Relative growth of pereiopods of the megalopa and early juveniles of *Portunus trituberculatus* (Miers, 1876) (Brachyura: Portunidae)

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**Abstract.**— We examined relative growth of the pereiopods in megalopae and first to fifth crab stages of *Portunus trituberculatus* (Miers, 1876) reared in the laboratory. Relative growth of the body parts in relation to a reference dimension (carapace length) was analyzed by the log-transformed allometric growth equation \( \ln y = \ln a + b \ln x \). Body proportions differed between the megalopae and juvenile crabs. In juveniles, the chela size showed isometric growth \((b = 1)\); the second to fourth pereiopods (walking legs) showed mainly negative growth \((b < 1)\) in length, and isometric growth \((b = 1)\) in width; and the width and length of the fifth pereiopod showed positive allometric growth \((b > 1)\). *Portunus trituberculatus* cling to drifting seaweeds using their chelae and pereiopods before undertaking a benthic life after the fourth or fifth crab stages; the chelipeds and fifth pereiopods are utilized mainly in the burying behavior of juveniles after settlement. Accordingly, the negative allometric growth in length of the walking legs likely represents an adaptation for a lifestyle shift from drifting seaweeds to benthos, whereas the isometric or positive allometric growth of the chelae and fifth pereiopods would be advantageous for the burying behavior of the crabs post-settlement.

**Key words:** Allometric growth, chelipeds, walking legs, settlement, swimming crab

**Introduction**

Ontogenetic changes in the body proportions of living organisms may be quantified through studies of relative growth, by which the growth rates of some body parts are determined in relation to a reference dimension. Analyses of relative growth in crustacean species have focused primarily on determining size at sexual maturity in brachyuran crabs through characterizations of secondary sexual characters, such as a wider abdomen in females and larger chelipeds in males (Hartnoll, 1974, 1978; Somerton, 1980; Ogawa *et al*., 2011).

Many benthic decapod crustaceans develop via a complex life cycle, comprising embryonic, meroplanktonic larval, and benthic juvenile to adult phases (Anger, 2001; Anger *et al*., 2015). The meroplanktonic larval phase of brachyuran crabs is generally composed of several zoeal stages and a megalopal stage (Anger, 2001; Anger *et al*., 2015). Zoeae swim using the first and second maxillipeds as they develop only rudimentary pereiopods, whereas megalopae have fully developed pereiopods and are able to swim using the pleopods under the abdomen; after molting to the first crab stage, the pleopods become reduced and the abdomen is positioned closer to the thoracic sternum as in the adult crab stage (Anger, 2001; Anger *et al*., 2015). Thus, in brachyuran crabs, behavior and morphology change in conjunction with lifestyle transitions during the early life stage. However, little is known about the quantitative changes in body proportions through the megalopal to juvenile stages of
brachyuran crabs, during which a habitat shift from the plankton to benthos community takes place (Kobayashi, 2001).

The swimming crab *Portunus trituberculatus* (Miers, 1876) is found in shallow water to depths of about 50 m, off the coasts of Japan, Korea, and China (Miyake, 1983). This species is an important fishery resource, with a global production of about 606,000 tonnes in 2014 (FAO, 2018). Adult females hatch their eggs from spring to summer, and the larvae develop through four planktonic zoeal stages and one megalopal stage, within about 3–4 weeks, before they metamorphose to the first crab stage (Hamasaki, 1996). Megalopae larvae and early juveniles of *P. trituberculatus* are frequently found on drifting macroalgae (*Sargassum* spp.) and eelgrass (*Zostera marina*) (Inoko & Takaba, 1978; Tanigawa, 2001), and the fourth and/or fifth crab stages are reportedly associated with eelgrass beds and tidal flats in shallower water (Oshima, 1938; Hamasaki, 1996; Tanigawa, 2001). Takeshima *et al.* (2017) examined the clinging and burying behaviors of megalopae and first to fifth crab stages of *P. trituberculatus*, using tanks with bottom substrates (naked polyethylene bottom, gravel or sand) and an attachment substrate (artificial seaweed) in the water column. They demonstrated that the proportion of individuals that exhibited clinging behavior increased from the megalopal to the first crab stage, peaked in the second and third crab stages, and declined through the fourth and fifth crab stages, whereas the proportion that buried in the bottom substrate linearly increased from the first or second to the fifth crab stages. Thus, in nature, megalopae and early juveniles of *P. trituberculatus* utilize drifting seaweeds as a vehicle before shifting to the benthos after the fourth crab stage. Takeshima *et al.* (2017) also documented that the species’ pereiopods (i.e., chelipeds and/or walking legs) are involved in both the clinging and burying behavior of the megalopae and early juveniles. However, relative growth of the pereiopods of *P. trituberculatus* has only been examined for the chelipeds to determine size at sexual maturity in males (Hamasaki, 1996).

The objective of our study was to examine the relative growth of the pereiopods in megalopae and early juveniles of *P. trituberculatus* to understand the morphological changes in this structure in relation to the species’ shift from plankton to the benthos during the early life stage.

### Materials and Methods

**Crab culture and sample collection**

To acquire the *P. trituberculatus* megalopae and juveniles used for measurement of body parts, newly hatched larvae were obtained from two females (carapace width: 206 and 212 mm), on May 10, 2012, following the method described by Dan *et al.* (2014). The larvae were cultured at the Tamano Laboratory of the National Research Institute of Fisheries and Environment of Inland Sea, Japan Fisheries Research and Education Agency, Tamano, Okayama, Japan. Larvae were stocked in a 14-kl concrete tank containing 10 kl of seawater (temperature, 25°C; salinity, 33) for the first zoeal to megalopal stages. Juveniles in the first to fifth crab stages were cultured in a 500-l polyethylene tank containing 500 l of seawater (temperature, 25°C; salinity, 33). To reduce cannibalism among the juvenile crabs, six fronds of artificial seaweed (Sakai Ovex Co., Fukui, Japan) were used as refugia; two fronds were placed on the tank bottom, and four were suspended vertically between the surface and bottom. The artificial seaweed had a plastic stem (diameter 7.5 mm, length 1 m) and leaves (diameter 1.0 mm, length 9 cm). Rotifers (*Brachionus plicatilis* species complex; 30 indiv. ml⁻¹) were fed to all zoeal stages; *Artemia* nauplii (0.3–4 indiv. ml⁻¹) were added from the third zoea to second crab stage; and a formula feed (Kurumaebi S-9, Higashimaru Co.,
Kagoshima, Japan) (5 g day$^{-1}$) was offered from the megalopa to the fifth crab stage. Megalopae and first to fifth crab stages in the intermolt period were collected from the culture tanks, then fixed with 10% neutral formalin for one day, and finally preserved in 70% ethanol solution until the morphological measurements were made.

**Morphological measurements**

The following morphological variables were measured using five individuals of each stage (Fig. 1): total carapace width (TCW) (Fig. 1: A-1 and B-1), carapace length (CL) (A-2 and

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**Fig. 1.** Measurements of *Portunus trituberculatus* megalopa (A, C, E and G) and juveniles (B, D, F and H). Carapace (A, B), total carapace width (1) and carapace length (2); chela (C, D), chela length (3) and chela height (4); second to fourth pereiopods (E, F) and fifth pereiopod (G, H), dactylus length (5), dactylus width (6), propodus length (7), propodus width (8), dactylus setae lengths 1–4 (9–12), and propodus setae lengths 1–4 (13–16).
B-2), crusher or cutter chela length (C-3 and D-3), crusher or cutter chela height (C-4 and D-4), dactylus length (DL) (E-5, F-5, G-5, and H-5), dactylus width (DW) (E-6, F-6, G-6, and H-6), propodus length (PL) (E-7, F-7, G-7, and H-7), propodus width (PW) (E-8, F-8, G-8, and H-8), and lengths of setae on the upper and lower margins of the dactylus (E-9–12, F-9–12, G-9–12, and H-9–12) and the propodus (E-13–16, F-13–16, G-13–16, and H-13–16) of the second to fifth pereiopods. Lengths of setae on the upper and lower margins were measured at two points: at the middle of the left and right parts, as divided at the widest part of the dactylus (DW) and propodus (PW). Measurements on the second to fifth pereiopods were made for the right and left ones of each sample, and the mean values were used for analyses. *Portunus trituberculatus* show heterochely of the crusher and cutter chelae (Hamasaki, 1996; Masunari *et al.*, 2015). Hence, a fine molar on the dactylus of the right chela was observed in the megalopal stage, and the right chelae of the juveniles were all crusher with a large molar on the dactylus, and the left chelae were all cutter (Fig. 2); consequently, the right and left chelae measurements were treated separately. All measurements were made using a stereomicroscope (Nikon SMZ800, Nikon Corp., Tokyo, Japan) equipped with a digital camera (Nikon DS-Fil) and an image-analyzing software system (Nikon NIS-Elements D 3.00, SP1 Build 455) with an accuracy of 0.01 mm, except the carapace of the third to fifth crab stages and the chelae of the fifth crab stage were measured using digital calipers (CD-S20C, Mitutoyo Corp., Kanagawa, Japan) with an accuracy of 0.01 mm.

**Statistical analysis**

Statistical analyses were performed using R version 3.3.2 (R Core Team, 2016), with a 5% significance level. The difference of the right and left chelae measurements was tested using
Welch’s *t*-test for data on megalopae, or an analysis of covariance for data on juveniles. Relative growth of the body parts in relation to a reference dimension (i.e. CL) was analyzed by the allometric growth equation (Huxley, 1932): \( y = ax^b \), where \( x \) is CL, \( y \) is the other body-part measurement, \( b \) is the allometric growth coefficient, and \( a \) is the initial growth constant. Parameters were estimated by applying the regression analysis to the log-transformed allometric growth equation \( \ln y = \ln a + b \ln x \). The relative growth patterns are defined as follows: \( b > 1 \), positive allometric growth, showing faster growth of \( y \) than \( x \); \( b = 1 \), isometric growth, showing the same growth rate for \( y \) and \( x \); and \( b < 1 \), negative allometric growth, showing slower growth of \( y \) than \( x \). The allometric growth coefficient, \( b \), was tested against the null hypothesis (\( H_0: b = 1 \)) using a *t*-test.

### Results

Measurements of the carapace and chelae are summarized for each stage, as mean ± standard deviation, in Table 1. There was no significant difference between the right and left chelae lengths in the megalopal stage (\( P = 0.3789 \)), but the right chela height was significantly greater than the left (\( P = 0.04094 \)). In the crab stages, the crusher (right) chelae was greater in both length (\( P = 0.00759 \)) and height (\( P =

| Stage | CL  | TCW | CrCL | CrCH | CtCL | CtCH |
|-------|-----|-----|------|------|------|------|
| MG    | 2.43 ± 0.26 | 1.87 ± 0.17 | 1.65 ± 0.05 | 0.51 ± 0.02 | 1.62 ± 0.05 | 0.49 ± 0.01 |
| C1    | 2.79 ± 0.05 | 4.67 ± 0.22 | 2.61 ± 0.06 | 0.79 ± 0.02 | 2.49 ± 0.09 | 0.73 ± 0.02 |
| C2    | 3.88 ± 0.24 | 7.18 ± 0.45 | 3.50 ± 0.24 | 1.04 ± 0.07 | 3.32 ± 0.21 | 0.95 ± 0.06 |
| C3    | 5.43 ± 0.23 | 10.21 ± 1.15 | 4.85 ± 0.33 | 1.47 ± 0.10 | 4.60 ± 0.45 | 1.35 ± 0.10 |
| C4    | 7.51 ± 0.34 | 14.58 ± 0.62 | 6.71 ± 0.47 | 2.02 ± 0.18 | 6.39 ± 0.26 | 1.80 ± 0.06 |
| C5    | 10.6 ± 1.05 | 21.22 ± 2.39 | 10.43 ± 1.23 | 2.78 ± 0.44 | 9.92 ± 1.14 | 2.73 ± 0.25 |

\( N = 5 \) for each stage. CL, carapace length; TCW, total carapace width; CrCL, crusher chela length; CrCH, crusher chela height; CtCL, cutter chela length; CtCH, cutter chela height.

![Fig. 3](image_url). Relative growth of total carapace width (TCW), crusher chela length (CrCL), crusher chela height (CrCH), cutter chela length (CtCL), and cutter chela height (CtCH) plotted against the carapace length (CL) of *Portunus trituberculatus*. MG, megalopa; C1–C5, first to fifth crab stages.
Measurements of the body parts are plotted against the reference dimension (i.e. CL) in logarithmic scales in Figs. 3 to 7. Results of the allometric growth analyses are summarized in Table 2. Measurements of the megalopae were not included in the relative growth analyses because the body proportions largely differed between the megalopal and juvenile crabs. The relative sizes of the body parts largely increased in the crab stages as compared with the megalopal stage; in particular, juvenile crabs had a wider carapace, and longer and denser setae on the pereiopods (Fig. 1).

The TCW showed positive allometric growth, and all chela dimensions showed isometric growth (Fig. 3, Table 2). The DL and PL of the second to fourth pereiopods showed negative allometric growth, except the DL of the fourth pereiopod showed isometric growth (Figs. 4–6, Table 2). The DW and PW of the second to fourth pereiopods showed isometric growth (Figs. 4–6, Table 2), and all dimensions of the fifth pereiopod showed positive allometric growth (Fig. 7, Table 2). Setae length on the second to fourth pereiopods showed negative allometric growth, except the third measurement positions of the dactylus of the second and fourth pereiopods displayed isometric growth (Figs. 4–7, Table 2).
Discussion

Megalopae of *P. trituberculatus* actively swim using their pleopods on the underside of the abdomen while folding their pereiopods alongside the carapace (K. Hamasaki, personal observation). In nature, both megalopae and early juveniles of *P. trituberculatus* may cling to drifting seaweeds, and this clinging behavior strengthens through to the first to third crab stages; however, megalopae cannot bury themselves in sand, and the burying behavior only strengthens through the first to fifth crab stages (Takeshima et al., 2017). The present study illustrates that the relative size of the pereiopods in relation to the CL is smaller in megalopa larvae than in juveniles of *P. trituberculatus*. Additionally, the megalopae exhibited fewer and shorter setae on the second to fifth pereiopods, likely related to their active swimming behavior at this stage. After molting to the first crab stage, the carapace width enlarged, and the TCW showed positive allometric growth toward the adult form.

The lengths and widths of the left and right chelae of the *P. trituberculatus* juveniles showed isometric growth. The chelipeds of *P. trituberculatus* are used for clinging to floating substrate as well as for burying in sand (Yamazaki, 1974; Takeshima et al., 2017); in brachy-
uran crabs, this body part is generally used for capturing prey and in agonistic behavior (Toba, 1989; Lee & Seed, 1992). Thus, the chelipeds are an essential structure enabling survival and growth of the crabs; accordingly, the chela dimensions showed isometric growth in the first to fifth crab stages of *P. trituberculatus*. Heterochely is known in *P. trituberculatus* (Hamasaki, 1996; Masunari et al., 2015). Hamasaki (1996) reported that 99% of artificially cultured crabs and 91% of wild crabs had a right crusher chela. In the present study, the laboratory-reared megalopae displayed a fine molar on the dactylus of the right cheliped, and the right and left chelae of juveniles showed distinct crusher and cutter morphs, respectively, with the right chelae larger than the left. Thus, the observations of the present study support those of Masunari et al. (2015), who first reported that heterochely (a right crusher) in *P. trituberculatus* begins in the megalopal stage.

The widths and lengths of the second to fourth pereiopods showed different patterns of relative growth; DW and PW showed isometric growth, whereas DL and PL principally showed negative allometric growth. The second to fourth pereiopods are involved in clinging to drifting macroalgae and walking on substrate (Takeshima et al., 2017). Relatively longer pereiopods might be advantageous for
clinging to seaweeds but disadvantageous for walking on substrate.

The fifth pereiopods of portunids, generally called swimming legs, typically have a wider dactylus and propodus than the other walking legs (i.e., the second and third pereiopods). Juveniles of *P. trituberculatus* can swim using the fifth pereiopods (K. Hamasaki, personal observation), and they bury themselves backwards in sand, using mainly the chelipeds and fifth pereiopods in conjunction with the other walking legs (Yamazaki, 1974). Moreover, if one of the chelipeds or fifth pereiopods is lost, the individual is not able to complete the burying behavior (Yamazaki, 1974). Burying behavior of *P. trituberculatus* juveniles occurs mainly during the daytime, and is presumably an important strategy to avoid predation by diurnal fishes (Takeshima et al., 2017). Thus, the fifth pereiopods are important legs for both swimming and burying, explaining why the dimensions of the fifth pereiopods of *P. trituberculatus* juveniles might show positive allometric growth.

The lengths of setae on the upper and lower margins of the second to fifth pereiopods showed mainly negative allometric growth. Relatively longer setae might generate larger lift-thrust forces (Vogel, 1994), thereby increasing the chance of finding drifting algae.
through a higher swimming ability during the first to second crab stages.

The present study reveals the relative growth patterns of the pereiopods in the megalopae and early juveniles of *P. trituberculatus* and highlights the usefulness of allometric growth analysis to understand the morphological changes of pereiopods in relation to settlement behavior during the early life stage from the plankton to the benthos in this species.

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