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journal or publication title	Biogeography : international journal of biogeography, phylogeny, taxonomy, ecology, biodiversity, evolution, and conservation biology
volume	20
page range	111-121
year	2018-09-20
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URL	<a href="http://id.nii.ac.jp/1342/00001968/">http://id.nii.ac.jp/1342/00001968/</a>

## Emigration behaviour, moulting and survival during the sea-to-land transition of land hermit crabs *Coenobita violascens* and *Coenobita rugosus* under laboratory conditions: Effects of salinity and riverine odours

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**Abstract:** *Coenobita rugosus* is distributed along the entire coast, and the distribution of *C. violascens* is restricted to the vicinity of rivers, mainly in the mangrove estuaries on southern Japanese islands. To infer the environmental cues affecting successful emigration from the sea to land on these species, we examined shell-wearing and landing behaviour, moulting and survival for laboratory-raised megalopae and early juveniles under different seawater conditions: 1) high salinity (34 ppt, control), 2) low salinity (24 ppt), and 3) high salinity (34 ppt) with riverine odours (mangrove riverine water). In *C. violascens*, reduced salinity and riverine odours stimulated shell-wearing activity, and riverine odours enhanced the landing activity. In *C. rugosus*, reduced salinity and riverine odours stimulated both shell-wearing and landing activities, and the magnitude of the effects was larger in response to reduced salinity than riverine odours. These seawater conditions also tended to enhance the moulting and survival of the animals. Salinity reductions widely occur along the shoreline due to the inflow of groundwater as well as river water. Riverine odours and reduced salinity should be cues for emigration from the sea to land by megalopae of *C. violascens* and *C. rugosus*, respectively, thereby characterizing the distributions of these species on the islands.

**Key words:** early life history; mangrove estuary; recruitment; terrestrial hermit crab

### Introduction

Terrestrial hermit crabs of the family Coenobitidae Dana, 1851 are distributed mainly in subtropical and tropical coastal regions (Hartnoll, 1988). The family Coenobitidae is composed of two genera: *Coenobita* Latreille, 1829, with approximately 17 species, and *Birgus* Leach, 1816, with only one species, *B. latro* (Linnaeus, 1767) (Hartnoll, 1988; Poupin, 1996; McLaughlin *et al.*, 2010; Rahayu *et al.*, 2016). Larvae of coenobitid crabs hatch on the shore, and they develop in the sea through several zoeal stages before their metamorphosis into the megalopal stage (Hartnoll, 1988; Nakasone, 2001; Hamasaki *et*

*al.*, 2014, 2015a). The megalopae immigrate to the coastal area, after which they carry gastropod shells and migrate onto land (Reese, 1968; Harvey, 1992; Brodie, 1999; Hamasaki *et al.*, 2011, 2014, 2015b, 2015c).

In Japan, six species, including *B. latro*, *Coenobita brevimanus* Dana, 1852, *C. cavipes* Stimpson, 1858, *C. purpureus* Stimpson, 1858, *C. rugosus* H. Milne-Edwards, 1837, and *C. violascens* Heller, 1862, are commonly found on the southern islands of the Ryukyu Archipelago (Nakasone, 1988, 2001; Asakura, 2004; Fujikawa *et al.*, 2017). We previously conducted surveys on the distributional characteristics of coenobitid crabs along the coasts of Ishigakijima Island and Iriomotejima Island in the Ryukyu Archipelago (Fujikawa *et al.*, 2017; Hama-

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saki *et al.*, 2017, 2018); the survey data revealed that *C. rugosus* was the dominant species occurring on the beach and in the vicinity along the entire coast of the islands, and the distribution of *C. violascens* was restricted to the vicinity of the river, mainly in the mangrove estuaries. Early juveniles were also found in the habitats of larger juveniles and adults of both species, indicating that *C. rugosus* and *C. violascens* complete their life cycles on land near the localities where they land.

It has been experimentally demonstrated that environmental stimuli such as salinity reductions and chemical cues, i.e., odours, derived from conspecific adults and/or nursery areas such as aquatic vegetation and biofilms in inshore areas affect settlement behaviour and accelerate the metamorphosis of the megalopal stage of many decapod crustacean species (Tankersley *et al.*, 1995; Anger, 2001, 2006; Forward *et al.*, 2001, 2003; Welch and Forward, 2001; Gebauer *et al.*, 2003; Epifanio and Cohen, 2016). It is therefore hypothesized that environmental cues that enhance successful emigration from the sea to land might differ between *C. violascens* and *C. rugosus*, which exhibit different distributional characteristics on the shoreline. The present study aimed to test this hypothesis by examining shell-wearing and landing behaviour, moulting, and survival of laboratory-raised megalopae and early juveniles of these species cultured in seawaters that present different salinity and mangrove riverine odour conditions.

## Materials and Methods

### Experimental animals

Ovigerous females of *C. violascens* and *C. rugosus* were captured during early July 2014 on Ishigakijima Island (24°23'N, 124°08'E). They were transferred to the laboratory at Tokyo University of Marine Science and Technology, Tokyo, where the air temperature was controlled at approximately 28°C and where they were maintained in tanks equipped with simulated land and sea areas (artificial

seawater, 34 ppt salinity; Sealife, Marinetech Co. Ltd., Tokyo, Japan) until hatching occurred, following the methods of Hamasaki *et al.* (2009) and Hamasaki (2011). After the larvae had hatched, all the female crabs were released back into their natural habitats.

The larvae of *C. violascens* that hatched from two females on July 25 (brood 1) and July 28, 2014 (brood 2), and those of *C. rugosus* from two females on July 18 (brood 1) and July 21, 2014 (brood 2), were stocked in four 1-l beakers at a density of 50 individuals beaker<sup>-1</sup> (approximately 28°C and 34 ppt salinity) and cultured until metamorphosis to the megalopal stage according to the methods of Hamasaki *et al.* (2013). The larvae from broods 1 and 2 metamorphosed into megalopae on August 10 and August 13, 2014, respectively, for *C. violascens* and on August 2 and August 5, 2014, respectively, for *C. rugosus*.

### Experimental seawater treatments

Three types of seawater were prepared: 1) high-salinity seawater (34 ppt), 2) low-salinity seawater (24 ppt), and 3) high-salinity seawater (34 ppt) that contained mangrove riverine odours. On Ishigakijima Island, the largest mangrove area extends from the mouth of the Naguragawa River on the western island; this area is a brackish estuary system called “Nagura Amparu”, with a tidal flat and mangrove tree area separated from the outer sea bay by a sandbank. *Coenobita violascens* is abundant on an inner tidal flat in the Nagura Amparu (Fujikawa *et al.*, 2017; Hamasaki *et al.*, 2017, 2018); therefore, mangrove riverine water collected from the Nagura Amparu was used in the present study. We did not prepare low-salinity seawater containing riverine odours because of the limited volume of available mangrove riverine water. The high-salinity condition was therefore considered a control treatment. High salinity was adjusted to 34 ppt because the salinity was recorded to be approximately 34–35 ppt in the open ocean around the Ryukyu Archipelago ([– 112 –](http://</a></p></div><div data-bbox=)

www1.kaiho.mlit.go.jp/KAN11/atlas/sal/). Low salinity was adjusted to 24 ppt considering the salinity fluctuations in accordance with a tidal cycle (approximately 0–34 psu) in the major river mouth of the Nagura Amparu (Kawachi and Ishikawa, 2008; Kawachi *et al.*, 2009) and the incidence of a few mortalities after 24 h in the megalopae of *C. violascens* and *C. rugosus* when they were abruptly transferred from 34 ppt to 20 ppt salinity conditions; however, no mortalities at 24 ppt occurred (Hamasaki *et al.*, unpublished data). Seawater with high and low salinity conditions was prepared using distilled water and artificial seawater salts. High-salinity seawater containing riverine odours was prepared by adding artificial seawater salts into the mangrove riverine water (22 ppt). It was collected during an ebb tide on July 6, 2014, from a small river mouth located in the southern part of the Nagura Amparu, after which the sample was transported to our laboratory. Organic materials and nutrients from mangrove swamps are exposed to the coastal area through river basins in the Nagura Amparu (Akamatsu *et al.*, 2002a, 2002b). Riverine water was stored at  $-60^{\circ}\text{C}$  until use for experiments. It has been reported that chemical cues that induce the megalopal metamorphosis of *Uca pugnax* (S. I. Smith, 1870) and *Panopeus herbstii* H. Milne-Edwards, 1834 maintained their activity even after freezing (O'Connor and Gregg, 1998; Andrews *et al.*, 2001).

### **Emigration behaviour and moulting**

The 0-day-old megalopae of *C. violascens* and *C. rugosus* were housed individually and cultured in transparent plastic containers (8 cm wide  $\times$  20 cm long  $\times$  6.5 cm high) equipped with an inclined simulated land surface (250 ml of coral sand; grain diameter = 0.5 mm) and designated test seawater (80 ml), as illustrated by Hamasaki *et al.* (2011). The gastropod shells of *Littoraria undulata* (Gray, 1839), which were easily collected at Ishigakijima Island, were provided for test individuals. Three sizes of cleaned gastropod shells (shell length, mean

$\pm$  standard deviation: small,  $4.0 \pm 0.1$  mm; medium,  $4.5 \pm 0.1$  mm; and large,  $5.0 \pm 0.1$  mm) were placed at the bottom of the sea area in each container because terrestrial hermit crab juveniles change their preference for larger shells in accordance with their growth (Hamasaki *et al.*, 2015c). A total of 16 animals, eight individuals from each brood, were used for each seawater treatment group for each species. The container was covered with 0.9 mm mesh-sized plankton netting to prevent the animals from escaping. The test containers were placed in 2-cm-deep water baths (38  $\times$  60  $\times$  7.5 cm) to maintain similar temperature and humidity environments among the culture containers. The photoperiod (13 h light:11 h dark) and temperature ( $\sim 28^{\circ}\text{C}$ ) in the culture room approximated the summer environment.

According to the methods of Hamasaki *et al.* (2013, 2014, 2015c), cultured animals were observed once a day in the morning until 44 days after metamorphosis into the megalopal stage for shell use (wearing or not), location (seawater or land), survival, and moulting. After the daily data collection, all seawaters of the test containers were renewed with designated treatment seawaters, and frozen mysid shrimps (Seahorse Ways Co. Ltd., Minamikyushu, Kagoshima, Japan) and freeze-dried polychaete (Kyorin Co. Ltd., Himeji, Hyogo, Japan) were given to the cultured animals as food in the seawater and on land, respectively. Feeding freeze-dried polychaete was initiated after the first landing event occurred for each cultured animal. The air temperature, seawater temperature and relative humidity recorded every 10 min with data loggers in the containers during the culture period were  $28.2 \pm 0.4^{\circ}\text{C}$  (mean  $\pm$  standard deviation),  $28.3 \pm 0.4^{\circ}\text{C}$ , and  $85.7 \pm 3.5\%$ , respectively. The salinity measured for the designated seawater treatment group after the daily data collection was  $34.4 \pm 0.8$  ppt,  $23.7 \pm 0.6$  ppt, and  $34.3 \pm 0.6$  ppt for the high-salinity seawater, low-salinity seawater, and high-salinity seawater containing riverine odours, respectively.

### Data analysis

Statistical analyses were performed using the R statistical software (R3.4.1; R Core Team, 2017) at a 5% significance level. Individual cultured animals were treated as replicates. A generalized linear mixed-effects model (GLMM) with binomial family (logit link; Zuur *et al.*, 2009) was used to evaluate the effects of test seawater treatments on the shell-wearing activity, landing activity, landing activity without a shell, and moulting of cultured animals. In these analyses, occurrence (1) or not (0) of the designated behaviour and moulting of individuals was a binary response variable, and the different seawater treatments (high salinity, low salinity, or high salinity with riverine odour conditions) represented the categorical explanatory variable. The animal age (number of days after metamorphosis into the megalopal stage) was also included as a continuous explanatory variable in the GLMM for evaluating the behavioural data. GLMM with the Poisson error distribution (log link) was applied to compare the intermoult periods (number of days) of the megalopae and first crabs among the test seawater treatments. The behavioural data were collected longitudinally for individual test animals (i.e., repeated measures data). Additionally, the test animals originated from two broods. Therefore, to account for a potential autocorrelation within individual animal data and animal origin (brood), the identity numbers of each animal and/or each brood were included in the GLMM as random intercept effects (Zuur *et al.*, 2009). The GLMM parameters (with standard errors, z-values with probabilities) were estimated using the *glmer* function implemented in the *lme4* package (Bates *et al.*, 2015). In the GLMM analyses, the coefficient estimate of the categorical explanatory variable is outputted for the respective treatments of low-salinity seawater and high-salinity seawater containing riverine odours, and it represents the change in the response variable relative to the baseline category (high-salinity seawater). To compare the animal survival among the test groups, a log-rank test was

performed with the *survdiff* function implemented in the survival package (Therneau, 2018).

### Results

The daily data on the numbers of animals that survived, the proportions of animals that exhibited shell-carrying activity, landing activity, and landing activity without a shell are shown in Fig. 1, and the

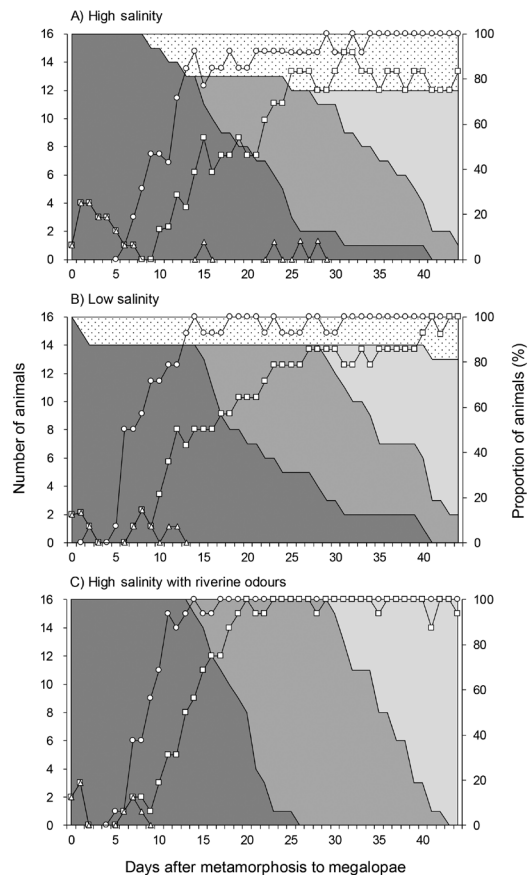


Fig. 1. The proportions of all surviving *Coenobita violascens* megalopae and early juveniles wearing shells ( $\circ$ ); the proportion of animals on land ( $\square$ ); the proportion of animals found on land that did not wear a shell ( $\Delta$ ); and the numbers of dead animals (dotted area), megalopae (dark grey area) and early juveniles (medium grey and light grey indicate the first and second crabs, respectively). The animals were cultured in containers with different seawater types: A, high salinity (34 ppt); B, low salinity (24 ppt); and C, high salinity (34 ppt) with riverine odours (mangrove river water).

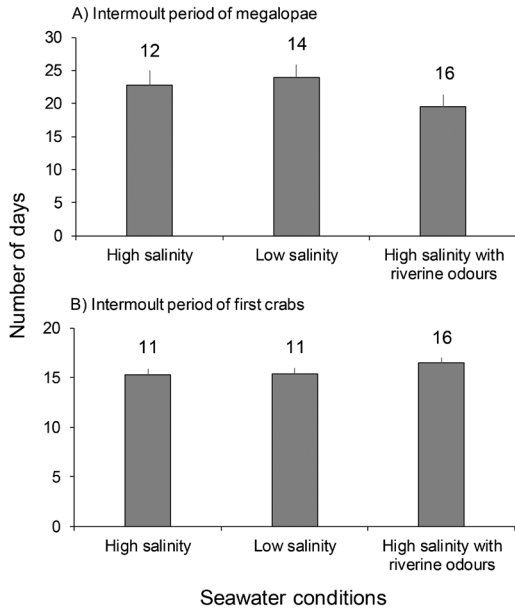


Fig. 2. Mean intermoult periods of the megalopae (A) and first crabs (B) of *Coenobita violascens* cultured in containers with different seawater types: high salinity (34 ppt), low salinity (24 ppt), and high salinity (34 ppt) with riverine odours (mangrove river water). The vertical bars indicate standard errors. The number on each bar indicates the sample size.

intermoult periods of animals are summarized in Fig. 2 for the megalopae and early juveniles of *C. violascens* cultured under different seawater treatments. Figures 3 and 4 also show similar data on animal behaviour and moulting for *C. rugosus*. The coefficient estimates in the GLMM evaluating the effects of seawater treatments (A, high salinity; B, low salinity; or C, high salinity with riverine odours) on the behaviour, moulting, and intermoult periods of the test animals are summarized in Table 1. The plus or minus signs of the coefficient estimates for the categorical explanatory variables (low salinity or high salinity with riverine odours) indicate a positive or negative effect, respectively, on the response variables compared with the baseline category (high salinity).

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Age significantly affected animal behaviour (Table 1); older animals exhibited higher activities for shell-wearing and landing, and landing activity without a shell was observed mainly in younger animals until they were ~10 days of age (Fig. 1). The proportions of animals wearing shells were significantly higher under low salinity and high salinity with riverine odour conditions compared with high salinity conditions, and the magnitude of effects (co-

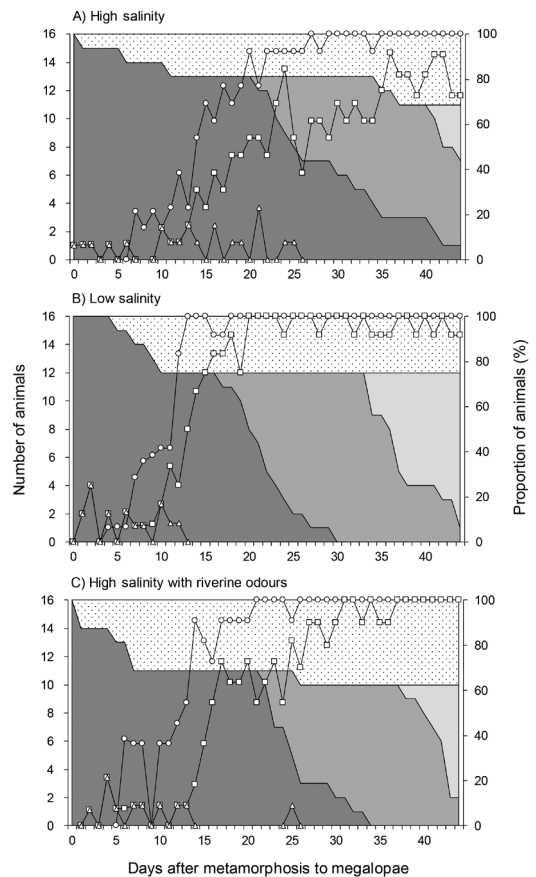


Fig. 3. The proportions of all surviving *Coenobita rugosus* megalopae and early juveniles wearing shells (○); the proportion of animals on land that did not wear a shell (□); and the proportion of animals found on land that did not wear a shell (Δ); and the numbers of dead animals (dotted area), megalopae (dark grey area) and early juveniles (medium grey and light grey indicate the first and second crabs, respectively). The animals were cultured in containers with different seawater types: A, high salinity (34 ppt); B, low salinity (24 ppt); and C, high salinity (34 ppt) with riverine odours (mangrove river water).



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Table 1. Evaluation of the effects of different seawater treatments (categorical explanatory variable; A, high salinity; B, low salinity; C, high salinity with riverine odours) and age (days after metamorphosis into megalopae) on the individual behavioural traits, moulting rates and intermoult periods of megalopae and early juveniles of the land hermit crabs *Coenobita violascens* and *C. rugosus*.

Species	Response variables	N	Coefficients	Estimates	SE	z values	P (> z )
<i>C. violascens</i>	Shell-wearing activity	1947	Intercept	-5.2469	0.7298	-7.189	< <b>0.0001</b>
			Treatment B	1.4804	0.5472	2.705	<b>0.0068</b>
			Treatment C	1.5044	0.5312	2.832	<b>0.0046</b>
			Age	0.4312	0.0268	16.090	< <b>0.0001</b>
	Landing activity	1947	Intercept	-4.3625	0.8000	-5.453	< <b>0.0001</b>
			Treatment B	0.4032	0.5744	0.702	0.4827
			Treatment C	1.6347	0.5593	2.923	<b>0.0035</b>
	Landing activity without a shell	1947	Intercept	-1.4699	0.3364	-4.370	< <b>0.0001</b>
			Treatment B	-0.6988	0.4187	-1.669	0.0951
			Treatment C	-0.9039	0.4233	-2.135	<b>0.0327</b>
	Moulting rate of megalopae	48	Intercept	-0.1554	0.0243	-6.406	< <b>0.0001</b>
			Treatment B	2.198	2.356	0.933	0.351
			Treatment C	1.089	1.083	1.006	0.314
	Moulting rate of first crabs	42	Intercept	20.070	1024	0.020	0.984
			Treatment B	3.363	2.522	1.317	0.188
			Treatment C	-1.141	1.410	-0.810	0.418
	Intermoult period of megalopae	42	Intercept	19.435	1182	0.016	0.987
			Treatment B	3.1630	0.1256	25.176	< <b>0.0001</b>
			Treatment C	0.0213	0.0818	0.261	0.794
	Intermoult period of first crabs	38	Intercept	-0.2015	0.0836	-2.409	<b>0.016</b>
Treatment B			2.7261	0.0772	35.33	< <b>0.0001</b>	
Treatment C			0.0059	0.1089	0.05	0.957	
<i>C. rugosus</i>	Shell-wearing activity	1652	Intercept	0.0773	0.0987	0.78	0.434
			Treatment B	-6.5085	0.5941	-10.956	< <b>0.0001</b>
			Treatment C	2.1478	0.4876	4.405	< <b>0.0001</b>
			Age	1.3869	0.4920	2.819	<b>0.0048</b>
	Landing activity	1652	Intercept	0.4372	0.0277	15.805	< <b>0.0001</b>
			Treatment B	-4.4338	0.3284	-13.502	< <b>0.0001</b>
			Treatment C	1.9706	0.3695	5.334	< <b>0.0001</b>
	Landing activity without a shell	1652	Intercept	1.1378	0.3762	3.024	<b>0.0025</b>
			Treatment B	0.1837	0.0090	20.361	< <b>0.0001</b>
			Treatment C	-2.1862	0.3689	-5.926	< <b>0.0001</b>
	Moulting rate of megalopae	48	Intercept	-0.2429	0.4403	-0.552	0.581
			Treatment B	-0.5208	0.4755	-1.095	0.273
			Age	-0.0867	0.0159	-5.454	< <b>0.0001</b>
	Moulting rate of first crabs	34	Intercept	0.7885	0.5394	1.462	0.144
			Treatment B	0.3102	0.7901	0.393	0.695
			Treatment C	0.0000	0.7628	0	1
	Intermoult period of megalopae	34	Intercept	-0.5596	0.6268	-0.893	0.3719
			Treatment B	2.9575	1.2181	2.428	<b>0.0152</b>
			Treatment C	1.5404	0.9226	1.670	0.095
	Intermoult period of first crabs	23	Intercept	3.3736	0.0558	60.44	< <b>0.0001</b>
Treatment B			-0.2527	0.0824	-3.07	<b>0.0022</b>	
Treatment C			-0.1050	0.0811	-1.30	0.1953	
Intermoult period of first crabs	23	Intercept	2.8708	0.1425	20.145	< <b>0.0001</b>	
		Treatment B	-0.1189	0.1436	-0.828	0.408	
		Treatment C	-0.1246	0.1467	-0.850	0.396	

Note: The data were analysed using a generalized linear mixed-effects model. The coefficient estimate of the categorical explanatory variable is outputted for treatment B and treatment C, and it represents the change in the response variable relative to the baseline category (treatment A). The bold values are significant.

efficient estimate) was similar between low salinity and high salinity with riverine odour treatments (Table 1). The riverine odours significantly stimulated landing activity and reduced landing activity without a shell, but low salinity did not significantly affect these activities (Table 1). In the high salinity, low salinity, and high salinity with riverine odour treatments, the moulting rates from the megalopae to the first crabs were 75%, 88%, and 100%, respectively; the rates from the first to second crabs were 92%, 79%, and 100%, respectively; and the final survival rates were 75%, 81%, and 100%, respectively. Thus, riverine odours tended to enhance the moulting and survival rates of animals, but the significant effects were not detected in the GLMM analyses (Table 1) or by a log-rank test for survival ( $\chi^2 = 4.6$ ,  $df = 2$ ,  $P = 0.0989$ ). However, the standard errors of the coefficient estimates for the high salinity with riverine odours (treatment C) were very large (Table 1), suggesting that the GLMM analyses did not successfully work because all animals moulted in this group. The intermoult periods (mean  $\pm$  standard error) of the megalopae were  $22.8 \pm 2.2$ ,  $23.9 \pm 2.0$ , and  $19.6 \pm 1.8$  days and of the first crabs were  $15.3 \pm 0.6$ ,  $15.4 \pm 0.6$ , and  $16.5 \pm 0.5$  days for high salinity, low salinity and high salinity with riverine odour groups, respectively (Fig. 2), and only the riverine odours had a significant effect on reducing the intermoult periods in the megalopal stage (Table 1).

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The shell-wearing and landing activities significantly increased with animal age (Table 1, Fig. 3). The proportions of animals that exhibited shell-wearing and landing activities were significantly higher under low salinity and high salinity with riverine odour conditions compared with high salinity conditions, and the magnitude of the effects (coefficient estimates) was larger in the low salinity treatment than in the high salinity with riverine odour treatment (Table 1). Naked animals on land appeared for

a longer period in the high salinity treatment compared with the other treatments (Fig. 3), but seawater conditions did not significantly affect the landing activity without a shell (Table 1). In the high salinity, low salinity, and high salinity with riverine odour treatments, the moulting rates from the megalopae to first crabs were 69%, 75%, and 69%; the rates from the first to second crabs were 36%, 92%, and 73%; and the final survival rates were 69%, 75%, and 63%, respectively. Thus, low-salinity seawater tended to enhance the moulting and survival rates of the animals, but significant effects were not detected in the GLMM analyses (Table 1) or by a log-rank test for survival ( $\chi^2 = 0.3$ ,  $df = 2$ ,  $P = 0.843$ ); however, for the low salinity treatment had a significant effect on increasing the moulting rates of the first crabs (Table 1). Intermoult periods (mean  $\pm$  standard error) of megalopae were  $29.2 \pm 2.2$ ,  $22.7 \pm 2.2$ , and  $26.3 \pm 2.5$  days, and those of first crabs were  $18.5 \pm 1.0$ ,  $15.6 \pm 0.6$ , and  $16.0 \pm 0.7$  days for high salinity, low

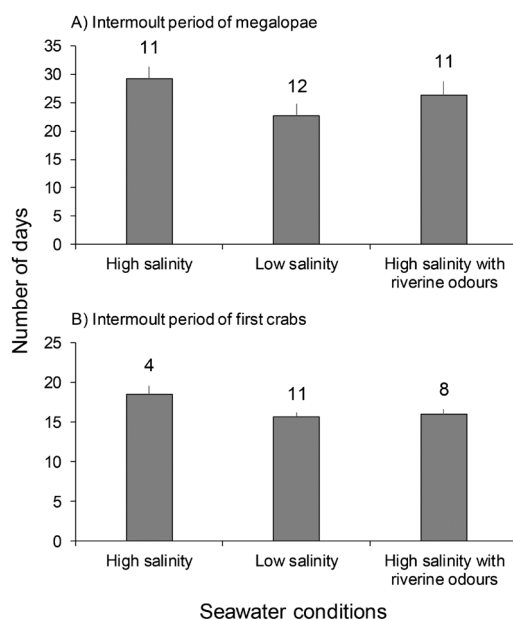


Fig. 4. Mean intermoult periods of the megalopae (A) and first crabs (B) of *Coenobita rugosus* cultured in containers with different seawater types: high salinity (34 ppt), low salinity (24 ppt), and high salinity (34 ppt) with riverine odours (mangrove river water). The vertical bars indicate standard errors. The number on each bar indicates the sample size.



salinity and high salinity with riverine odour groups, respectively (Fig. 4), and only the low-salinity seawater had a significant effect on reducing the intermoult periods in the megalopal stage (Table 1).

## Discussion

The results of the present study demonstrated that salinity reduction and riverine odours significantly affected the emigration behaviour and moulting of *C. violascens* and *C. rugosus* during the sea-to-land transition. For *C. violascens*, low salinity and riverine odours stimulated shell-wearing activity, and riverine odours enhanced landing activity. For *C. rugosus*, low salinity and riverine odours stimulated both shell-wearing and landing activities, and the magnitude of the effects was larger under low-salinity than riverine odour conditions. Riverine odours and low salinity tended to result in high moulting and survival rates of *C. violascens* and *C. rugosus*, respectively. The intermoult periods of *C. violascens* and *C. rugosus* megalopae were also significantly shorter under the riverine odours and low-salinity conditions, respectively, and those of the first crabs were not influenced by seawater conditions. Megalopae of terrestrial hermit crabs moult to first crabs after migrating onto land (Reese, 1968; Harvey, 1992; Brodie, 1999; Hamasaki *et al.*, 2011, 2014, 2015c). Therefore, megalopal moulting was accelerated under seawater conditions, which stimulated the landing activity of animals of both species.

In the present study, some naked megalopae without shells were observed on land, and they particularly occurred in high salinity groups in both species. It has been considered that larvae of terrestrial hermit crabs migrate offshore during the early zoeal stage and then immigrate to inshore regions in the megalopal stage (Hamasaki *et al.*, 2015b; Fujikawa *et al.*, 2018). Early megalopae of the terrestrial hermit crabs are active swimmers, which is likely associated with their immigration behaviour to inshore habitats where they settle (Hamasaki *et al.*, 2015b;

Fujikawa *et al.*, 2018). To infer the mechanisms of recruitment to mangrove estuaries by *C. violascens*, we previously investigated the settlement behaviour in 0-day-old to 6-day-old megalopae in the containers with the same three types of seawater as those used in the present study (Fujikawa *et al.*, 2018). The experiment demonstrated that low salinity decreased the swimming activity and enhanced the walking activity at the bottom, i.e., the conditions stimulated the settlement behaviour of megalopae, but riverine odours did not affect these activities. Therefore, active swimming behaviour might lead megalopae to mislanding without carrying shells under high salinity conditions in the limited space of the culture containers, whereas low salinity and riverine odours enhanced the shell-wearing activity of the megalopae, leading to successful emigration from sea to land.

The present study highlighted that environmental cues that stimulate emigration behaviour and moulting during the sea-to-land transition differed between *C. violascens* and *C. rugosus*. Mangrove riverine odours led the megalopae and early juveniles of *C. violascens* to migrate onto land, and salinity reduction was a strong environmental cue that enhanced landing behaviour of *C. rugosus* megalopae and early juveniles. Salinity reductions, which induced the settlement behaviour of megalopae (Fujikawa *et al.*, 2018), might widely occur along the shoreline due to the inflow of groundwater as well as river water on Ishigakijima Island (Tottori *et al.*, 2004). The megalopae of *C. rugosus* might settle on the seashore by detecting salinity reductions; afterward, they might migrate onto land near the settlement place and spend their entire life cycles there, causing *C. rugosus* to be the dominant species occurring on the beach and in the vicinity along the entire coasts of the island (Fujikawa *et al.*, 2017; Hamasaki *et al.*, 2017, 2018).

On the other hand, the distribution of *C. violascens* was restricted to the vicinity of the river, mainly in the mangrove estuaries (Fujikawa *et al.*, 2017; Hamasaki *et al.*, 2017, 2018). In the Nagura Ampa-

ru, which is a brackish estuary system with a tidal flat and mangrove tree area where *C. violascens* is most abundant on Ishigakijima Island (Fujikawa *et al.*, 2017; Hamasaki *et al.*, 2017, 2018), brachyuran megalopae are recruited to the tidal lagoon through a main estuary mouth before dawn near the new moon periods for a short time during the flooding tide under high salinity conditions (approximately 34 psu) (Kawachi *et al.*, 2009). The megalopae of *C. violascens* exhibit nocturnal swimming behaviour (Fujikawa *et al.*, 2018). Therefore, it could be inferred that the settlement of *C. violascens* megalopae is stimulated under low salinity conditions during the ebb tide near the river mouth area; afterward, they enter the tidal lagoon by swimming during the nocturnal flooding tide when the salinity is increasing, and their landing behaviour is stimulated by chemical cues (riverine odours) there. To further explain the recruitment mechanisms of *C. violascens*, we should understand how megalopae find suitable habitats, i.e., mangrove estuaries, where they settle and migrate onto land. It has been demonstrated that chemical cues such as seagrass odour from inshore habitats affect the orientation of megalopae of the blue crab *Callinectes sapidus* Rathbun, 1896 (Diaz *et al.*, 1999; Forward *et al.*, 2003; Epifanio and Cohen, 2016). The megalopae of *C. violascens* might utilize the increased but diffusing concentrations of chemical cues from the mangrove areas to the coastal region as an orientation cue to find a direction for the settlement habitat. To better understand the recruitment mechanisms of terrestrial hermit crabs, additional studies will be needed to investigate the effects of inshore odours on megalopal orientation in the sea.

### Acknowledgements

We would like to thank the Okinawa Prefectural Board of Education and the Agency for Culture Affairs, Ministry of Education, Culture, Sports, Science and Technology of Japan for permission to

collect the land hermit crabs (Licence Certificate No. 4-2058). We thank the members of the laboratory for helping with the laboratory work. We are also grateful to the editor and an anonymous reviewer for valuable comments and suggestions, which have improved the manuscript. This study was supported by Grants-in-Aid for Scientific Research B24310171 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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(Received May 14, 2018; Accepted July 31, 2018)