Growth and elemental composition (C, N, P) during larval developmental stages of mass-cultured swimming crab *Portunus trituberculatus*

B. K. Lim, K. Hirayama

Graduate School of Marine Science and Engineering, Nagasaki University, 1–14 Bunkyo-machi, Nagasaki 852, Japan

ABSTRACT· Larval rearing of the swimming crab *Portunus trituberculatus* for mass production was carried out from the first zoeal stage to the first crab stage. Larvae were collected during 9 rearing trials conducted either at an average water temperature of 23 °C (19 to 22 d) or at 28 °C (14 to 15 d). The threshold temperature for the swimming crab larvae to metamorphose was estimated at 13.4 °C. Effective cumulative water temperature (day-degrees) for the hatched larvae to molt to the zoea 2 stage was ca 43 °C; to zoea 3, ca 72 °C; to zoea 4, ca 103 °C; to megalopa, ca 144 °C; and to the first crab stage, ca 199 °C. After each molt the larvae grew exponentially and exhibited a 2.4 to 2.5 times increase in fresh and dry body weights, C, N, P and energy. The water component of fresh body weight was 79 to 80 % in all larval stages, and a ratio of ca 25 : 5 . 1 of C : N : P was noted in all larval stages.

INTRODUCTION

The distribution of swimming crab *Portunus trituberculatus* extends from the Tsugaru Straits, between Honshu and Hokkaido of Japan, southward to Hong Kong. In Japan this species is one of the most important commercial fisheries resources. Larvae were first successfully cultured about 50 yr ago and presently are raised in various parts of Japan for restocking purposes. The production has increased steadily, and in 1988 about 48.6 million individuals of young crab were produced (Anonymous 1990).

The major chemical constituents of many crustaceans have been previously described (Childress & Nygaard 1974, Parsons et al. 1984). With respect to decapod larvae, Dawirs (1980) analyzed the C, N and H contents of all larval stages of *Pagurus bernhardus* and *Carcinus maenas*, Hirota & Fukuda (1985) measured the elemental composition of larvae of a pelagic brachyuran crab species, and Anger (1988) conducted similar studies on *Inachus dorsettensis* larvae. Knowledge of these components allows one to estimate the biomass and production of larvae in pelagic ecosystems of adjacent waters.

We previously analyzed the nitrogen and phosphorus contents of swimming crab larvae as part of a nutrient budget calculation in the larval rearing tanks (Lim & Hirayama 1990). Although the swimming crab has been extensively studied with respect to, e.g., larval production techniques (Kurata 1983), oxygen consumption (Tamanoi et al. 1979, Morioka et al. 1988), metamorphosis of internal organs during the proecdysial period (Hamazaki 1989) and developmental processes of internal organs during metamorphosis in the larval periods (Nakamura 1990), its growth patterns and chemical constituents are still largely unknown.

The present study describes larval growth of mass-cultured swimming crabs from the first zoeal stage to the first crab stage by measuring fresh and dry body weights, and by analyzing the carbon, nitrogen and phosphorus content. The threshold temperature for development and thermal constant of the larvae, and effective cumulative water temperatures (day-degrees) for molting to each larval stage, were also determined.

MATERIALS AND METHODS

Handling of larvae. Egg-carrying crabs were dredged from the Sea of Ariake adjacent to Shimabara. Each egg-laden crab was placed into a black polycarbonate tank (1 m³) and allowed to spawn overnight. The hatched larvae were transferred into a rearing tank, and cultivated through the 4 zoeal stages (Z_1 to

Z_4), the megalopal stage (M), and to the first crab stage (C_1). The routine feeding regimen consisted of rotifers for Z_1, Z_2 and Z_3, followed by nauplii of brine shrimp for Z_4 and M, and minced clam, minced shrimp and artificial diets (Kyowa Hakko Kogyo Co., Ltd.) for M and C_1. Phytoplankton, chlorella (*Nannochloropsis* sp.), diatoms (*Chaetoceros* sp.), and chemical fertilizers were also introduced into the tanks. The larval rearing was conducted at the Saga Prefectural Sea Farming Center during June and July in 1986 and 1987, and at the Nagasaki City Aquacultural Center during July and August in 1988 and 1989. In Saga a circular 100 m^3 tank was used, while in Nagasaki a rectangular 70 m^3 tank was employed.

Sampling of larvae. Larval growth was studied during 9 trials: 1 in 1986, 3 in 1987, 2 in 1988, and 3 in 1989. Almost all of the larvae for each trial were obtained from a single brood crab. The larvae were collected with a plankton net every morning during the rearing period. They were placed on filter paper to absorb the surficial water before measuring their fresh body weight with an electronic balance. At each sampling, ca 100 mg of larvae (total fresh body weight) were collected. To measure dry body weight and chemical constituents, the larvae were wrapped in a sheet of aluminum foil, placed into a 20 ml vial containing silica gel, and stored at $-20\,°C$ for 3 mo. The silica gel was replaced several times during this period. Dry body weight measurements were repeated until a constant weight was obtained.

Observation of larval rearing. In order to distinguish among the larval stages, a portion of the sampled larvae were individually observed either with a microscope or with the naked eye throughout the rearing period. The staging differentiation was based on criteria established by Kurata (1983). Since the larvae in each trial normally molted simultaneously overnight, almost all individuals were at the same stage. In exceptional cases, the larvae were considered to be in a new stage if more than 80 % of the observed larvae had molted. Water temperature was measured twice a day, in the morning and in the afternoon, and was averaged to yield the daily mean water temperature. The threshold temperature for development and the thermal constant were calculated using the theory of heat summation known as Réaumur's law (Murai & Ito 1977). The effective cumulative water temperature (day-degrees) was calculated by summing the daily differences between daily mean water temperature and the threshold temperature for development. One day-degree refers to a mean water temperature 1 degree above the threshold temperature for development, lasting for 1 d. The threshold temperature is the lowest temperature at which development through a given stage can be completed. The needed summation

of heat up to a selected end point is called the thermal constant for that set of processes.

Elemental analyses. Analysis of carbon, nitrogen and phosphorus was conducted on dried larvae. The carbon and nitrogen content was analyzed with a C–N corder (Yanagimoto MT-500). Phosphorus content was determined by hydrolyzing the samples to polyphosphate and orthophosphate with hydrochloric acid after drying and baking (Solórzano & Sharp 1980), and then applying the molybdate method using a spectrophotometer (Hitachi 150–20). Chemical analysis was not performed in 1986. Energy of the larvae was calculated from the N-corrected formula given in Salonen et al. (1976).

RESULTS

Larval production and growth

Table 1 presents the cumulative data from the 9 larval rearing trials. The larvae took ca 14 to 22 d to develop from the first zoeal stage to the first crab stage. These larvae can be roughly divided into 2 groups, based on mean water temperature: those reared at 23 °C (Trial nos. 1 to 4), and those at 28 °C (Trial nos. 5 to 9). There was an obvious difference in the survival rate among these trials, ranging from 0.9 to 48.3 %. Even in the rearing tank with the lowest survival rate (Trial 5), more than 10^4 C_1 individuals were harvested. Salinity in these trials fluctuated between 31 and 35 ppt during the rearing period. Other data regarding the larval production and parameters of water quality have been previously reported (see Lim & Hirayama 1990).

Fig. 1 shows the typical larval growth (Z_1 to C_1) measured (as fresh body weight) in 2 rearing trials (Trials 4 and 7), which were conducted at 2 different water temperatures. The growth rate observed in each trial is expressed as an equation, shown in Table 1. The larvae in Trials 1 to 4, which were reared at the lower water temperature, had a longer rearing period, and also exhibited a lower growth rate (slope *b*). These equations were used to compare larval growth between rearing trials. Ordinarily, the hatched larvae did not grow linearly. Larval growth can be expressed as a 'stepped function', that is, after molting the larvae gained weight exponentially, but displayed almost no weight gain during each larval stage (Fig. 1). The duration of each stage of larvae reared at 23 °C was 3 d for Z_1 and Z_2, 3 to 4 d for Z_3, 4 to 5 d for Z_4, and 5 to 7 d for M. At 28 °C, the larvae required 2 d each for Z_1, Z_2 and Z_3, 3 d for Z_4, and 4 d for M. The larvae achieved C_1 19 to 20 d after hatching at 23 °C, and 14 d at 28 °C.

The thermal constant (*K*) and threshold temperature for development (*t*) to the first crab stage were calcu-

Table 1. *Portunus trituberculatus*. Data from the 9 larval rearing trials. Trials 1 to 4 were conducted at the Saga Prefectural Sea Farming Center with a 100 m^3 tank, Trials 5 to 9 at the Nagasaki City Aquacultural Center with a 70 m^3 tank. Y = fresh body weight (μg ind.$^{-1}$); X = no. of days after hatching

| Year, Trial no.		Rearing period (d)	No. of crabs produced ($\times$ 10^4)	Survival rate (%)	Range of water temperature (°C)	Formula for larval growth ($\ln Y = \ln a + bX$)	R^2
1986	1	19	84.0	24.1	23–25	$\ln Y = 3.836 + 0.267\ X$	0.982
1987	2	21	24.2	7.0	22–25	$\ln Y = 3.784 + 0.263\ X$	0.967
	3	22	107.0	48.3	22–25	$\ln Y = 4.111 + 0.239\ X$	0.974
	4	22	46.5	11.6	22–24	$\ln Y = 4.358 + 0.214\ X$	0.962
1988	5	15	1.2	0.9	27–28	$\ln Y = 4.237 + 0.347\ X$	0.982
	6	15	35.4	15.5	27–28	$\ln Y = 4.223 + 0.329\ X$	0.965
1989	7	15	107.4	44.0	27–28	$\ln Y = 4.147 + 0.335\ X$	0.957
	8	14	54.0	41.5	27–28	$\ln Y = 4.206 + 0.341\ X$	0.961
	9	14	56.6	34.7	27–28	$\ln Y = 4.149 + 0.332\ X$	0.959
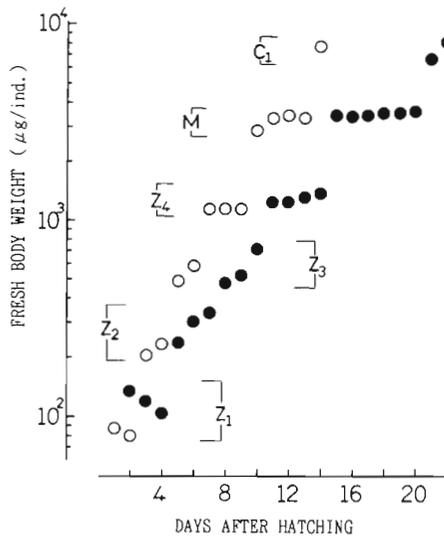
Fig. 1. *Portunus trituberculatus*. Typical larval growth (fresh body weight) during 2 trials from the first zoeal stage to the first crab stage. (●): Trial 4, conducted at 23 °C; (○): Trial 7, conducted at 28 °C. Developmental stages: Z, zoeal; M, megalopal; C, crab

lated from the regression equation given in Table 2. The calculated values K and t were 198.9 °C and 13.4 °C, respectively. Fig. 2 shows the average effective cumulative water temperatures required for the hatched larvae to molt to each new stage. The relationship between these 2 variables can be described as hyperbolic. That is, the intervals between the effective cumulative water temperatures became larger as the larvae developed from Z_2 to C_1. The effective cumulative water temperature required for achieving each new larval stage was calculated from the following equation:

$$1/S = 0.0742 + 18.3467/T_c \quad (R^2 = 0.999)$$

where S is a numerical expression of each stage, for which Z_2, Z_3, Z_4, M und C_1 equal 2, 3, 4, 5 and 6, respectively, and T_c = cumulative water temperature. The effective cumulative water temperatures required for the hatched larvae to molt to Z_2, Z_3, Z_4, M and C_1 were ca 43, 72, 103, 144 and 199 °C, respectively.

A linear relationship between fresh body weight and dry body weight was noted (Fig. 3). The slope was ca 1;

Table 2. *Portunus trituberculatus*. Calculation of the thermal constant (K) and the threshold temperature for development (t) of larvae. Mean water temperature (T) is the average daily water temperature during the period for reaching the first crab stage (C_1); D = period for reaching C_1; V = velocity of development

Variable	Trial no.								
	1	2	3	4	5	6	7	8	9
T (°C)	23.70	23.15	23.38	23.18	27.57	27.61	27.87	27.48	27.44
D (d)	19	20	20	21	14	14	14	14	14
V (1/D)	0.053	0.050	0.050	0.048	0.071	0.071	0.071	0.071	0.071

Regression between mean water temperature and velocity development: $V = a + bT = -0.0674 + 0.00503T$ ($R^2 = 0.993$)
Calculation of thermal constant: $K = 1/b = 198.9$ °C
Calculation of threshold temperature for development: $V = 0$, $t = |a| / |b| = 0.0674/0.00503 = 13.4$ °C
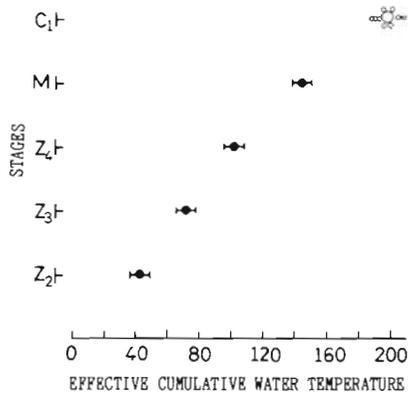
Fig. 2. *Portunus trituberculatus.* Effective cumulative water temperatures (day-degrees) required for achieving the molting stages. Filled circles represent arithmetic means; the larger open circle represents the thermal constant, and the 9 smaller open circles represent the daily effective cumulative temperatures up to the first crab stage in the 9 trials
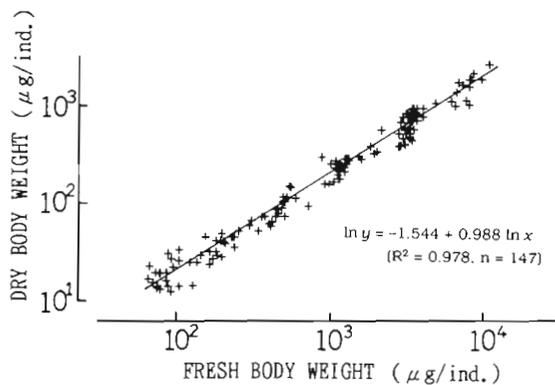

Fig. 3. *Portunus trituberculatus.* Linear relationship between fresh body weight and dry body weight from the first zoeal stage to the first crab stage in the 9 trials
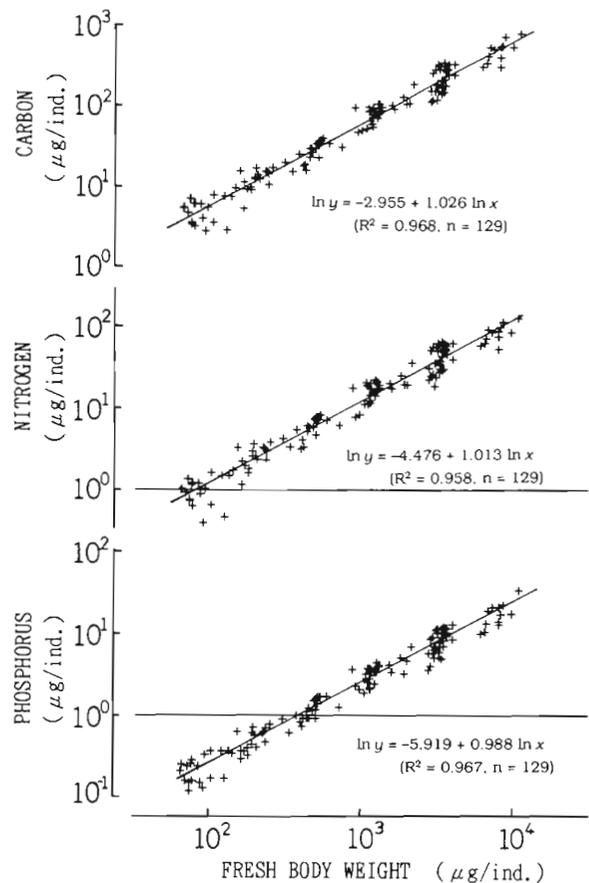

Fig. 4. *Portunus trituberculatus.* Linear relationships between fresh body weight and carbon, nitrogen, and phosphorus contents throughout larval development during the 9 trials

thus there was a proportional relationship between these 2 variables. Linear regressions for fresh body weight vs 3 chemical constituents (C, N, P) were also determined (Fig. 4). The slope of these linear regressions was also ca 1

Body weights, chemical constituents, and energy of each larval stage

Arithmetic means and standard deviations for body weights, chemical constituents, and energy of each larval stage were calculated from the 9 trials. The calculated data are presented in Table 3. Regression relationships between the larval stages and these variables after natural logarithmic transformation can be expressed as follows (all values in µg ind.$^{-1}$, except for energy, in J ind.$^{-1}$):

Fresh weight: $\ln W_f = 3.518 + 0.905\,S$ ($R^2 = 0.999$)
Dry weight: $\ln W_d = 1.897 + 0.907\,S$ ($R^2 = 0.999$)
Carbon: $\ln C = 0.786 + 0.914\,S$ ($R^2 = 0.999$)
Nitrogen: $\ln N = -0.810 + 0.911\,S$ ($R^2 = 0.999$)
Phosphorus: $\ln P = -2.299 + 0.879\,S$ ($R^2 = 0.999$)
Energy: $\ln E = -2.559 + 0.910\,S$ ($R^2 = 0.999$)

where S represents the larval stages, transformed into numerical values as mentioned above. After each molt there was a ca 2.4 to 2.5 ($e^{0.88}$ to $e^{0.91}$) times increase in each of the above variables. The water component of fresh body weight was 79 to 80 % in all larval stages. The average percentage of dry body weight for the 3 chemical constituents was: 34.3 %, C; 6.9 %, N; and 1.4 %, P Ratios of C : N, C : P and N : P at all larval stages ranged from 4.6 to 5.6, 23.8 to 27.1 and 4.4 to 5.6, respectively. The C : N : P ratio was ca 25 : 5 1. Energy values fluctuated between 9.9 and 13.4 J mg^{-1} dry body weight.

DISCUSSION

The larval survival rate was significantly different among these 9 rearing trials (Table 1), although the

Table 3. *Portunus trituberculatus*. Means ± SD of individual fresh body weight (W_f), dry body weight (W_d), water content (H_2O), chemical constituents (C, N, P), proportion (%) of elements in dry body weight, ratios of the elements, and energy for each larval stage. Z: zoeal stage; M: megalopal stage; C: crab stage; n: number of replicate analyses

Variables	Stages						n
	Z_1	Z_2	Z_3	Z_4	M	C_1	
W_f (µg ind.$^{-1}$)	90.2 ± 18.8	200.3 ± 25.3	482.4 ± 28.9	1234.6 ± 197.7	3281.4 ± 225.3	7794.5 ± 1148.4	9
W_d (µg ind.$^{-1}$)	18.0 ± 4.1	41.2 ± 6.9	95.1 ± 15.7	240.2 ± 31.2	641.9 ± 55.1	1648.5 ± 406.7	9
H_2O (%)	79.7 ± 4.1	79.4 ± 2.7	80.3 ± 3.3	80.4 ± 1.5	80.4 ± 1.1	79.0 ± 3.4	9
C (µg ind.$^{-1}$)	5.6 ± 1.2	13.9 ± 1.6	33.2 ± 5.0	85.6 ± 6.9	224.8 ± 21.3	527.4 ± 140.2	8
(% W_d)	33.1 ± 8.0	34.2 ± 6.3	37.1 ± 9.0	35.8 ± 2.6	35.1 ± 2.6	30.5 ± 2.3	8
N (µg ind.$^{-1}$)	1.0 ± 0.2	2.9 ± 0.3	6.7 ± 0.5	18.7 ± 2.0	47.4 ± 6.9	93.5 ± 20.4	8
(% W_d)	6.2 ± 1.2	7.1 ± 1.2	7.5 ± 1.3	7.9 ± 1.4	7.4 ± 1.0	5.5 ± 0.3	8
P (µg ind.$^{-1}$)	0.24 ± 0.05	0.61 ± 0.13	1.40 ± 0.30	3.36 ± 0.42	8.63 ± 0.72	19.68 ± 5.24	8
(% W_d)	1.43 ± 0.33	1.52 ± 0.43	1.57 ± 0.45	1.40 ± 0.14	1.35 ± 0.04	1.14 ± 0.14	8
C:N	5.4 ± 0.7	4.8 ± 0.3	5.0 ± 0.4	4.6 ± 0.5	4.8 ± 0.5	5.6 ± 0.4	8
C:P	23.8 ± 6.9	23.2 ± 3.5	24.0 ± 2.0	25.7 ± 2.1	26.1 ± 1.8	27.1 ± 2.8	8
N:P	4.4 ± 0.9	4.8 ± 0.9	4.9 ± 0.8	5.6 ± 0.8	5.5 ± 0.7	4.9 ± 0.7	8
J ind.$^{-1}$	0.19 ± 0.06	0.47 ± 0.08	1.19 ± 0.31	2.97 ± 0.18	7.76 ± 0.88	17.24 ± 4.95	8
J mg^{-1}	11.4 ± 4.1	11.9 ± 3.2	13.4 ± 5.0	12.5 ± 1.3	12.1 ± 1.3	9.9 ± 1.0	8

routine feeding procedures were similar. The lower survival rates in Trials 2 and 5 were attributed to the mass mortality that occurred during molting to the megalopal stage. This period is termed the 'critical period' of swimming crab larval development. This phenomenon also occurs during larval development of other decapod species (Hartnoll & Mohamedeen 1987, Sulkin & McKeen 1989, Mene et al. 1991). Ammonia and nitrite levels in the water during the trials fluctuated below the larval resistance limit reported by Mawatari & Hirayama (1975). In addition, detrimental handling of the brood crabs could not be identified as a cause of mass mortality when larval vitality during starvation experiments was tested among the hatched larvae from 6 individual brood crabs (unpubl. data). Nevertheless, the larvae in the present study did not significantly differ among trials with respect to their weights and chemical constituents at each larval stage, and thus they can be expressed by regression equations with an R^2 value (coefficient of determination) of more than 95 %. Once the larvae molt, they gain a constant exponential increment at each larval stage.

Water temperature was the only obvious environmental factor which varied among these trials. The period of larval development was shorter at a higher rearing water temperature (Fig. 1). The larvae of *Macrobrachium rosenbergii* (Gomez Diaz 1987), *Cancer magister* (Sulkin & McKeen 1989) and *Necora puber* (Mene et al. 1991) also have shorter periods of early larval development at higher water temperatures. Figs. 1 & 2 suggest that larval stage durations become longer as larvae develop from Z_1 to C_1. This phenomenon has

also been found in other decapod species: *Hyas araneus* (Anger 1983) and *Inachus dorsettensis* (Anger 1988).

Harding et al. (1983) noted that larval hatching of *Homarus americanus* usually begins only after water temperatures rise above 12.5 °C, and that the larvae require even warmer water to complete development. A number of mathematical formulae have been used in an attempt to express the relationship between temperature and the velocity of biological processes in many organisms. Réaumur's law is such an equation (Murai & Ito 1977). Since molt staging of the swimming crab larvae was significantly related to the effective water temperature, it is thought that the relationship holds not only for cultured populations, but also for those in coastal waters.

The larval stages of the swimming crab exhibited exponential growth after each molting, as mentioned above. This is similar to the larval growth of *Pagurus bernhardus* in zoeal stages, but the megalopal larvae of this species does not metamorphose exponentially to the first crab stage (Anger 1989), whereas the megalopal larvae of the swimming crab does. The described pattern of growth differs from that found in *Carcinus maenas* (Dawirs 1983), *Hyas coarctatus* (Jacobi & Anger 1985), *H. araneus* (Anger & Jacobi 1985) and *Inachus dorsettensis* (Anger 1988). These species seem to have no exponential growth after molting. The variation may be due to species and habitat differences. The dry body weight of swimming crab larvae was also measured at the megalopal stage by Hirota & Fukuda (1985) and at all larval stages by

Morioka et al. (1988). The number of specimens meas-
ured by these authors was much smaller than in our
study. In spite of the small numbers and the fact that
these authors used a different drying method, the body
weights reported are almost identical. The range of
values for water content and percentages of chemical
constituents relative to dry body weight in all larval
stages of swimming crab generally approximate those
of all the zooplankton groups analyzed by Curl (1962),
Beers (1966) and Omori (1969). The phosphorus con-
tents (% of dry body weight) of many groups of zoo-
plankton (0.1 to 1.2 %) are comparatively smaller than
those of swimming crab larvae. Omori (1969) also cal-
culated the C : N ratio of various zooplankton samples;
they ranged from 3 to 8. The C : N ratios of the swim-
ming crab at all larval stages were also distributed
within this range (Table 3). The C · N ratio is consid-
ered an indicator of the lipid . protein ratio, and has
been reported to decline under starvation (Ikeda 1974,
Sekiguchi 1975). However, the pattern of daily fluctua-
tions in the ratios varied considerably among the trials;
the conspicuous cyclical changes which have been
described in *I. dorsettensis* larvae (Anger 1988) were
not detected.

The swimming crab larvae were reared using the
'community culture method' on a large scale. Thus, it
could be said that these hatched larvae grew and
metamorphosed to the first crab stage under 'semi-
natural conditions', despite the fact that the system was
closed. Consequently, the findings under these condi-
tions can be considered an adequate description of the
growth pattern and biochemical changes of swimming
crab larvae during their ordinary development.

LITERATURE CITED

Anger, K. (1983). Temperature and the larval development of
Hyas araneus L. (Decapoda: Majidae); extrapolation of
laboratory data to field conditions. J. exp. mar. Biol. Ecol.
69: 203–215

Anger, K. (1988). Growth and elemental composition (C, N, H)
in *Inachus dorsettensis* (Decapoda: Majidae) larvae reared
in the laboratory. Mar. Biol. 99: 255–260

Anger, K. (1989). Growth and exuvial loss during larval and
early juvenile development of the hermit crab *Pagurus
bernhardus* reared in the laboratory. Mar Biol. 103:
503–511

Anger, K., Jacobi. C. C. (1985). Respiration and growth of
Hyas araneus L. larvae (Decapoda: Majidae) from hatch-
ing to metamorphosis. J. exp. mar Biol. Ecol. 88: 257–270

Anonymous (1990). National records of larval production, larval
obtaining and restocking in 1988. Fisheries Agency, Japan
Sea Farming Fisheries Association, Tokyo (in Japanese)

Beers, J. R. (1966). Studies on the chemical composition of
major zooplankton groups in the Sargasso Sea off Ber-
muda. Limnol. Oceanogr. 11 520–528

Childress, J. J., Nygaard, M. (1974). Chemical composition
and buoyancy of midwater crustaceans as function of
depth of occurrence off southern California. Mar. Biol. 27:
225–238

Curl, H. (1962). Standing crops of carbon, nitrogen and phos-
phorus and transfer between trophic levels, in continental
shelf waters south of New York. Rapp. P.-v. Réun. Cons.
int. Explor. Mer 153: 183–189

Dawirs, R. R. (1980). Elemental composition (C, N, H) in larval
and crab-1 stages of *Pagurus bernhardus* (Decapoda:
Paguridae) and *Carcinus maenas* (Decapoda: Portunidae).
Mar. Biol. 57: 17–23

Dawirs, R. R. (1983). Respiration, energy balance and develop-
ment during growth and starvation of *Carcinus maenas* L.
larvae (Decapoda: Portunidae). J. exp. mar. Biol. Ecol. 69:
203–215

Gomez Diaz, G. (1987). Effect of environmental embryonic
temperature on larval development of *Macrobrachium
rosenbergii* (De Man). J. exp. mar. Biol. Ecol. 114: 39–47

Hamazaki, K. (1989). Metamorphosing in metabolism organs
of the proecdysial larvae of the swimming crab *Portunus
trituberculatus*. Saibaigiken 17: 87–91 (in Japanese)

Harding, G. C., Drinkwater, K. F., Vass, W. P. (1983). Factors
influencing the size of American lobster (*Homarus
americanus*) stocks along the Atlantic coast of Nova Scotia,
Gulf of St. Lawrence, and Gulf of Maine: a new synthesis.
Can. J. Fish. Aquat. Sci. 40: 168–184

Hartnoll, R. G., Mohamedeen, H. (1987). Laboratory growth of
the larvae of six British crabs. J. exp. mar Biol. Ecol. 107:
155–170

Hirota, R., Fukuda, Y (1985). Dry weight and chemical com-
position of the larval forms of crabs (Decapoda: Brachy-
ura). Bull. Plankton Soc. Japan 32: 149–153

Ikeda, T (1974). Nutritional ecology of marine zooplankton.
Mem. Fac. Fish. Hokkaido Univ. 22: 1–97

Jacobi, C. C., Anger, K. (1985). Growth and respiration during
the larval development of *Hyas coarctatus* (Decapoda:
Majidae). Mar Biol. 87: 173–180

Kurata, H. (ed.) (1983). Techniques of mass production of the
blue crab *Portunus trituberculatus* seedling. Larval pro-
duction workshop of the blue crab. Fisheries Propagation
and Aquaculture, Vol. 32. Japan Fisheries Resource Con-
servation Association, Tokyo (in Japanese)

Lim, B. K., Hirayama, K. (1990). Nitrogen and phosphorus
budgets in larval rearing tanks for mass production of
swimming crab *Portunus trituberculatus*. In: Hirano, R.,
Hanyu, I. (eds.) Proceedings of the Second Asian Fisheries
Forum, Tokyo, Japan, 17–22 April 1989. The Asian
Fisheries Society, Manila, p. 177–180

Mawatari. K., Hirayama, K. (1975). Studies on resistibility of
some marine animals at various larval stages to ammonia,
nitrite and nitrate (in Japanese). Bull. Fac. Fish. Nagasaki
Univ. 39: 1–6

Merie. L., Alvarez-Ossorio, M. T., González-Gurriarán, E.,
Valdés, L. (1991). Effects of temperature and salinity on
larval development of *Necora puber* (Brachyura: Por-
tunidae). Mar. Biol. 108: 73–81

Morioka, Y., Kitajima, C., Hayashida, G. (1988). Oxygen con-
sumption, growth and calculated food requirement of the
swimming crab *Portunus trituberculatus* in its early develop-
mental stage. Bull. Jap. Soc. Sci. Fish. 54: 1137–1141

Murai, M., Ito, Y. (eds.) (1977). Study on animal ecology issues
2. Kokin Shoin, Tokyo, p. 445–450 (in Japanese)

Nakamura, K. (1990). Organogenesis during metamorphosis

in the swimming crab *Portunus trituberculatus*. Bull. Jap. Soc. Sci. Fish. 56: 1561–1564

Omori, M. (1969). Weight and chemical composition of some important oceanic zooplankton in the North Pacific Ocean. Mar. Biol. 3: 4–10

Parsons, T. R., Takahashi, M., Hargrave, B. (eds.) (1984). Biological oceanographic processes, 3rd edn. Pergamon Press, New York, p. 50–54

Salonen, K., Sarvala, J., Hakala, I., Viljanen, M.-L. (1976). The relation of energy and organic carbon in aquatic invertebrates. Limnol. Oceanogr. 21: 724–730

Sekiguchi, H. (1975). The survival of larval fishes. Kaiyo Kagaku (Marine Sciences), Vol. 7(12). Symposium – 73, Studies on fish population 7: 36–41 (in Japanese)

Solórzano, L., Sharp, J. H. (1980). Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol. Oceanogr. 25: 754–758

Sulkin, S. D., McKeen, G. L. (1989). Laboratory study of survival and duration of individual zoeal stages as a function of temperature in the brachyuran crab *Cancer magister*. Mar. Biol. 103: 31–37

Tamanoi, S., Hirayama, K., Oka, M. (1979). Respiration rate of larvae of the swimming crab *Portunus trituberculatus*. Bull. Fac. Fish. Nagasaki Univ. 47: 21–26 (in Japanese)

This article was submitted to the editor