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Post-embryonic development in *Archaeomysis vulgaris* (Nakazawa, 1910) reared in laboratory: Growth and sexual differentiation

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Abstract

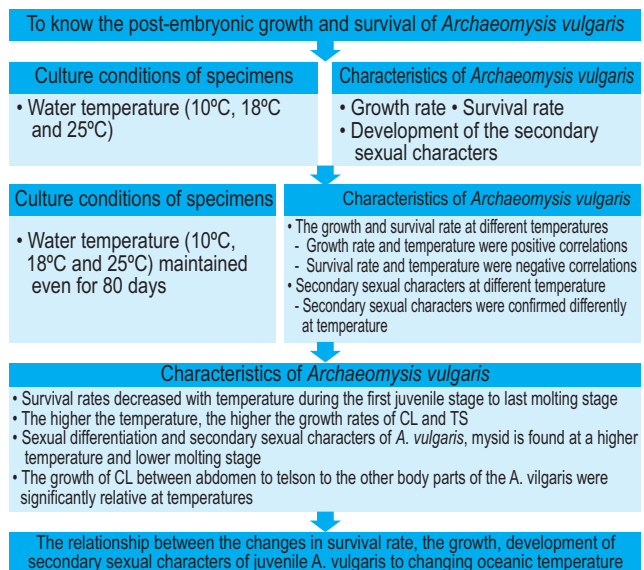
Aim: This study will help to understand the changes in survival rate and growth of juvenile *A. vulgaris* due to changing oceanic temperature. This study proved that water temperature among environmental factors affects *A. vulgaris*'s developing period of secondary sexual characters, molting frequency, daily growth, and growth ratio of post-embryonic, especially in higher water temperature.

Methodology: *Archaeomysis vulgaris* in South Korea were reared and analyzed under controlled condition of at 10, 18, 25°C, (33-34 psu; 12/12 L/D) from hatching through a series of instars. Each specimen was individually maintained during the intermolt period, percentage increment and growth rate based on laboratory rearing.

Results: This experiment result indicated the position of sexual molt variation related to water temperature followed by the statement of these species sexual characters appeared at 4th juvenile stage at 10°C, 6th juvenile stage at 18°C and 7th juvenile stage at 25°C. Survival rates increased with temperature during the first juvenile stage to last molting stage. The growth rate of *A. vulgaris* at 10°C, 18°C and 25°C was 0.05, 0.09 and 0.17 mm/daily. Intermolt period and the growth rate of mean carapace length were inversely correlated with temperature. The growth of carapace length between abdomen to telson to the other body parts of the *A. vulgaris* were significantly relative at 10°C, 18°C and 25°C. In the 6th period.

Interpretation: This research will help to understand the relationship between the changes in survival rate, the growth of juvenile *A. vulgaris* to changing oceanic temperature. The following research will present a strategy to maintain the population of *A. vulgaris* by using shorter cycle, where it participates in reproduction at lower temperature or delay the growth for increasing survival ratio.

Key words: *Archaeomysis vulgaris*, Epibenthic, Mysidacea, Sexual characters, Survival ratio



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Introduction

Mysidae, a macro-plankton, is usually found in the shallow ocean of surf-zone and an intertidal area which exhibits an epibenthic life. As a principle feed for large crustacea, Mysidae occupies an important position within food web in the coastal ecosystem (Brown and Talbot, 1972; Lasiak, 1983; Rossouw, 1983). Post-embryonic growth and development of Mysidae are essential for ecological and biological study. In particular, the reproductive cycle and post-embryonic growth are important to understand the lifespan and population structure (Ma *et al.*, 2001; Hong and Oh, 1989). The post-embryonic growth of Mysidae is examined to understand their reproductive characters and generations cycle in laboratory or field-collection (Mauchline, 1980). In a study on the post-embryonic growth of epibenthic mysid, Sudo (2003) reposted the embryonic growth of *Acanthomysis robusta* related to temperature changes and Yamada *et al.* (1995) investigated the survival and growth depending on feed control of *Acanthomysis mitsukurii*. Theories on the embryonic growth of *Archaeomysis* sp. which are connected to same genus target species in this study, such as Hanamura's (1997) morphological study that carapace length by phases of embryonic growth, the study on taxonomical characters by phases of embryonic growth of *A. kokuboi* and *A. japonica*, by Takahashi and Kawaguchi (1996) and Jawed's (1973) study which differences in growths with environmental factors of *A. grednitzkii* and *Neomysis awatschensis*. Although most of the studies have been analyzed by relying on mass rearing in laboratory and field collection or embryonic growth and life cycle by individual rearing (Mauchline, 1980). There was study of sexual differentiation, survival, absolute and relative growth by using exuviae of post-embryonic growth of *A. kokuboi* in South Korea (Ma *et al.*, 2001). *A. vulgaris* was studied for embryonic growth through field collection by Mastudaria *et al.* (1952) and morphological features by embryonic growths by Nonomura *et al.* (2005) in Japan.

This study analyzes intermolt period, survival rate of each molting stages, daily growth and relative growth through individual rearing experiment for understanding the embryonic growth of *A. vulgaris* with relative dominance among Mysidae in the west coast of Korea.

Materials and Methods

Collection and culture conditions of specimens : Ovigerous females of *Archaeomysis vulgaris* was collected in the surf zone of Hakampo beach, on the west coast of South Korea. They were gradually acclimatized to each experimental temperature three days. Following condition was maintained during the experiment until the juveniles hatched: regime was 6:18 L:D, they were fed *ad libitum* with < 24-hr old *Artemia* sp., nauplii and water was replaced every 2 days.

With the advancement in experiment, each ovigerous female released average 35 juveniles which were divided into three groups by same brood to expose to three temperature regimes (10°C, 18°C and 25°C). The juveniles were kept individually in 100 ml glass beakers in three different controlled temperature incubators under 12:12 L:D regime during the experiment. They were fed *ad libitum* with <24-hr old *Artemia* sp. nauplii and water was replaced every 2 days. All incubators were checked twice a day to check exuvia.

Date collection : The total length (TL), carapace length (CL) and telson length (TS) were determined by measuring the exuvia for obtaining more accurate measurements by reducing stress due to handling. The measurements were recorded as digital images by image analysis software (Image-pro Plus 3.0) with a stereomicroscope (Fig. 1).

Development of secondary sexual characters in each incubators were divided into three stages: Stage I juveniles showed should no differentiation. Stage II juveniles were developed oostegites (female) and 2nd ramus of pleopods (male). Stage III juveniles developed into marsupium (female) and

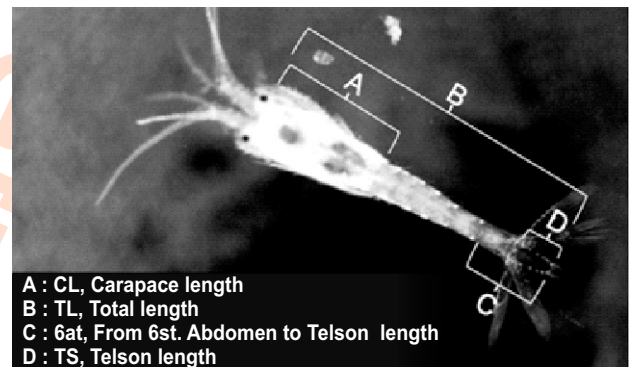


Fig. 1 : Morphometric measurements of *Archaeomysis vulgaris* (Nakazawa, 1910).

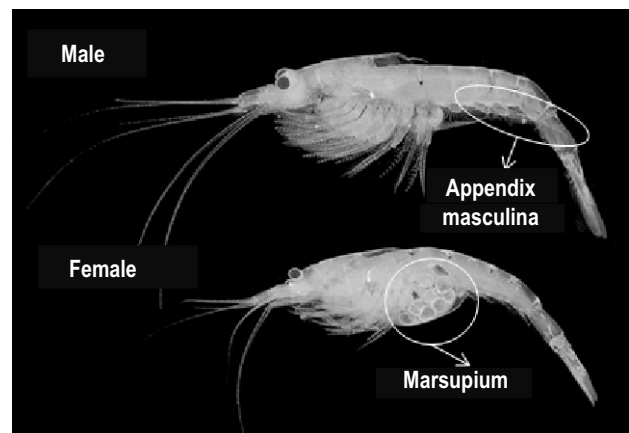


Fig. 2 : Secondary sexual characters of male and female *Archaeomysis vulgaris*.

appendix masculine (male) (Fig. 2). The growth of juveniles data was collected in each incubator with growth rate of carapace length, a day after releasing each molt stages, intermolt period and sexual moult. The growth results were analyzed based on the body part-growth of post-embryonic and relative growth between carapace length. The observation was carried until all specimens in a series were dead.

Statistics analyses : All data were checked with Komogrov-Smirnov for normality and homogeneity of variance. The effect of temperature was tested for significance using one-way analysis of variance. A chi-square test was used for comparing the percentage surviving of individuals until given instars and daily growth rates at three temperature regimes. A Pearson correlation analysis was determined between daily growth rates and temperature of each incubator. Statistical correlation analysis of Pearson product moments was accomplished using SPSS ver. 15. All box plots were show as mean, standard error and standard deviation.

Results and Discussion

The survival duration at each temperature of rearing are as follows: 80 days at 10°C, 71 days at 18°C and 45 days at 25°C. After 40 days, the survival rate at each temperature are as follows: over 80% at 10°C and 18°C and relatively low rate of 40%, at 25°C, after which the survival rate decreased abruptly. This result is consistent with the fact that the survival duration of crustacean is inversely proportional to temperature: higher the temperature, shorter the duration (Fig. 3).

At 10°C, over 80% of survival rate was observed until 6th molting stage, and then survival rate decreased rapidly. At 18°C, the survival ratio was about 80% until 5th molting stage and decreased rapidly at the end of the term. At 25°C, the ratio reduced to 72% until 5th and 8th molting stage but later decreased by 13% at each molting stage. The decrease in mode of survival ratio was same at 10°C and 18°C, except for 25°C (Fig. 4). Survival rate of *Archaeomysis vulgaris* juveniles showed best survival at 10°C. However, more individuals reached later instars at higher temperature (25°C) (Fig. 4). It has been reported that in general, the crustacean survival period is shorter when the temperature rises and longer when the temperature decreases (Rudolf and Isabelle, 1982; Minagawa, 1990; Domingues *et al.*, 1999; McKenney, 1987). Growth studies of *A. kokuboi* by Ma *et al.* (2001) and *Leptomys lingura* and *Hemimysis speluncula* by Gaudy and Guerin (1979) also reported that temperature, duration of survival and growth were readjusted and the intermolt period was inversely proportional.

Both males and females exhibited secondary sexual characters after going through 6th and 7th molting stage at 18°C and 25°C. After 8th and 9th molting stage at 18°C and 25°C, the oostegites of females developed into marsupium, and the appendix masculine of males developed enough to mate and

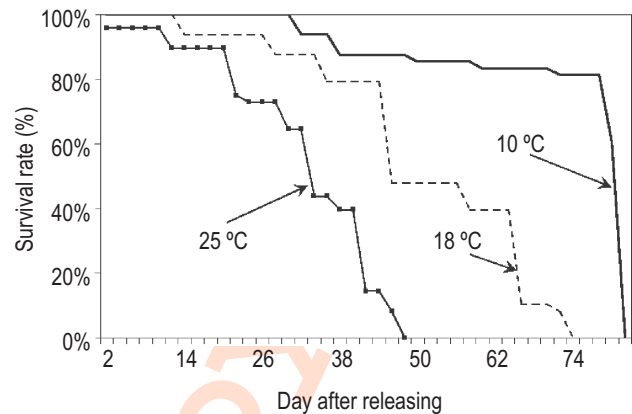


Fig. 3 : Survival rates plotted against time of post-embryonic *Archaeomysis vulgaris* reared at three temperatures.

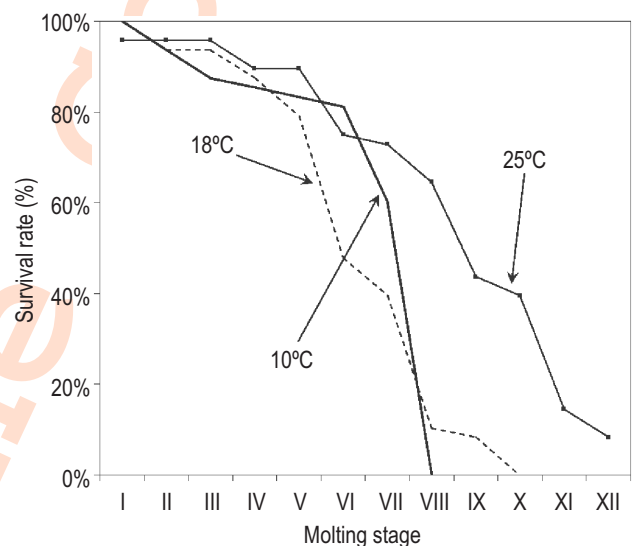


Fig. 4 : Survival rates at each molting stage of post-embryonic *Archaeomysis vulgaris* reared at three temperatures.

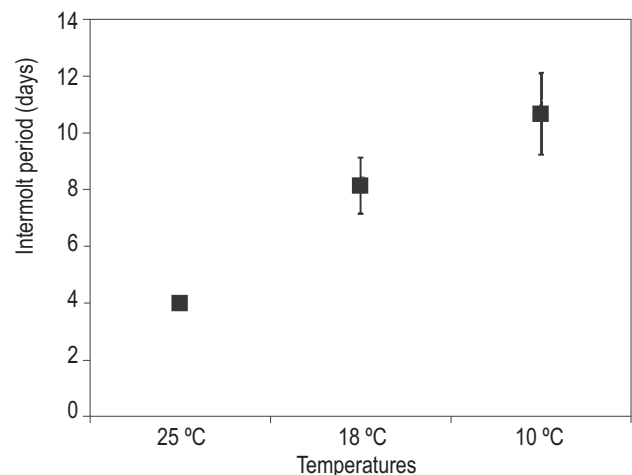


Fig. 5 : Morphometric measurements taken on *Archaeomysis vulgaris* (Nakazawa, 1910).

Table 1 : Duration of each development stage, carapace length and development of secondary sexual characters of post-embryonic *Archaeomysis vulgaris* reared at three temperatures

Generations	Development stages	Intermolt period (days)			Carapace length (mm)		Development of secondary sexual characters	
		Instars	Total	Mean	Standard deviation	Instars	Female	Male
10°C	I	15.8	15.8	0.96	±0.017	0.96	no differentiation	no differentiation
	II	15.4	31.2	1.11	±0.018	0.14	no differentiation	no differentiation
	III	7.5	38.7	1.26	±0.018	0.16	no differentiation	no differentiation
	IV	10.6	49.3	1.52	±0.020	0.25	oostegites	2 nd ramus of pleopods
	V	9.9	59.2	1.83	±0.018	0.32	oostegites	2 nd ramus of pleopods
	VI	12.3	71.5	2.27	±0.018	0.44	oostegites	2 nd ramus of pleopods
	VII	8.3	79.8	2.66	±0.017	0.39	oostegites	2 nd ramus of pleopods
18°C	I	5.8	5.8	0.95	±0.023	0.95	no differentiation	no differentiation
	II	8.1	13.9	1.07	±0.022	0.13	no differentiation	no differentiation
	III	8.3	22.2	1.24	±0.024	0.17	no differentiation	no differentiation
	IV	6.0	28.2	1.52	±0.021	0.27	no differentiation	no differentiation
	V	8.1	36.3	1.82	±0.024	0.30	no differentiation	no differentiation
	VI	9.4	45.7	2.25	±0.021	0.44	oostegites	2 nd ramus of pleopods
	VII	11.6	57.3	2.64	±0.027	0.39	oostegites	2 nd ramus of pleopods
	VIII	8.5	65.8	3.03	±0.018	0.38	marsupium	appendix masculine
25°C	IX	5.2	71.0	3.48	±0.013	0.45	marsupium	appendix masculine
	I	1.7	1.7	1.17	±0.010	1.17	no differentiation	no differentiation
	II	3.1	4.8	1.20	±0.013	0.03	no differentiation	no differentiation
	III	3.6	8.4	1.31	±0.013	0.10	no differentiation	no differentiation
	IV	4.3	12.7	1.53	±0.016	0.22	no differentiation	no differentiation
	V	4.6	17.3	1.78	±0.015	0.25	no differentiation	no differentiation
	VI	3.7	21.0	2.01	±0.020	0.23	no differentiation	no differentiation
	VII	4.0	25.0	2.27	±0.018	0.26	oostegites	2 nd ramus of pleopods
	VIII	4.6	29.6	2.49	±0.021	0.21	oostegites	2 nd ramus of pleopods
	IX	4.3	33.9	2.78	±0.015	0.29	marsupium	appendix masculina
	X	3.5	37.4	3.00	±0.014	0.22	marsupium	appendix masculina
	XI	3.7	41.1	3.28	±0.036	0.28	marsupium	appendix masculina
XII	4.4	45.5	3.55	±0.036	0.27	marsupium	appendix masculina	

reproduce. At 10°C, individuals developed male appendix masculine and marsupium were not observed. Sexual differentiation and secondary sexual characters of *A. vulgaris*, mysid was found at a higher temperature and lower molting stage. After oostegites, 2nd ramus of pleopods at 18°C and 25°C and then two molting stages, the marsupium and appendix masculine are matured enough to participate in reproduction. Nakasawa (1910) reported that in *A. vulgaris* the number of pre-puberty (development secondary sexual characters) instars varied between generations, maturing at different times of the year, and it is likely that limited variation within species is fairly general. Although this study showed difference in the position of sexual moult between temperatures, the effects of temperature on the total number of pre-puberty moults could not be examined since observations at a lower temperature (10°C) were not possible. However, different stages of sexual moult may influence the position of puberty instar, indicating that the position of both sexual and puberty moults can vary between individuals in relation to temperature changes in the field.

According to the result, in crustaceans, higher the temperature, shorter is the intermolt period and vice-versa, including Mysidae. Furthermore, the intermolt period was also shorter when temperature was high: 10.7 ±1.4 days at 10°C; 8.1 ±1.0 days at 18°C; 4.0 ±0.2 days at 25°C. However, there was no statistical outstanding difference between 18°C and 10°C series (Fig. 5).

The daily growth of *A. vulgaris* CL and TS depending on rearing temperatures are as follows: CL: 0.07 ±0.01/day and TS: 0.05 ±0.01/day at 10°C; CL: 0.11 ±0.01/day and TS: 0.09 ±0.01/day at 18°C; CL: 0.14 ±0.01/day and TS: 0.05 ±0.01/day at 25°C, suggesting that higher the temperature, higher the growth rates of CL and TS. However, no statistical difference was found in daily growth of CL between 18°C and 10°C (Fig. 6).

The carapace was released from marsupium at 25°C, after 20 days its length was about 2.8 mm. It had a marsupium of female and appendix masculina of male. It took about 26 days to be 2.8 mm at 18°C, 40 days at 10°C. Intermolt period increased

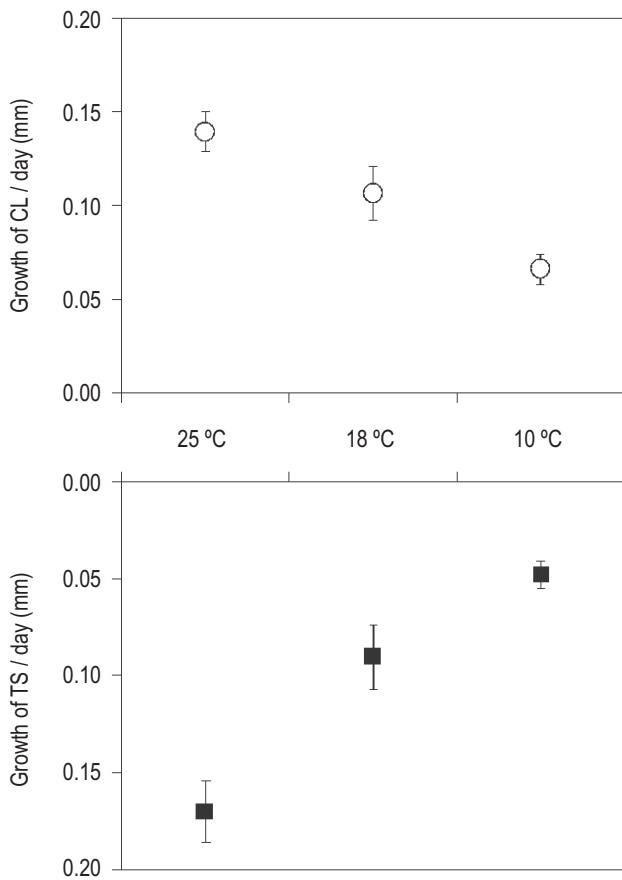


Fig. 6 : Daily growth rate of post-embryonic *Archaeomysis vulgaris* reared at three temperatures.

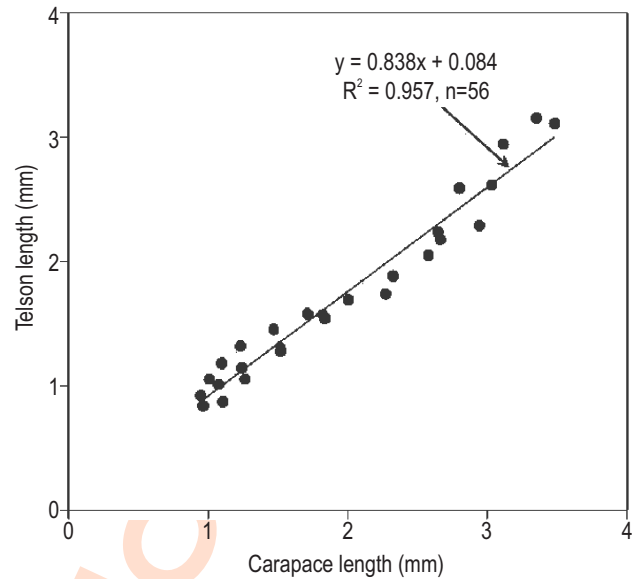


Fig. 8 : Relationship between carapace length and telson length of post-embryonic *Archaeomysis vulgaris*.

steadily with premoult size at all temperature. This is in confirmation with the previous studies conducted on crustaceans (Mauchline, 1976, 1977; Hartnoll, 1982). As most study allows the fact of carapace length tends to be longer at a lower temperature. This lengthening research consistently reported for large number of crustacean study (Hartnoll, 1982; Mohamedeen and Hartnoll, 1989). The position of sexual moult varied at different temperature. First external sexual differentiation in females and males appeared at 4th moult at lowest temperature, and 2nd-3th moult appeared at higher temperature than previous moult. This demonstrates that the onset of sexual differentiation can be affected by changes in ambient temperature. The full development of marsupium or appendix masculine, the puberty instar was consistent after 8th moult at 18°C and 10°C. The results revealed that the period of marsupium and appendix masculine were developed enough to take part in reproduction among *A. vulgaris* population inhabiting the west coast of Korea, followed by summer, spring generation and overwintering generation (Fig. 7).

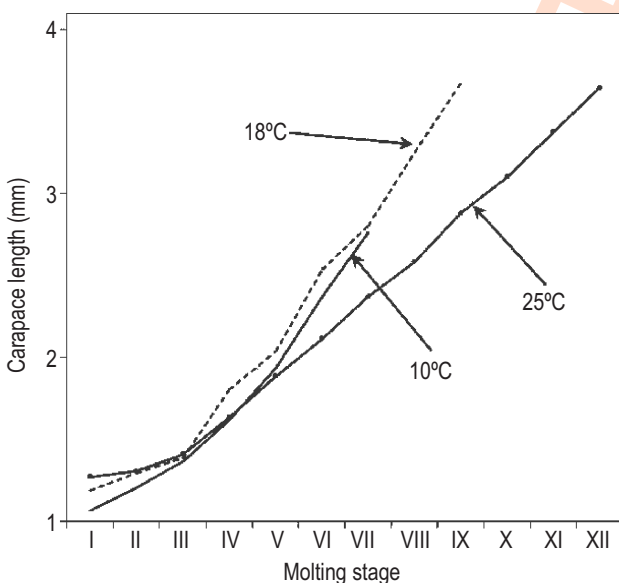


Fig. 7 : Sequence of molting stages plotted against carapace length of post-embryonic *Archaeomysis vulgaris* reared at three temperatures.

The daily growth rate of *A. vulgaris* CL and TS were higher at high temperature. As a result, it was found that among juveniles of *A. vulgaris*, inhabiting the west coast of Korea, actively recruited at high-temperature water takes a shorter time to reproduce, than any other temperature group.

CL and TS of 56 individuals among *A. vulgaris* were randomly measured and compared to show that the CL and TS ratio was 1: 0.838, and factors were closely correlated (Pearson's correlation $R^2 > 0.5$, $p < 0.05$). From the growth analysis using *A. vulgaris* CL, it is reported that precise result values were not easily found due to some factors such as transformation, damage and loss of carapace. However, the growth study of *A. kokuboi* by Ma et al. (2001) and *Neomysis integer* by Astthorsson and Ralph

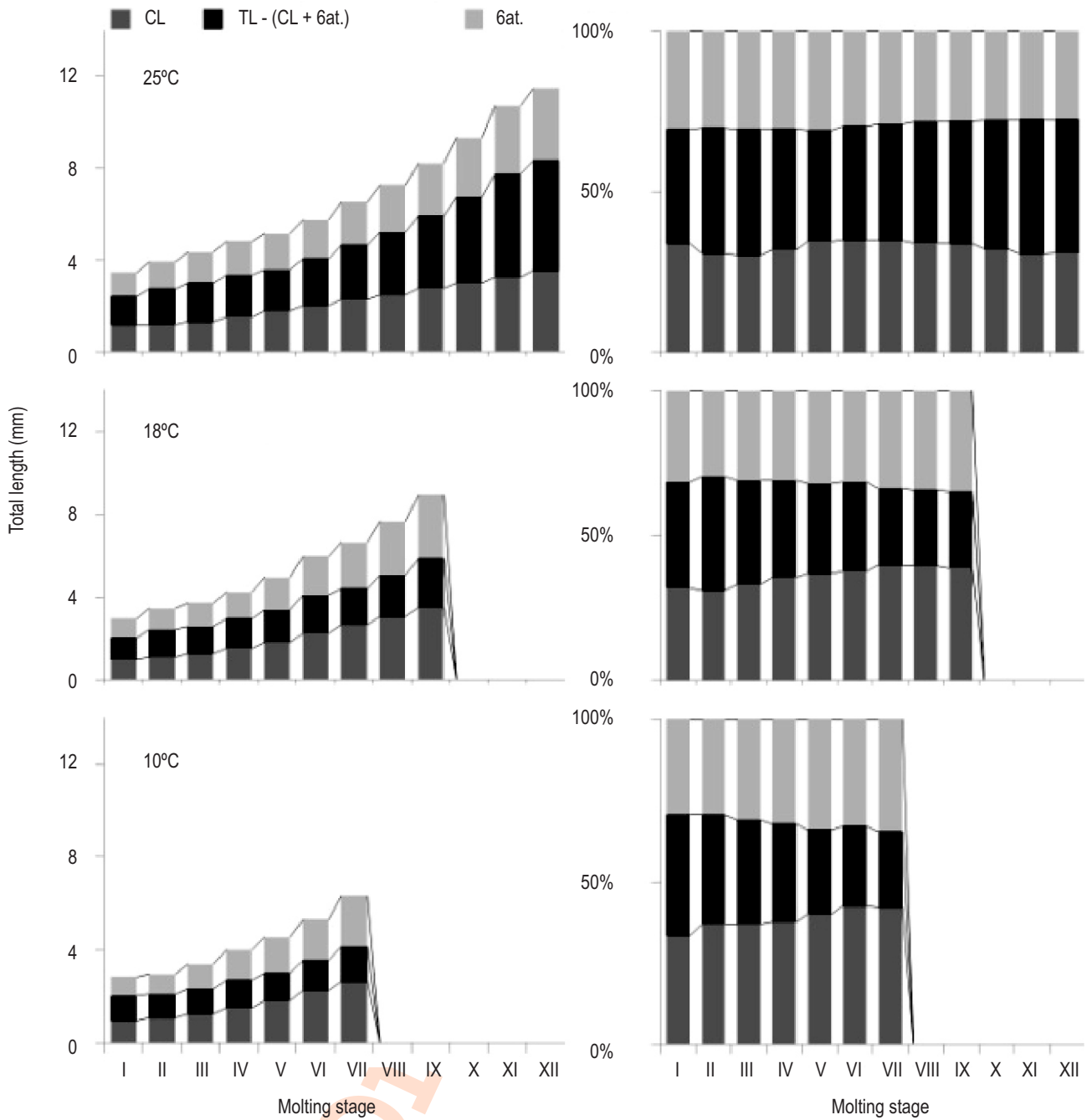


Fig. 9 : Relative growth of carapace length, 6at. (from 6th abdomen to telson) and other body parts of post-embryonic *Archaeomysis vulgaris* reared at three temperatures.

(1984) reported that it is convenient to measure TS as a measuring part for growth analysis. Thus, the study suggests that TS be used as a material for growth study of mysidae, including *A. vulgaris* (Fig. 8).

The post-embryonic growth of mysidae, including *A. vulgaris* varied according to each part at distinct temperatures. At

some point, even among *A. vulgaris* population, some individuals recruited at winter and spring were determined to have CL and 6at (From 6st. abdomen to telson legh). morphologically larger than other parts. With this critical research, breeding experiments of *A. vulgaris* showed that the peeling interval remained almost equilibrium at all temperature intervals, consistent with the study of isochronal development of Miller *et al.* (1977)(Fig. 9).

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