Zone D contained mainly rice fields mixed with some residential areas. According to our adult distribution survey, C. dehaani adults were frequently found in the vegetated area along the riverbank during the survey period, whereas C. haematocheir adults migrated between the riverbank and inland areas. Chiromantes dehaani is the most water-dependent of all sesarmid crabs (Matsuda & Kamihogi 2004, Ito et al. 2011). Furthermore, the results of this study indicated that C. dehaani has low resistance to dry conditions. The revetment area contains some appropriate habitats for C. dehaani along the riverside and levee, where we observed active C. dehaani individuals. Therefore, C. dehaani might return to the revetment area in response to substances derived from the small population of conspecific adults or other environmental factors. However, C. dehaani randomly selects settlement areas and might recruit along the riverside without using chemical signals. The settlement cues of C. dehaani and C. haematocheir should be investigated in future research that includes areas where these species are not distributed.

Other factors affecting the settlement of megalopae

The settlement season of megalopae differed between Chiromantes dehaani, which settled from mid-August to early October, and Chiromantes haematocheir, which settled from early September to early November. The difference in settlement season between species is related to differences in breeding season and larval period. During the breeding season of C. haematocheir, reproductive individuals appear from early June to mid-September, and the incubation period is 24–27 days. On the other hand, reproductive individuals of C. dehaani are observed from the end of May to the end of August, and their incubation period is 18 to 21 days (Hashiguchi & Miyake 1967b). Furthermore, the larval period of C. haematocheir is approximately 1 month in duration (Oh et al. 2007), which is approximately 1 week longer than that of C. dehaani (Baba & Miyata 1971). This is because C. haematocheir zoae transform into megalopae via five zoea stages, whereas C. dehaani has only four zoea stages (Cuesta et al. 2006). Thus, C. haematocheir release their larvae into the river later, and consequently, the megalopae settle in the riverbank habitat later, compared with C. dehaani.

Due to tidal effects, C. haematocheir and C. dehaani megalopae showed differing settlement patterns. Chiromantes haematocheir megalopae returned primarily on the spring tide. In many estuarine crabs, newly hatched larvae are transported to coastal waters and then return to the estuary after metamorphosis into megalopa larvae (Epifanio & Cohen 2016). However, megalopae larvae are prevented from returning by rip currents and strong horizontal currents near the mouths of estuaries (Tankersley et al. 1995). Megalopa larvae utilize marine transport systems, such as onshore-flowing residual bottom currents (Dittel & Epifanio 1982, Epifanio et al. 1988), wind-driven exchange (Goodrich et al. 1989, Little & Epifanio 1991), and selective tidal stream transport (STST; Tankersley & Forward 1994, Forward et al. 2003), to overcome the reverse flow and enter estuaries from coastal waters. Among
these transport systems, STST during flood tides is used by many crabs (Epifanio & Cohen 2016). Megalopae ascend into the water column during the flood tide to promote their return to estuaries, and then descend to near the bottom during the ebb tide to prevent transport away from the estuary (Forward et al. 2003, Lee et al. 2005). By repeating these vertical movements rhythmically, megalopae can enter an estuary from coastal waters (Little & Epifanio 1991). Previous studies have reported that the number of sesarmid crab megalopae, including those of *C. haematocheir*, increases in estuaries during the spring tide (Hashimoto 1968, Paula et al. 2001, Ragionieri et al. 2015). Therefore, *C. haematocheir* larvae transported to the coastal area are assumed to have reached the estuary area via STST on the day of the spring tide when the tidal range was greatest. However, the tidal amplitude in the Sea of Japan is relatively small. Therefore, the migration of megalopae may be influenced by other factors synchronizing with the tidal rhythm, such as the lunar cycle, which is strongly associated with tidal fluctuations. Brachyuran crab shows exhibit cyclic behaviors, such as larval release by adults and settlement of larvae, which are synchronized with the lunar cycle (Saigusa & Hidaka 1978, Boylan & Wenner 1993, Hasek & Rabalais 2001, Saigusa et al. 2003). In addition, a strong relationship between the zoea release by *C. haematocheir* and the lunar cycle has been noted in several areas in Ishikawa Prefecture, including the study site (Murakami unpublished date). Such adult *C. haematocheir* behavior synchronized with the lunar cycle may also be observed in its megalopae, although further studies are required to test this hypothesis.

The tidal effect on the settlement was investigated for *C. dehaani*, but its megalopae did not show a notable difference between spring and neap tides, but instead they settled mostly on August 16. For some estuarine crabs, larvae remain in the estuary and never migrate to the coastal area (Epifanio & Cohen 2016). The white-fingered mud crab *Rhithropanopeus harrisii* completes its life cycle within the estuary (Forward 2009). The larvae of *R. harrisii* remain in the estuary immediately after hatching by rising to the surface during the flood tide and descending to near the bottom during the ebb tide (Cronin & Forward 1983, Forward 2009). Organisms that complete their entire life history in an estuarine habitat represent the evolution of marine organisms that have adapted to freshwater and extended their range upstream (Bilton et al. 2002). Such organisms adapt to brackish and even freshwater by acquiring a wide range of salinity tolerance mechanisms (Pearse 1927). The larvae of *R. harrisii*, a species that completes its life history in estuaries, could survive to a minimum salinity of 2, showing adaptation for survival in low-salinity environments (Hasek & Rabalais 2001). According to a laboratory experiment conducted by Saigusa (1981), the larvae of *C. dehaani* survived more than twice as long in freshwater as those of *C. haematocheir*. Laboratory experiments on the salinity tolerance of zoea during their metamorphosis into megalopa showed that *C. haematocheir* larvae were able to metamorphose in the range of 17.0–25.5%, whereas *C. dehaani* larvae developed over a wider salinity range (8.5–34.0%) (Murakami unpublished data). Thus, *C. dehaani* larvae are more tolerant of both high and low salinities than are *C. haematocheir* larvae and therefore are better adapted to brackish water habitats with low salinity. Newly hatched *C. dehaani* larvae may complete their life cycle in estuaries, and this species showed smaller tidal effects on settlement than did *C. haematocheir*.

In previous studies, megalopae have been limited to genus-level identification due to the difficulty of identifying megalopae based on their external form (Epifanio et al. 1988, Hasek & Rabalais 2001). This study clarified the differences in the settlement patterns of *Chiromantes* spp., which have sympatric ranges, via genetic identification. Such genetic analysis could be further applied to juvenile crabs and smaller zoea larvae. Recently, numerous studies have been conducted to elucidate the number of species of phytoplankton and zooplankton in the ocean using metagenomics analysis (de Vargas et al. 2015, Bryant & Arehart 2019). Such techniques may be applied to research on the distribution of zoea larvae in marine and brackish waters. In the future, the use of molecular biological approaches will enable comprehensive elucidation of the ecology of *Chiromantes* spp. and thereby contribute to the conservation of these species.

**Acknowledgements**

We thank Assistant Professor Osamu Nakayachi (Pref. Univ. Ishikawa) for his technical advice on DNA analysis. This study was supported financially by the TaKaRa Harmonist Fund (2018).

**References**

Adamczewska AM, Morris S (2000) Locomotion, respiratory physiology, and energetics of amphibious and terrestrial crabs. Physiol Biochem Zool 73: 706–725.

Anger K (2006) Contributions of larval biology to crustacean research: a review. Invert Reprod Develop 49: 175–205.

Baba K, Miyata K (1971) Larval development of *Hemimetasoma caecum* (Crustacea, Brachyura) reared in the laboratory. Mem Fac Educ, Kumamoto Univ 19: 54–64.

Bilton DT, Paula J. Bishop JDD (2002) Dispersal, genetic differentiation and speciation in estuarine organisms. Estuar Coast Shelf Sci 55: 937–952.

Bryant PJ, Arehart TE (2019) Diversity and life-cycle analysis of Pacific Ocean zooplankton by videomicroscopy and DNA barcoding: Hydrozoa. PLoS One 14.

Boylan JM, Wenner EL (1993) Settlement of brachyuran megalopae in a South Carolina, USA, estuary. Mar Ecol Prog Ser: 237–246.
Cannici S, Mostert B, Fratini S, McQuaid CD, Porri F (2019) Recruitment limitation and competent settlement of sesarmid crab larvae within East African mangrove forests. Mar Ecol Prog Ser 626: 123–133.

Castejón D, Rotllant G, Guerao G (2019) Factors influencing successful settlement and metamorphosis of the common spider crab *Maja brachydactyla* Balss, 1922 (Brachyura: Majidae): Impacts of larval density, adult exudates and different substrates. Aquaculture 501: 374–381.

Cronin TW, Forward Jr RB (1983) Vertical migration rhythms of newly hatched larvae of the estuarine crab, *Rhithropanopeus harrissii*. Biol Bull 165: 139–153.

Cuesta JA, Guerao G, Liu HC, Schubart CD (2006) Morphology of the first zoal stages of eleven Sesarmidae (Crustacea, Brachyura, Thoracotremata) from the Indo-West Pacific, with a summary of familial larval characters. Invertibr Reprod Dev 49: 151–173.

de Noronha Campos KL, Abrunhosa FA, de Brito Simiti DDJ (2016) Triggering settlement behaviour and metamorphosis of the burrowing ghost shrimp, *Lepidopthis halimus siriiboia* (Callianassidae): do cues matter? Mar Freshwater Res 67: 291–300.

de Vargas C, Audic S, Henry N, Decelle J, Mahé F, Logares R, et al. (2015) Eukaryotic plankton diversity in the sunlit ocean. Science 348: 1261605.

Dittel AI, Epifanio CE (1982) Seasonal abundance and vertical distribution of crab larvae in Delaware Bay. Estuaries 5: 197–202.

Epifanio CE, Little KT, Rowe PM (1988) Dispersal and recruitment of fiddler crab larvae in the Delaware River estuary. Mar Ecol Prog Ser 43: 181–188.

Epifanio CE, Cohen JH (2016) Behavioral adaptations in larvae of brachyuran crabs: a review. J Exp Mar Biol Ecol 482: 85–105.

Forward Jr RB (2009) Larval biology of the crab *Rhithropanopeus harrissii* (Gould): a synthesis. Biol Bull 216: 243–256.

Forward Jr RB, Tankersley RA, Ritschosf D (2001) Cues for metamorphosis of brachyuran crabs: an overview. Am Zool 41: 1108–1122.

Forward RB, Tankersley RA, Welch JM (2003) Selective tidal-stream transport of the blue crab *Callinectes sapidus*: an overview. Bull Mar Zool 41: 347–365.

Gebauer P, Paschke K, Anger K (2002) Metamorphosis in a semiterrestrial crab, *Sesarma curacoaense*: intra-and interspecific settlement cues from adult odors. J Exp Mar Biol Ecol 268: 1–12.

Goodrich DM, van Montfrans J, Orth RJ (1989) Blue crab megalop influx to Chesapeake Bay: evidence for a wind-driven. Estuar Coast Shelf Sci 29: 247–260.

Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. Mar Ecol Prog Ser 146: 265–282.

Hartnoll RG, Broderick AC, Godley BJ, Musick S, Pearson M, Stroud SA, Saunders KE. (2010) Reproduction in the land crab *Johnsgarthia lagostoma* on Ascension Island. JCrustacean Biol 30: 83–92.

Hasek BE, Rabalais NN (2001) Settlement patterns of brachyuran megalopae in a Louisiana estuary. Estuaries 24: 796.

Hashiguchi Y, Miyake S (1967a) Ecological studies of marsh crabs, *Sesarma spp*. I: hibernation, crabs’ habitats and parasitic animals in winter. Sci Bull Fac Agric, Kyushu Univ 23: 67–80. (in Japanese with English abstract).

Hashiguchi Y, Miyake S (1967b) Ecological studies of marsh crabs, *Sesarma spp*. II: habitats, copulation and egg-bearing season. Sci Bull Fac Agric, Kyushu Univ 23: 81–89. (in Japanese with English abstract).

Hashimoto H (1968) Ecological distribution of the megalops of the terrestrial sesarmid crabs in the river. Bull Fac Educ, Shizuoka Univ Nat Sci Ser 19: 55–63. (in Japanese with English abstract).

Hicks JW (1985) The breeding behavior and migrations of the terrestrial crab *Gecarcoidea natalis* (Decapoda: Brachyura). Aust J Zool 33: 127–142.

Hunt HL, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. Mar Ecol Prog Ser 155: 269–301.

Ito S, Suzuki T, Kominami Y (2011) Fruit foraging and seed dispersal by land crabs in a temperate coastal forest. Jpn J Ecol 61: 123–131. (in Japanese with English abstract).

Japan Meteorological Agency (2017) Tyouseki, kaimensuu no data tyouhou Kanazawa [A tidal table at the Kanazawa Port, Ishikawa Prefecture, Japan]. Available at: https://www.data.jma.go.jp/gmd/kaiyou/db/tide/suisan/suisan.php?stn=T1 (accessed on 12 July 2020) (in Japanese).

Kobayashi S (2000) Distribution pattern and ecology of brachyuran crabs in the riverine environment: their significance in the ecosystem and present condition. Ecol Civil Eng 3: 113–130. (in Japanese with English abstract).

Kobayashi S (2006) Environmental condition in the settlement area and nursery ground of the Japanese mitten crab *Eriocheir japonica* (de Haan) in the tidal river area. Ecol. Civil. Eng. 8: 133–146. (in Japanese with English abstract).

Lee JT, Coleman RA, Jones MB (2005) Vertical migration during tidal transport of megalopae of *Necora puber* in coastal shallow waters during daytime. Estuar Coast Shelf Sci 65: 396–404.

Little KT, Epifanio CE (1991) Mechanism for the re-invasion of an estuary by two species of brachyuran megalopae. Mar Ecol Prog Ser 68: 235–242.

Marco-Herrero E, González-Gordillo JI, Cuesta JA (2014) Morphology of the megalopa of the mud crab, *Rhithropanopeus harrissii* (Gould, 1841) (Decapoda, Brachyura, Panopeidae), identified by DNA barcode. Helgol Mar Res 68: 201.

Matsuda T, Kamihogi A (2004) A study of a relationship between the medium of terrestrial crabs and environment characteristic around an artificial shore in Sennan, Osaka. Landscape Res Jpn 67: 537–542. (in Japanese with English abstract).

Moksnes PO, Pihl L, Van Montfrans J (1998) Predation on post-larvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism. Mar Ecol Prog Ser 166: 211–225.

Ng PKL, Guinot D (2001) On the land crabs of the genus Discoplax A. Milne Edwards, 1867 (Crustacea: Decapoda: Brachyura: Gecarcinidae), with description of a new cavernicolous species from the Philippines. Raffl Bull Zool 49: 311–388.

Oh SM, Chung DE, Ko HS (2007) Complete larval development of *Chiromantes haematocheir* (Crustacea: Decapoda: Brachy-
ura: Grapsidae), with a key to seven sesarminid zoeas in Korea. Anim Syst, Evol Diversity 23: 9–20.
Okano A, Urade T, Kamihogi A (2016) A study of construction and maintenance of the ecological path for the conservation of semi-terrestrial crab. Landscape Res Jpn 79: 697–702. (in Japanese with English abstract).
Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool’s guide to PCR, version 2.0. Univ Hawaii, Honolulu.
Paula J, Dray T, Queiroga H (2001) Interaction of offshore and inshore processes controlling settlement of brachyuran megalopae in Saco mangrove creek, Inhaca Island (South Mozambique). Mar Ecol Prog Ser 215: 251–260.
Paula J, Mendes RN, Mwaluma J, Raedig C, Emmerson W (2003) Combined effects of temperature and salinity on larval development of the mangrove crab Parasesarma catenata Orman, 1897 (Brachyura: Sesarmidae). Western Indian Ocean J Mar Sci 2: 57–63.
Pawlik JR (1992) Chemical ecology of the settlement of benthic marine invertebrates. Oceanogr Mar Biol Ann Rev 30: 273–335.
Pearse AS (1927) The migration of animals from the ocean into freshwater and land habitats. Am Nat 61: 466–476.
Perkins-Visser E, Wolcott TG, Wolcott DL (1996) Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (Calinecetes sapidus) Rathbun. J Exp Mar Biol Ecol 198: 155–173.
Pineda J, Porri F, Starczak V, Blythe J (2010) Causes of decoupling between larval supply and settlement and consequences for understanding recruitment and population connectivity. J Exp Mar Biol Ecol 392: 9–21.
Radulovici AE, Sainte-Marie B, Dufresne F (2009) DNA barcoding of marine crustaceans from the Estuary and Gulf of St Lawrence: a regional-scale approach. Mol Ecol Resour 9: 181–187.
Ragionieri L, Fratini S, Cannicci S (2015) Temporal patterns of megalopal settlement in different areas of an East African mangrove forest (Gazi Bay, Kenya). Hydrobiologia 749: 183–195.
Raupach MJ, Barco A, Steinke D, Beermann J, Laakmann S, Mohrbeck I, Neumann H, Kihara TC, Pointner K, Radulovici A, Segelken-VOigt A, Wesse C, Knebelbsberger T (2015) The application of DNA barcodes for the identification of marine crustaceans from the North Sea and adjacent regions. PLoS One 10: e10139421.
Rodriguez SR, Ojeda FP, Inestrosa NC (1993) Settlement of benthic marine invertebrates. Mar Ecol Prog Ser 97: 193–207.
Saigusa M, Hidaka T (1978) Semilunar rhythm in the zoea-release activity of the land crabs Sesarma. Oecologia 37: 163–176.
Saigusa M (1981) Adaptive significance of a semilunar rhythm in the terrestrial crab Sesarma. Biol Bull 160: 311–321.
Saigusa M, Okochi T, Iketi S (2003) Nocturnal occurrence, and synchrony with tidal and lunar cycles, in the invertebrate assemblage of a subtropical estuary. Acta Oecologica 24: S191–S204.
Sakurai N, Lim BK, Koshikawa Y, Uchikawa T, Karaki Y (2001) Improving creatures living environment by using a new type of concrete panel. Proc Civil Eng Ocean 17: 153–158. (in Japanese with English abstract).
Suzuki S (1981) The life history of Sesarma (Holometopus) haematocheir (H. Milne Edwards) in the Miura peninsula. Res Crustacea 11: 51–65.
Tankersley RA, Forward JR RB (1994) Endogenous swimming rhythms in estuarine crab megalopae: implications for flood-tide transport. Mar Biol 118: 415–423.
Tankersley RA, McKelvey LM, Forward Jr RB (1995) Responses of estuarine crab megalopae to pressure, salinity and light: implications for flood-tide transport. Mar Biol 122: 391–400.
Wada K (1983) Spatial distributions and population structures in Scopimera globosa and Ilyoplax pusillus (Decapoda: Ocypodidae). Publ Seto Mar Biol Lab 27: 281–291.
Washitani Y, Kamihogi A (2010) The distributional change of the semi-terrestrial crabs in coastal forest around a reclaimed shore. Landscape Res Jpn 73: 513–518. (in Japanese with English abstract).
Yamanishi H (2013) Infection rates of larval lung flukes, Paragonimus ohirai, in the brackish water crabs, Sesarma dehaani, from Kikuyajima Sandbank in the Maruyama river in Hyogo prefecture from 2010 to 2013. Med Entomol Zool 65: 157–162. (in Japanese with English abstract).
Yoshida M (1961) Daily rhythmic activity of a land-crab Sesarma intermedia (DE HAAAN). Jpn J Ecol 11: 160–162. (in Japanese with English abstract).