Tolerance of low salinity by larvae in six terrestrial hermit crab species (Decapoda: Anomura: Coenobitidae)

Katsuyuki Hamasaki, Ei Saeki, Kotomi Mizuta, Masaru Tanabe, Ikumi Yamazaki, Tetsuya Sanda, Shunsuke Fujikawa, Shigeki Dan, Shuichi Kitada

**Abstract.**—Salinity is an important ecological factor affecting larval survival and development of coastal and estuarine decapod crustaceans. We investigated the low salinity tolerance limits of larvae in the six terrestrial hermit crab species, the coconut crab *Birgus latro*, and the land hermit crabs *Coenobita brevimanus*, *C. cavipes*, *C. purpureus*, *C. rugosus*, and *C. violascens* to infer their early life history strategies. Zoaeeae and megalopae were exposed to six different salinity levels ranging from 5–30 ppt with intervals of 5 ppt for 24 h, and the median lethal salinity (MLS) was estimated as the salinity at which 50% of test larvae died. The MLS estimates were lowest in the first zoaeae, increased during the zoeal stage, and declined in the megalopal stage in all species. Early zoaeae and megalopae were euryhaline and later zoaeae stenohaline, suggesting that coenobitids exhibit a larval export strategy towards the offshore (oceanic) marine waters. Interspecific variation was evident in the salinity tolerance of the first zoaeae, probably reflecting the salinity conditions at the species-specific larval hatching place. In contrast, the low salinity tolerance ability of megalopae did not differ among species, suggesting that coenobitid megalopae might require similar habitats for the settlement and initiation of benthic life.

**Key words:** Coenobitidae, coconut crab, land hermit crab, early life history, mesopelagic larva

**Electronic supplementary material.**—The online version of this article contains supplementary material at https://www.jstage.jst.go.jp/article/crustacea/47/0/47_101/_article

**Introduction**

Estuarine decapod crustaceans with meroplanktonic larvae principally show two early life history strategies (Strathmann, 1982; Anger, 2001): 1) retention strategy–larvae develop in or close to the parental habitat within estuaries where low and unstable salinities frequently occur and 2) export strategy–zoeal larvae ontogenetically migrate towards coastal or offshore marine areas for development in more stable and higher salinity conditions, and megalopal larvae finally return to the estuarine habitat for initiating the benthic life.

In species using the retention strategy, all larval stages are better adopted to low salinity conditions, whereas in species with the export strategy, the low salinity tolerance limit for survival is lowest in newly hatched larvae, that increases during the zoeal stage and declines in a megalopal stage. In these case, early zoaeae and megalopae are euryhaline and later zoeal stages are stenohaline (Costlow et al., 1966; Cronin, 1982; Anger, 1991; Charmantier et al., 2002; Anger et al., 2008). Furthermore, interspecific variation is known for the low salinity tolerance of newly hatched larvae in phylogenetically related species with export strategy.
Crustacean Research 47

Anger et al., 2008; Smith et al., 2014), for instance, in congeneric sesarmid crabs of the genus Armases Abele, 1992, the low salinity tolerance limit for survival was lower in *A. roberti* H. Milne Edwards, 1853 larvae that hatch in riverine water than *A. ricordi* H. Milne Edwards, 1853 larvae that hatch in estuarine sea (Anger et al., 2008). Information of the low salinity tolerance for larval survival could thus assist us in inferring the early life-history traits of decapod crustacean species.

Terrestrial hermit crabs of the family Coenobitidae Dana, 1851 are composed of approximately 17 land hermit crab species in the genus Coenobita Latreille, 1829 and the coconut crab Birgus latro (Linnaeus, 1767) (Hartnoll, 1988; Poupin, 1996; McLaughlin et al., 2010; Rahayu et al., 2016). They are mainly distributed in subtropical and tropical coastal regions (Hartnoll, 1988). Coenobitid crabs are terrestrial except during the larval phase. Females hatch their eggs on shores, and the larvae spend a planktonic life in the sea through several zoeal stages before they metamorphose into the megalopal stage (Hartnoll, 1988; Nakasone, 2001; Hamasaki et al., 2015a). The megalopae acquire empty gastropod shells and then migrate onto land (Reese, 1968; Harvey, 1992; Brodie, 1999; Hamasaki et al., 2015b).

In Japan, six coenobitid species, including *B. latro*, *C. brevimanus* Dana, 1852, *C. cavipes* Stimpson, 1858, *C. purpureus* Stimpson, 1858, *C. rugosus* H. Milne-Edwards, 1837, and *C. violascens* Heller, 1862, commonly occur on the southern islands of the Ryukyu Archipelago (Okinawa Prefectural Board of Education, 1987, 2006; Nakasone, 1988, 2001; Asakura, 2004; Fujikawa et al., 2017). Hamasaki et al. (2015a) demonstrated that larvae of these six coenobitid species could metamorphose into megalopae, showing high survival rates when they were cultured in vessels with artificial seawater of 34 ppt salinity. However, very little is known about the low salinity tolerance of larvae of terrestrial hermit crab species.

As a basis for understanding the early life history strategies of terrestrial hermit crab species, the aim of the present study was to evaluate the low salinity tolerance limits in the zoeal and megalopal stages of six coenobitid species that occur in Japan by testing larval survival responses to different salinity media in short-term exposure experiments.

### Materials and Methods

**Larval source**

Culture experiments were conducted in the laboratory at Tokyo University of Marine Science and Technology during the reproductive season of each species from 2012 to 2015. Ovigerous females of *B. latro*, *C. brevimanus*, *C. cavipes*, *C. purpureus*, *C. rugosus*, and *C. violascens* were captured by hand during late June to early July on Hatomajima Island (24°28′N, 123°49′E) and Ishigakijima Island (24°23–31′N, 124°07–18′E), Okinawa Prefecture, southern Japan. They were transferred to the laboratory where the air temperature was controlled at 28°C and maintained in tanks equipped with simulated land and sea areas (artificial seawater, 34 ppt salinity; Sealife, Marinetech Co. Ltd., Tokyo, Japan) until hatching occurred according to the methods of Hamasaki et al. (2009) and Hamasaki (2011). After larvae hatched, all female crabs were released back into the natural habitats.

Females hatched their eggs into seawater in tanks at night, and rotifers of the *Brachionus plicatilis* species complex were fed to newly hatched larvae in these tanks in the early morning (5 am). Newly hatched larvae were then transferred (11 am) and cultured with *Artemia* and rotifers in 5-l or 30-l tanks (28°C and 34 ppt salinity) according to Hamasaki et al. (2011, 2013a). Larval culture was conducted twice for each species using larvae hatched from different females.
Salinity tolerance experiments
Six salinity levels, ranging 5–30 ppt with intervals of 5 ppt, were used to determine the low salinity tolerance limits of larvae. Test seawater was prepared by adding artificial seawater salts to distilled water. Intermoult periods of coenobitid zoeae generally increased from 3 to 7 days with advancing stages, and they mostly metamorphose to megalopae through the sixth zoeae in *C. cavipes* and fourth zoeae in other species under culture conditions similar to the present study (Hamasaki et al. 2015a). Salinity tolerance experiments were conducted twice for the first to sixth zoeae (*C. cavipes*) or the first to fourth zoeae (other species) and megalopae (all species) using larvae from different culture lots of each species. The first zoeae and megalopae were subjected to experiments in the afternoon of the first day of these stages. It was difficult to identify the zoeal stages by observation with the naked eye; therefore, ten zoeae were sampled from the culture tanks every morning, and their larval stages were microscopically determined based on their morphological characteristics (Reese & Kinzie, 1968; Shokita & Yamashiro, 1986; Nakasone, 1988; Hamasaki et al., 2014; Kato et al., 2015). Experiments were also started in the afternoon every 2–7 days when the moulting rates to successive zoeal stages (second to sixth zoeae), exceeded 70%. The ages of test larvae are summarized in Table S1 in the electronic supplementary material. In some culture lots of larvae, experiments using megalopae took 4 and 5 days (two lots of *C. cavipes*) and 2 days (each one lot of *C. rugosus* and *C. purpureus*) because moulting synchronicity was somewhat low when larvae metamorphosed into the megalopal stage.

When salinity tolerance experiments were initiated, larvae were collected from the culture tanks and rinsed once with the designated salinity seawater in 1-L beakers. They were then stocked individually in six-well cell culture plates containing 9-ml of test seawater in each cell. The test wells did not contain any foods for larvae. Five culture plates (*N* = 30) were principally prepared for each test salinity level in each larval stage. Culture plates were wrapped in cellophane and set in the temperature-controlled chamber (28°C and 14 light: 10 dark cycle) for 24 h. Individual larvae were then microscopically observed and dead animals counted. The zoeal stage was determined for each test larva, and when the zoea was not in the targeted stage (being still in the previous stage), they were excluded from data analysis. The mean number of larvae for each test salinity level ranged from 19–30 individuals (Table S1).

Statistical analysis
Statistical analyses were performed using the R statistical software (R3.4.1; R Core Team, 2017). To evaluate the low salinity tolerance limits of respective larval stages of each species, the median lethal salinity (MLS) was estimated as the salinity at which 50% of the test larvae died. The MLS values with standard errors were estimated using the LD50 function (Hill method) implemented in the HelpersMG package (Girondot, 2017). In the fourth zoeal stage of culture lot no. 2 of *B. latro*, survival dropped from 100% at 25 ppt to 0% at 20 ppt within one unit of salinity level (5 ppt interval), and the MLS value was not estimated in a reliable manner, while showing a very large standard error. In this case, the MLS was calculated as the midpoint value between these two salinity levels. A linear mixed-effects model (LMM) (Zuur et al., 2009) was used to compare the MLS estimates among the larval stages and species. In the LMM analysis, larval stages and species were the categorical explanatory variables, and the culture lot number of larvae of each species was included as a random intercept effect because the data were collected longitudinally in each culture lot (i.e., repeated-measures data) (Zuur et al., 2009). Statistical significance of the explanatory variable was
evaluated using the *lme* and *anova* (marginal type) functions in the nlme package (Pinheiro et al., 2018). To evaluate the degree of interspecific variation of low salinity tolerance traits of larvae in comparison with newly hatched larvae (first zoeae), relationships between mean MLS values of the first zoeae and the successive larval stages (second to fourth zoeae and megalopae) were analysed by linear regressions using data from all species. The significance level was set at $P<0.05$.

### Results

Survival rates of respective zoeal stages and megalopal stage when exposed to different salinity media for 24 h are shown in Fig. S1 in the electronic supplementary material. The MLS estimates with standard errors for respective larval stages from two culture lots of each species are summarized in Table S1 in the electronic supplementary material. The mean MLS values with standard deviations are illustrated for respective larval stages of each species in Fig. 1. The relationships between mean MLS values of the first zoeae and the successive larval stages (second to fourth zoeae and megalopae) are also shown in Fig. 2.

The MLS estimates were significantly different among larval stages ($F = 19.97; DF = 6, 46; P<0.0001$) and species ($F = 24.85; DF = 5, 6; P = 0.0006$). The mean MLS values of the first
zoeae were lowest in *C. cavipes* (8.2 ± 0.4 ppt), followed by *C. violascens* (11.0 ± 1.3 ppt), *C. brevimanus* (11.7 ± 2.1 ppt), *C. rugosus* (12.1 ± 2.8 ppt), and *C. purpureus* (15.4 ± 0.9 ppt) and highest in *B. latro* (16.8 ± 2.8 ppt). The MLS estimates ontogenetically changed in all species; they increased with the zoeal development and then declined at the megalopal stage (Fig. 1). The linear regression equations between mean MLS values of the first zoeae and the second, third and fourth zoeae were statistically significant ($R^2 = 0.865–0.931; P = 0.0018–0.0071$), and the slope of the equation decreased from second zoeae (1.305) through third zoeae (1.082) and fourth zoeae (0.858), whereas the equation between those of the first zoeae and megalopae was not statistically significant ($R^2 = 0.376; P = 0.1958$) (Fig. 2).

**Discussion**

Our results highlighted the similar salinity tolerance patterns of larvae in six terrestrial hermit crab species; *B. latro, C. brevimanus, C. cavipes, C. rugosus, C. purpureus, and C. violascens*. The low salinity tolerance limit for survival was lowest in the first zoeae and increased during the zoeal stage and decreased in the megalopal stage for all six species. This ontogenetic pattern in the larval salinity tolerance (i.e., early zoeae and megalopae being euryhaline and later zoeae stenohaline), suggests that these coenobitid crabs may exhibit the larval export strategy (Anger, 1991; Charmantier *et al.*, 2002; Anger *et al.*, 2008). The larval export strategy has also previously been suggested for *B. latro* (Hamasaki *et al.*, 2013b). The spectral sensitivity of zooplankton has been considered to match the spectral distribution of light in the environment to maximize photon capture (sensitivity hypothesis) (Cohen & Forward, 2002; Forward, 1988, 2009). Hamasaki *et al.* (2013b) examined the phototactic behaviour to different light wavelengths of the zoeal and megalopal stages of laboratory-reared *B. latro* larvae, and assuming the sensitivity hypothesis, they suggest that after hatching on the shores, *B. latro* zoeae may migrate to the upper layer of offshore/oceanic waters.

Interspecific variation was evident, and three groups were identified in the low salinity tolerance limit of newly hatched larvae of coenobitid crabs in the present study: 1) strongly euryhaline, *C. cavipes* (8 ppt); 2) moderately euryhaline, *C. violascens, C. brevimanus, and C. rugosus* (11–12 ppt); and 3) weekly euryha-
line, *C. purpureus* and *B. latro* (15–17 ppt). The interspecific variation in the low salinity tolerance ability of newly hatched larvae of coenobitid crabs may be related to salinity conditions at larval hatching locations as reported for congenic sesarmid crabs, as the low salinity tolerance ability was greater in *A. roberti* larvae that hatch in riverine water than *A. riciordi* larvae that hatch in estuarine conditions (Anger et al., 2008). The general habitats of coenobitid crabs on the coasts of islands in the Ryukyu Archipelago, Japan, could be summarized as follows: *C. cavipes*, coastal and inland forests (Okinawa Prefectural Board of Education, 1987, 2006; Nakasone, 1988, 2001; Doi et al., 2018; Hamasaki et al., 2018); *C. violascens*, vicinity of the river, mainly in mangrove estuaries (Okinawa Prefectural Board of Education, 1987, 2006; Nakasone, 1988; Fujikawa et al., 2017; Hamasaki et al., 2017, 2018); *C. brevimanus*, coastal forests (Okinawa Prefectural Board of Education, 1987; Tsuru et al., 2018); *C. rugosus*, beach and along the entire coast, including river mouth areas (Okinawa Prefectural Board of Education, 1987, 2006; Nakasone, 1988, 2001; Fujikawa et al., 2017; Tsuru et al., 2018); *C. purpureus*, sandy beach and coastal forests (Okinawa Prefectural Board of Education, 1987, 2006; Nakasone, 1988, 2001; Fujikawa et al., 2017; Tsuru et al., 2018); and *B. latro*, limestone shore and inland (Sato & Yoseda, 2013; Oka et al., 2016; Fujikawa et al., 2017; Tsuru et al., 2018). Larval hatching locations have previously been reported for several coenobitid species: in the Ryukyu Archipelago, *C. cavipes* released larvae during both nocturnal flood and ebb tides at the bank of the river mouth (Doi et al., 2018); *C. violascens* released larvae during or after the nocturnal high tides in the mangrove forests (Doi et al., 2016); and *C. rugosus* and *C. purpureus* released larvae around high tide on the sandy beach (Nakasone, 2001); and in Vanuatu, Niue and Christmas Island, *B. latro* released larvae on the coastlines (Schiller et al., 1992). Considering the low salinity tolerance ability of newly hatched larvae, general habitats and previously reported hatching locations of coenobitid crabs, it can be inferred that 1) strongly euryhaline and most inland-dwelling *C. cavipes* might have opportunities to release larvae in the upper river banks, 2) the moderately euryhaline trait of *C. violascens* and *C. rugosus* may be an adaptation to their estuarine habitats where reduced and unstable salinities frequently occur depending on local weather conditions, and coastal-forest dwelling *C. brevimanus* may release larvae along the estuarine coasts, and 3) weekly euryhaline trait of *C. purpureus* and *B. latro* may be related to larval release behaviours on the coasts in more stable and higher salinity conditions.

Our linear regression analyses between MLS values of the first zoeae and successive larval stages suggested that the degree of interspecific variation in larval salinity tolerances weakened during the zoeal stage, probably reflecting the larval export strategy towards the similar offshore/oceanic environments in later zoeal stages of coenobitid crabs. The present study also demonstrated that interspecific variation was not observed in the low salinity tolerance ability at the megalopal stage, suggesting that coenobitid megalopae require similar habitats for the settlement and initiation of the benthic life-history stage. Fujikawa et al. (2018a) examined settlement behaviour in laboratory-raised early megalopae of *C. violascens* under different seawater conditions: 1) offshore salinity (34 ppt, control), 2) offshore salinity (34 ppt) with inshore odours (riverine water), and 3) inshore salinity (24 ppt). They demonstrated that inshore odours did not affect swimming and walking activities, whereas the inshore salinity conditions decreased swimming activity and enhanced walking activity (i.e., stimulated the settlement behaviour of megalopae). Fujikawa et al. (2018b) also examined behaviour for landing in laboratory-raised megalopae and early juveniles of *C. violascens* and *C. rugosus*.
under the same seawater conditions as employed by Fujikawa et al. (2018a). In *C. violascens*, reduced salinity and riverine odours stimulated shell-wearing activity, and riverine odours enhanced landing activity. In *C. rugosus*, reduced salinity and riverine odours stimulated both shell-wearing and landing activities, and the magnitude of effects was greater in reduced salinity than riverine odours. Riverine odours may thus play an important role in emigration from sea to land by mangrove-dwelling *C. violascens*, while reduced salinity conditions may stimulate settlement landing behaviour in *C violascens* and *C. rugosus*. Salinity reductions widely occur along the shoreline due to the inflow of ground water as well as river water in the islands (Tottori et al., 2004). Therefore, these may act as common environmental cues for inducing settlement of coenobitid megalopae near or on the shoreline.

The present study demonstrated ontogenetic changes in low salinity tolerance limits of coenobitid zoeae and megalopae typical for species employing the larval export strategy. To elucidate the larval dispersal ability of coenobitid crabs at sea, further study will need to examine the effects of different salinities on survival and development of larvae under feeding conditions.

**Acknowledgements**

We would like to acknowledge 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 (License Certificate No. 4–1997 and No. 4–2058). We thank the members of the laboratory for helping with laboratory work. We are also grateful to the editor and reviewers for valuable comments and suggestions, which have improved the manuscript. This study was supported by Grants-in-Aid for Scientific Research B24310171 to KH and C18K05781 to SK from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

**Literature Cited**


Fujikawa, S., Hamasaki, K., Dan, S., & Kitada, S., 2018b. 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. Biogeography, 20: 111–121.


Reese, E. S., & Kinzie, R. A. III, 1968. The larval development of the coconut or robber crab Birgus latro (L.) in the laboratory (Anomura, Paguridea). Crustaceana Supplement, 2:
117–144.

**Addresses**
(KH) (ES) (KM) (MT) (IY) (TS) (SD)
(SK) Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Konan, Minato, Tokyo 108–8477, Japan
(TS) Present address: Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Japan Fisheries Research and Education Agency, Fukai-Ota, Ishigaki, Okinawa 907–0451, Japan

**E-mail address of corresponding author**
(KH) hamak@kaiyodai.ac.jp