Thesis for the Degree of Master of Science

Population structure and life history of *Neomysis awatschensis* (Brandt, 1851) (Crustacea: Mysidae) in Jeju Island, Korea

by

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[제주도에 서식하는 까막곤쟁이의 개체군 구조와 생활사]

Advisor: Prof. Won Gyu Park

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배재용

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요 약

이 논문은 한국 제주도에 서식하는 까막곤쟁이(*Neomysis awatschensis*)의 개체군 구조와 생활사를 연구하였다. 까막곤쟁이는 제주도의 얕은 내만에서 2013년 5월부터 2014년 5월까지 월별로 채집되었으며 채집 시 수온과 염분은 측정되었고, 오조리 내만의 수온변화는 수온기록계를 이용하여 관측되었다. 곤쟁이의 크기는 갑각장과 평형석 직경을 측정하였다. 생활사는 6단계로 분류하였고, 보육낭 내 유생은 발달 단계에 따라 구분하였다. 제주 오조리 내만의 수온범위는 -0.27에서 35.67°C 였다. 수온은 1월에 -0.27°C 로 가장 낮았고, 8월에 35.67°C 로 가장 높았다. 갑각장과 평형석 직경의 범위는 각각 0.137에서 2.842mm, 30.7에서 199μm 였고, 평형석 직경은 갑각장과 유의한 상관관계가 있었다. 수온이 증가할 때 성숙개체들의 갑각장은 감소하였고, 수온이 감소할 때 성숙개체의 갑각장은 증가하였다. 암컷의 갑각장은 수컷의 갑각장보다 컸으며, 암컷의 비율이 수컷의 비율보다 높았다. 포란 암컷과 어린 개체는 연중 출현하였으며, 어린 개체의 풍도는 다른 달에 비해 5월, 7월, 12월 그리고 2월에 높았다. 보육낭 내 유생의 수는 포란 암컷의 갑각장 크기에 비례하였다. 결론적으로 이 연구는 제주도 오조리에 서식하는 *N. awatschensis*가 1년동안 연중 산란하지만 4번의 주요 산란군이 있다는 것을 알아냈으며, 평형석 직경은 곤쟁이목의 갑각장을 대신할 수 있다.

I. Introduction

Mysids are highly adaptive crustaceans inhabiting a wide variety of aquatic environments from freshwaters to saline water (Laprise & Dodson, 1993; McKenney & Celestial, 1995). Estuarine and coastal waters have fluctuating gradients of physicochemical characteristics which put estuarine mysids under physiological and ecological stress (Ysebaert & Herman, 2002). The life history and population structure of mysids are influenced by changing environmental conditions such as temperature, salinity and nutrients (Mauchline, 1980). Thus, the population status of mysids may be resulted from changing environments.

The members in the genus *Neomysis* are abundant in coastal and estuarine environments of world oceans (Mauchline, 1980), probably due to their high tolerance on temperature and salinity (Tattersall & Tattersall, 1951; Pezzack & Corey, 1982) and opportunistic omnivorous feeding habits (Fockedey & Mees, 1999). Mysids are abundant, and play an important ecological role in their distribution ranges, being consumed by numerous fish species (Toda *et al.*, 1982; Yamada *et al.*, 2007). *Neomysis awatschensis* (Brandt, 1851) is broadly distributed in temperate and subarctic region from 35°N to 59°N (Ii, 1964; Holmquist, 1973; Petryashev, 2002). This species is also euryhaline and eurythermal myisd (Tattersall & Tattersall, 1951; Pezzack & Corey, 1982). This sciaphilic species commonly lives in the shallow depth in estuarine and coastal waters which have fluctuating gradients of physicochemical characteristics such as temperature and salinity (Toda *et al.*, 1983; Ysebaert & Herman, 2002; Yamada *et al.*, 2007).

Jeju Island is located at the southern part of Korean waters. An embayment of Ojo-ri, where the research was conducted, is located at the eastern coast of Jeju Island. This region is affected by the Tsushima Warm Current throughout the year (Affan & Lee, 2004). The water temperature in the embayment is more influenced by air temperature because of shallow water depth (mean 1.2 m) and enclosed geographical feature being, connected to coastal sea by a narrow channel (Oh, 2004). The freshwater irregularly flows in this area from nearby land.

Environmental factors such as temperature and salinity and geography may affect the population structure and life history of *N. awatschensis*. Water temperature influences various components such as growth, maturity and reproduction (Mauchaline, 1980). *N. awatschensis* rapidly grows and matures, and has shorter life span in warm water regions (Toda *et al*., 1982; Yan, 1982). Brood size decreases in cold water temperature (Toda *et al*., 1984). The reproduction of *N. awatschensis* depends on water temperature. Tropical and subtropical mysids breed continuously whereas temperate mysid stops breeding in winter season (Toda *et al*., 1982; Baldo *et al*., 2001; Hanamura *et al*., 2009). Salinity also leads to change growth rates, maturity and fecundity (Toda *et al*., 1984; McKenney & Celestial 1995; Fockedey *et al*., 2005). The number of eggs or larvae carried by *Neomysis japonica* was smaller in a low salinity than in a high salinity (Ishikawa & Oshima, 1951). Body length and daily growth rate of *N. awatschensis* decreased with decreasing salinity (Jee *et al*., 2001). *N. awatschensis* has been reported with the different numbers of generations at various locations. Six generations in a year were reported at Akkeshi-ko estuary, Japan (Yamada *et al*., 2007) and four generations (reported as *N. intermedia*) were found at Kasumigaura Lake, Japan (Toda *et al*., 1982).

The objectives of the present study are to investigate the life-history and the population structure of *N. awatschensis* in the Ojo-ri embayment, Jeju Island.

II. Materials and methods

2.1 Research site and sampling

An embayment of Ojo-ri is located at 33°27'N, 126°54'E in the eastern coast of Jeju Island, Korea (Fig. 1). This embayment is approximately 1.2 m deep and 1.54 km^2 wide. The north of Ojo-ri embayment is connected to the coastal sea by the Seongsan water gate.

Neomysis awatschensis was monthly collected from May 2013 to May 2014 using a hand net (mesh size: 500 μm) from approximately 0.3-0.5 m depths in the Ojo-ri embayment (33°27'N, 126°54'E). Temperature and salinity were simultaneously measured during the sampling in situ. Also, onset data loggers (HOBO) were established to record water temperature with 10 minute intervals from July 2013 to May 2014. Specimens were mainly collected at low tide and preserved in 70% ethanol.

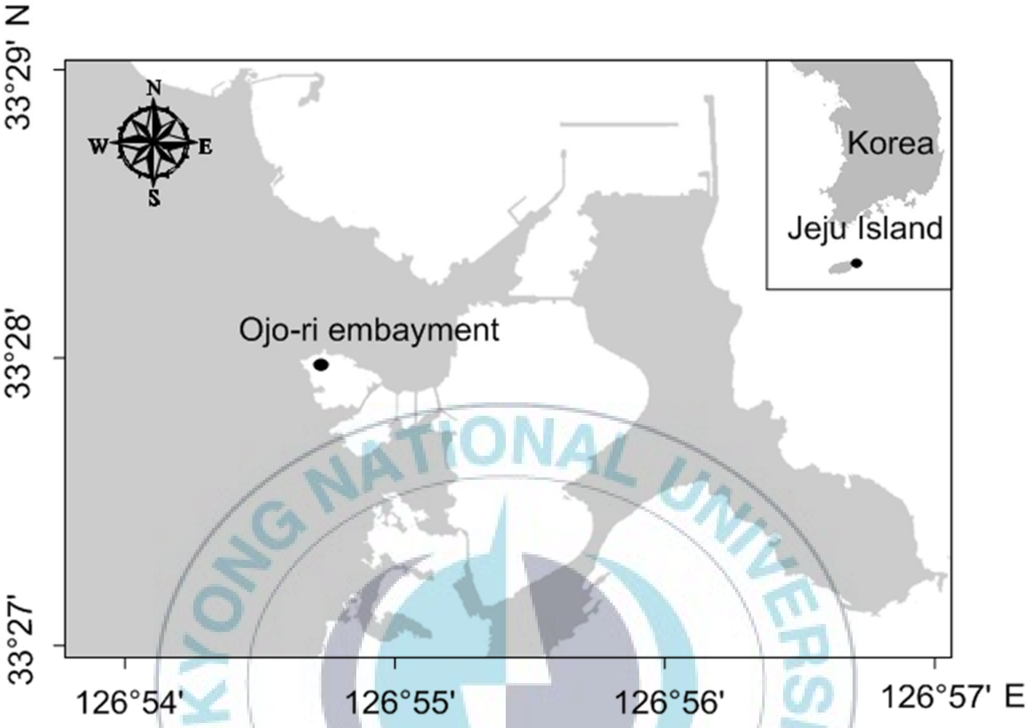

Fig. 1. Sampling location in the Ojo-ri embayment of Jeju Island, Korea.

2.2 Demographic and size structure

Carapace length (CL) and statolith diameter (SD) were measured up to 100 specimens, which were randomly selected, if specimen were more than 100 specimens. Measurements were taken under stereo-microscope equipped with an image analysing system (Olympus SXZ10, Active measure, Japan). CL was measured from the posterior margin of the right eye socket to the mid-point of the posterior margin of the carapace. SD was measured along the long and short axes. The relationship between CL and SD was examined by regression analysis using the following allometric equation.

$$CL = a + b\,(SD)$$

The specimens were identified to species level (Jo & Ma, 1996) and then classified to six demographic categories on the basis of their reproductive and sexual characteristics, following Mauchline (1980): (1) juveniles (JUV) without secondary sexual characteristics; (2) immature males (IM) with developing secondary sexual characteristics (4th pleopod); (3) immature females (IF) with a undeveloped marsupium; (4) mature males (MM) with completely developed secondary sexual characteristics; (5) mature females (MF) with completely developed secondary sexual characteristics and an empty marsupium; and (6) brooding females (BR), corresponding to mature females with eggs or larvae in the marsupium.

2.3 Population dynamics

Length-frequency histograms were figured with a 0.25 mm size-class interval, following Sturges rules (Sturges, 1926). Normality of distributions was tested with a Ryan-Joiner test, using the Minitab 12.1 (Minitab Inc., 1998). Histograms were smoothed with a moving average to rule out non-significant peaks. A modal decomposition of distributions was made according to Bhattacharya's method (1967), using FISAT Ⅱ software (FAO, Rome, Italy). The number of modes, means and standard deviation of the carapace length of mysids were computed.

2.4 Sex ratio

Sex ratio (number of mature males vs. number of mature females) was examined following Lejeusne & Chevaldonné (2005) and Delgado *et al*. (2013). To identify the difference of relative proportions of both sexes, χ^2 test was used.

2.5 Size at sexual maturity

To determine the size at sexual maturity of the population, the proportion of sexual maturity was calculated by the following equation.

$$P_i = 1/\ [1 - \exp\ (a_i \times CL_i + b_i)]$$

where P_i was the predicted proportion of mature females ($P_i = P_f$) or males ($P_i = P_m$), a_i and b_i, the estimated coefficients of the logistic equation, and CL_i, the carapace length of females (CL_f) or males (CL_m). The parameter estimation of this equation was made by regression analysis of variables P and CL after linearization. Size at sexual maturity $CL_{i(50)}$ was the carapace length, 50% of total population was sexual maturity. It was calculated by the following equation (Campbell, 1985)

$$CL_{i(50)} = -\ (b_i\ /\ a_i)$$

2.6 Marsupial contents

The number of embryos and larvae in the marsupium of female was counted. Embryonic and larval development were classified into three developing stages, following Wittmann (1981): (1) embryonic stage (no distinguished appendages), (2) nauplioid stage (thoracic appendages in development) and (3) post-nauplioid stage (thoracic appendages fully developed and distinct stalked eyes). The relationship between brood size and brooding female size was estimated using regression analysis. The brood size and CL of brooding females were natural log transformed so that the data conform closely to the assumptions of linear regression regarding homogeneity of error variance over the range equation. Linear regression test was used to find the relation between CL and brood size with following equation.

$$ln\ (\text{Brood size}) = a + b\ ln\ (\text{CL})$$

where, a was intercept and b was slope.

III. Results

3.1 Field observations

Neomysis awatschensis was found in the entire sampling months, except in September 2013. Water temperature increased from February 2013 (10.1°C) to August (32.3°C) and then decreased slowly to January 2014 (7°C) (Fig. 2A). Water temperature range of data logger was wider than water temperature of sampling area (– 0.27 to 35.67°C) (Fig. 2A). Salinity in the area varied with the time of the day and sampling months, ranging from 6 to 28 psu (Fig. 2B).

3.2 Relationship between carapace length and statolith diameter

The carapace length ranged from 0.137 to 2.842 mm. The statolith diameter ranged from 30.7 to 199 µm. SD increased with increasing CL. SD was significantly related with CL (n= 1199, $r^2 = 0.805$, $p < 0.001$) (Fig.3). SD of mysids can be an alternative measurement of CL

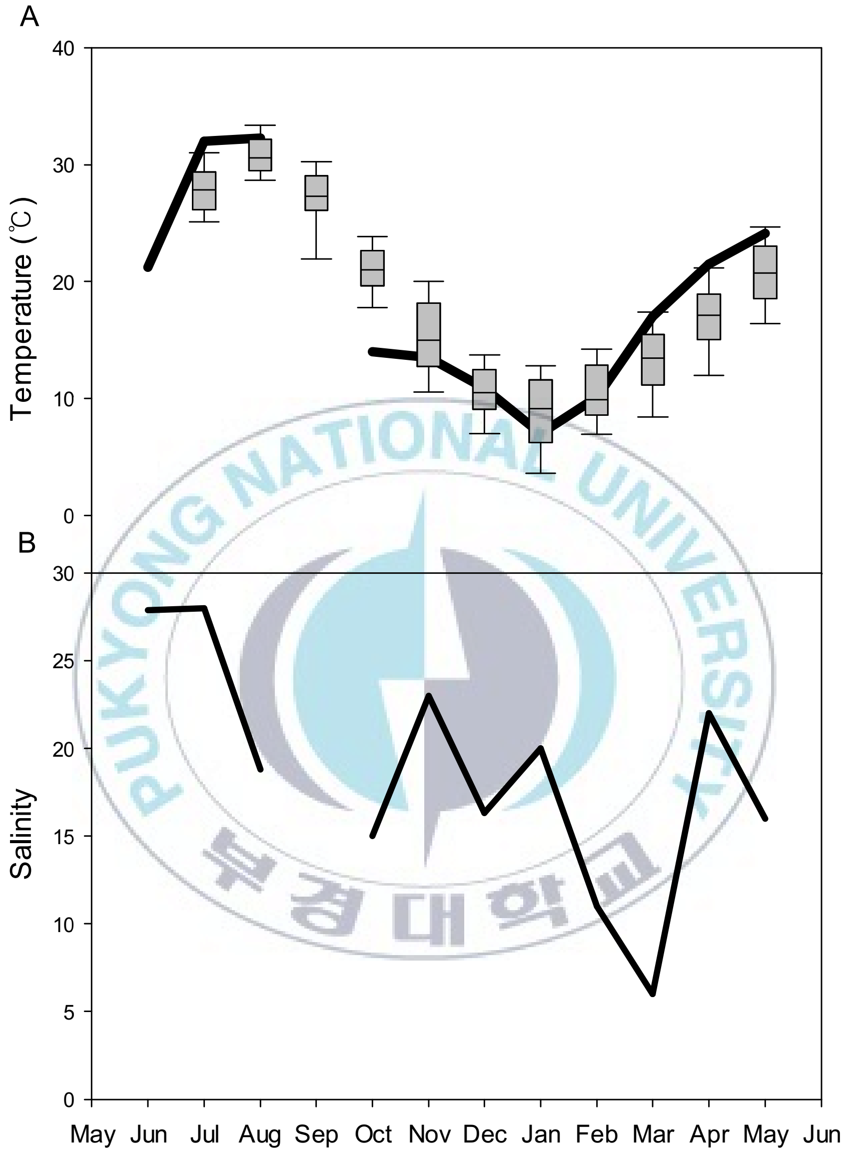

Fig. 2. Temperature (A) and salinity (B) in the Ojo-ri embayment of Jeju Island, Korea. Black solid line: sampling site , Box plot: Data logger.

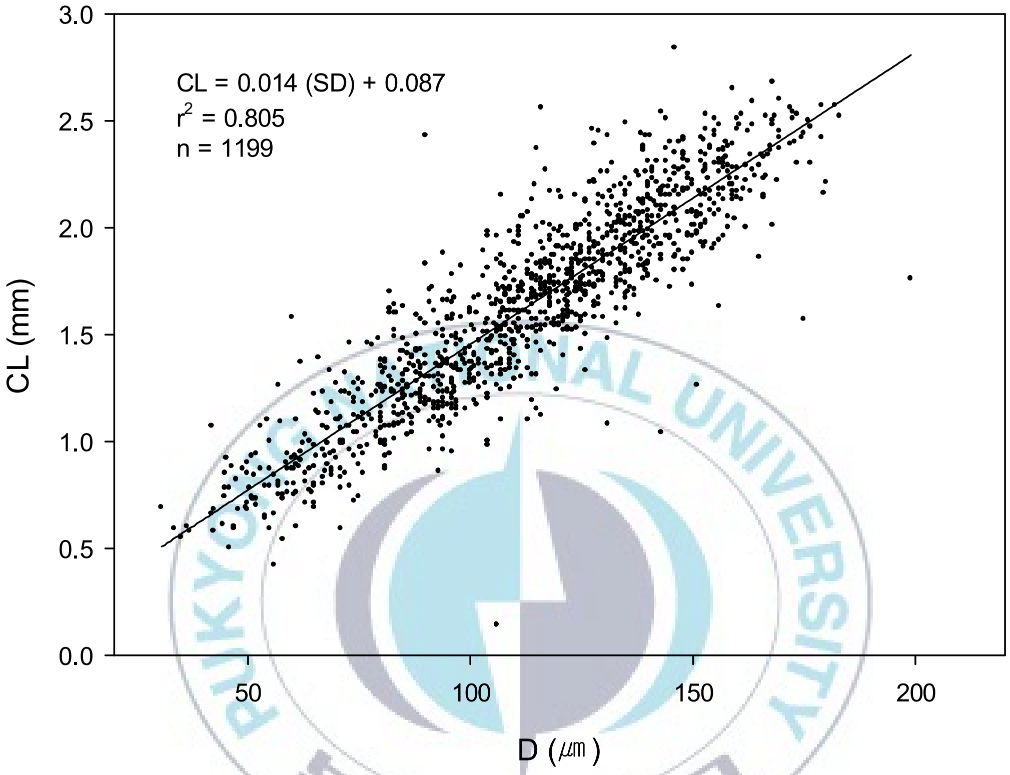

Fig. 3. Linear regression analysis between carapace length (CL) and statolith diameter (SD) of *Neomysis awatschensis* (Brandt, 1851).

3.3 Demographic and size structure

The relative proportion of demographic categories in the population changed monthly (Fig. 4). Brooding females and juvenile were observed throughout the year. The proportion of brooding females was relatively higher in October 2013 (24%) than other sampling months, followed by April 2014 (22%), May 2013 (18%) and June 2013 (17%). The proportion of juvenile was relatively higher in May 2014 (37%) than other sampling months, followed by December 2013 (31%), March 2014 (26%), February 2014 (24%) and July 2013 (16%). Four spawning groups were produced per year, with their reproduction on May, July, December and February.

The mean CL of mature females was larger than that of mature males (t = 2.11; $p < 0.05$) (Fig. 5). The mean CL of adult females and males was the largest in January and December (female: 2.340 ± 0.029 mm, male: 2.084 ± 0.059 mm), while it was the smallest in August (female: 1.676 ± 0.025 mm, male: 1.563 ± 0.023 mm) (Fig. 5). The mean CL of mature males and females was significantly correlated with the temperature variations (Spearman's rank correlation coefficient, mature females: $n = 11$, $r_s = -0.809$, $p < 0.01$; mature males: $n = 11$, $r_s = -0.618$, $p < 0.05$). The mean CL of immature females and males was the largest in January (female: 1.811 ± 0.05 mm, male: 1.795 ± 0.068 mm), while it was the smallest in November (female: 1.31 ± 0.028 mm) and in July (male: 1.255 ± 0.02 mm). The mean CL of juveniles was the largest in June (1.257 ± 0.046 mm), while it was the smallest in May 2014 (0.805 ± 0.029 mm). The mean CL of immature individuals and juveniles was not significant with temperature.

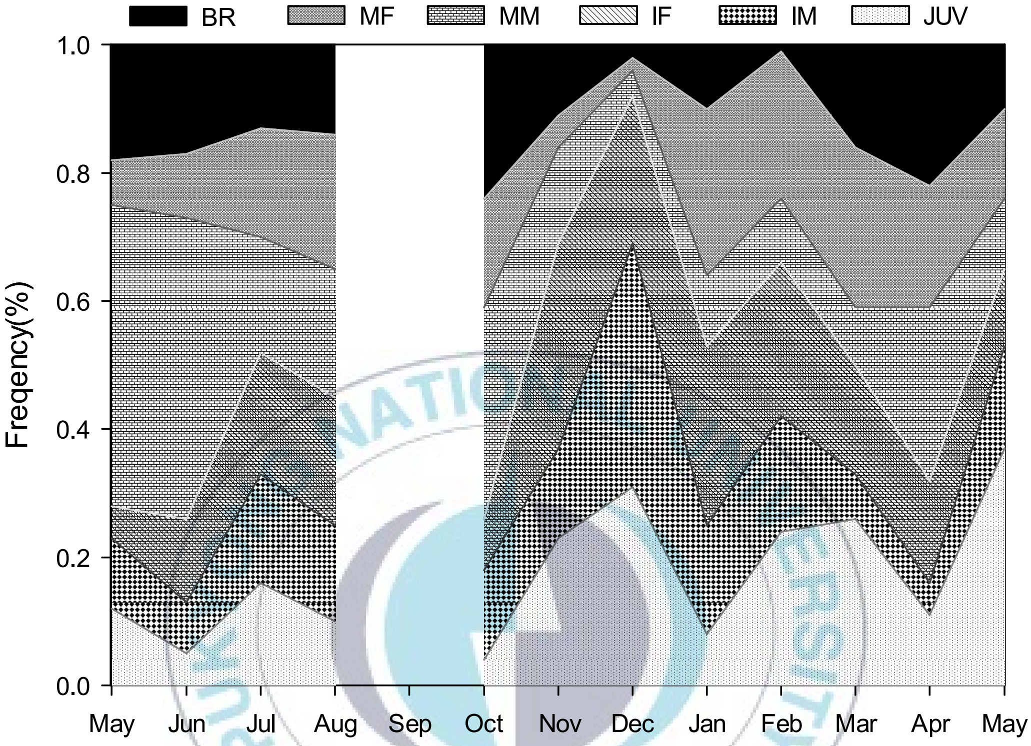

Fig. 4. Demographic structure of *Neomysis awatschensis* (Brandt, 1851) shown by % frequency of individual number (JUV: Juvenile, IF: Immature female, IM: Immature male, MM: Mature male, MF: Mature female, BR: Brooding female).

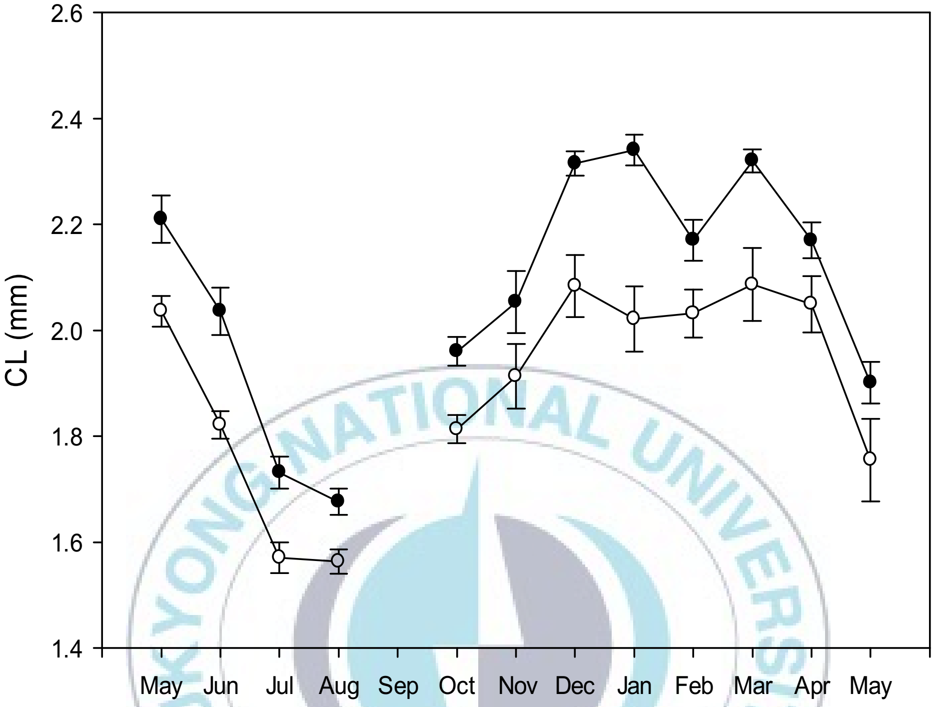

Fig. 5. Monthly variation of the mean size (CL) of mature females and males of *Neomysis awatschensis* (Brandt, 1851). Black circle: Mature females, White circle: Mature males

3.4 Population structure

One or two modes of the carapace length of *Neomysis awatschensis* were found in all sampling months (Fig. 6 and Table 1). Two modes were in January 2014. The mean of the small mode was 1.43 ± 0.24 mm, and that of large mode was 2.13 ± 0.34 mm. The new mode (0.95 ± 0.19 mm) occurred in February 2014. Although brooding females of this large mode in February was very few, the small mode (1.04 ± 0.30 mm) occurred in March 2014. A small mode (0.81 ± 0.16 mm) and a large mode (2.05 ± 0.24 mm) were in April 2014. The mean of the small mode in May 2014 (1.03 ± 0.29 mm) was higher than that of small mode in April. A small mode (1.34 ± 0.32 mm) and a large mode (2.02 ± 0.30 mm) in May 2013 One mode was in June, July and August 2013. The mean of mode (1.87 ± 0.33 mm) in June 2013 was higher than that of small mode in May 2013. The mean of mode in July (1.42 ± 0.34 mm) was similar to that of mode in August 2013 (1.52 ± 0.28 mm). Two modes were in October, November and December 2013. The mean of smaller mode in October 2013 was 1.17 ± 0.19 mm, and that of large mode was 1.88 ± 0.24 mm. The mean of small (1.26 ± 0.34 mm) and large mode (2.03 ± 0.20 mm) in November 2013 was higher than that of small and large mode in November 2013. A small mode (0.86 ± 0.13 mm) and a large mode (1.27 ± 0.38 mm) were in December 2013.

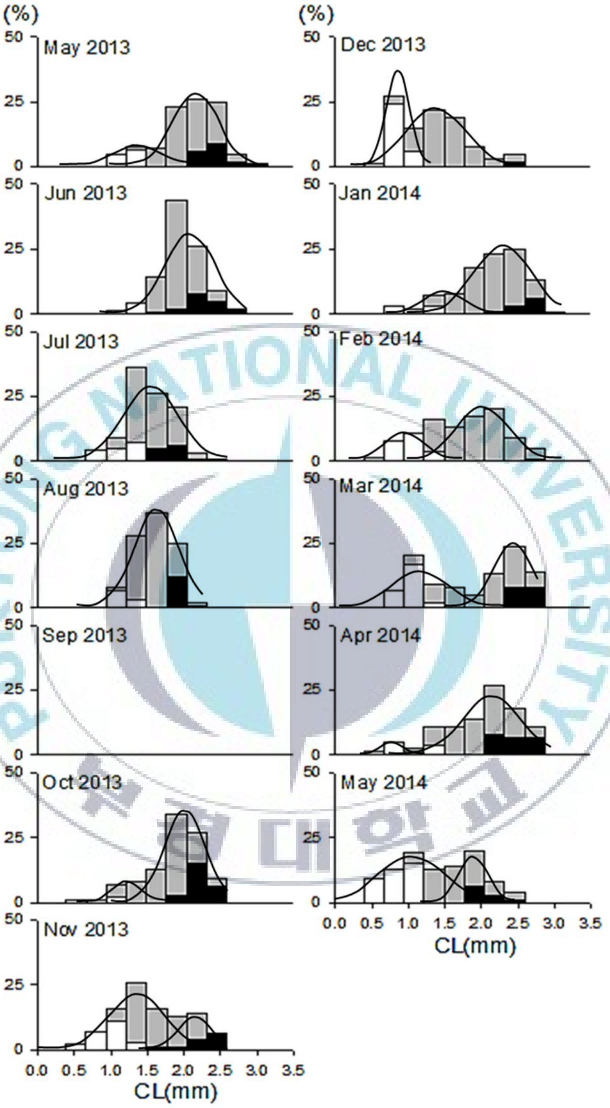

Fig. 6. Size frequency distributions of *Neomysis awatschensis* (Brandt, 1851) from May 2013 to May 2014. White bar: Juvenile, Black bar: Brooding females, Gray bar: Others.

Table 1. The number of modes, means and standard deviation of the carapace length of *Neomysis awatschensis* (Brandt, 1851).

Month/Year	Mode	Means (mm)	S.D.
May 2013	1	1.34	0.32
	2	2.02	0.30
Jun 2013	1	1.87	0.33
Jul 2013	1	1.42	0.34
Aug 2013	1	1.52	0.28
Oct 2013	1	1.17	0.19
	2	1.88	0.24
Nov 2013	1	1.26	0.34
	2	2.03	0.20
Dec 2013	1	0.86	0.13
	2	1.27	0.38
Jan 2014	1	1.43	0.24
	2	2.13	0.34
Feb 2014	1	0.95	0.19
	2	1.85	0.34
Mar 2014	1	1.04	0.18
	2	2.26	0.29
Apr 2014	1	0.81	0.16
	2	2.03	0.44
May 2014	1	1.03	0.29
	2	1.76	0.20

3.5 Sex ratio

Sex ratio over the whole sampling period was 0.73 with monthly variations ranging from 0.2 to 1.9 (Fig. 7). The proportion of mature females was lower than mature males in May and June in 2013 (35 and 36%), whereas that of mature females was higher than mature males in other months (more than 50%). In March 2014, the proportion of mature females was the highest among sampling period (82%). Females were more abundant than males in most of the sample periods (χ^2 test, $df = 11$, $\chi^2 = 54.45$ $p < 0.01$). However, sex ratio in each month was not significantly correlated with temperature or salinity (Spearman's rank correlation coefficient, temperature: $n = 11$, $r_s = -0.082$, $p = 0.811$, salinity: $n = 11$, $r_s = -0.527$, $p = 0.096$).

3.6 Size at sexual maturity

The proportion of mature individuals was fitted to carapace length by a logistic function, such as: $P_m = 1/ [1 - \exp (7.06 \times CL_m - 11.44)]$ ($r^2 = 0.976$, $p < 0.01$) for mature males and $P_f = 1/ [1 - \exp (8.17 \times CL_f - 14.05)]$ ($r^2 = 0.957$, $p < 0.01$) for mature females (including brooding females). The size of 50% sexual maturity was $CL_{m(50)}$ = 1.62 mm for males and $CL_{f(50)}$ = 1.72 mm for females.

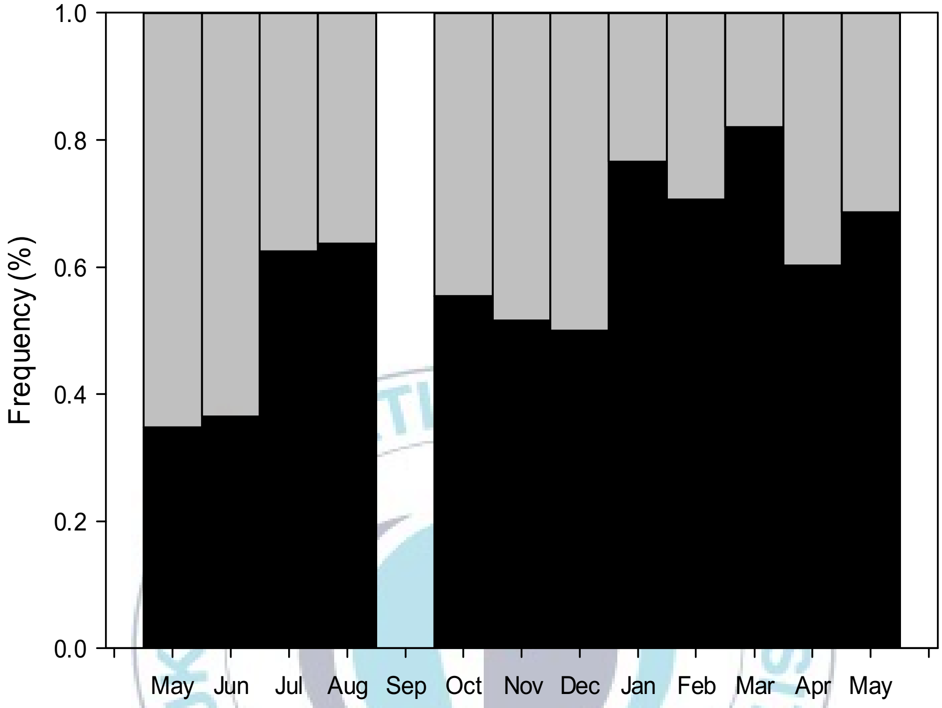

Fig. 7. Variations of sex ratio in *Neomysis awatschensis* (Brandt, 1851) (number of mature males vs. number of mature females). Black bar: Mature females; Gray bar: Mature males.

3.7 Marsupial contents

The number of embryos or larvae ranged between 1 and 36 individuals per marsupium during the study period (Fig. 8). The mean brood size was the highest in January 2014 (maximum: 23.1 individual marsupium^{-1}), while it was the lowest in August 2013 (minimum: 7.66 individual marsupium^{-1}). The brood size was monthly variations that corresponded to the variations of mean carapace length of brooding female relative to temperature fluctuations (Spearman's rank correlation coefficient, $r_s = -0.733$, $p < 0.01$).

The log-transformed values of CL and number of embryos or larvae in the females with embryonic and nauplioid stage, except post-nauplioid stage, showed statistically significant linear relationships (Embryonic stage: $n = 141$, $r^2 = 0.432$, $p < 0.001$, Nauplioid stage: $n = 250$, $r^2 = 0.148$, $p < 0.001$, Post-nauplioid stage: $n = 39$, $r^2 = 0.034$, $p > 0.260$). When larvae grew in the marsupium, the brood sizes of brooding females decreased (Fig. 9). However, in the embryonic and nauplioid stage, the slope of regressions was significantly different (ANCOVA, $F = 9.80$, $df = 1$, $p < 0.01$). The number of eggs or larvae in the embryonic and nauplioid stage was not significantly different.

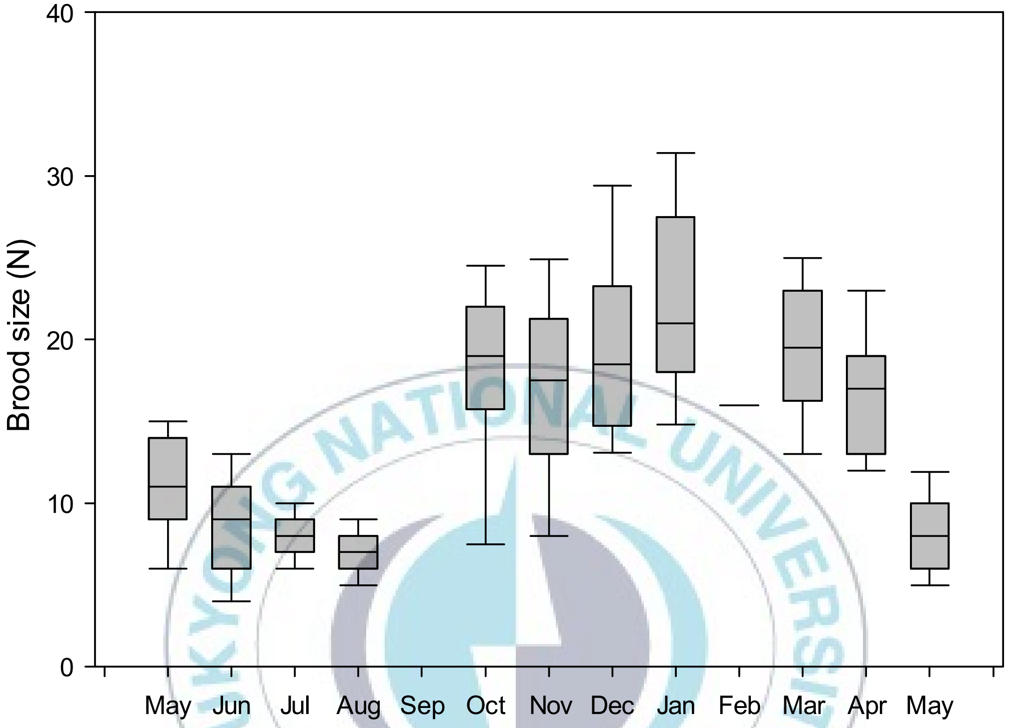

Fig. 8. Monthly variation of the mean brood size of brooding females in *Neomysis awatschensis* (Brandt, 1851).

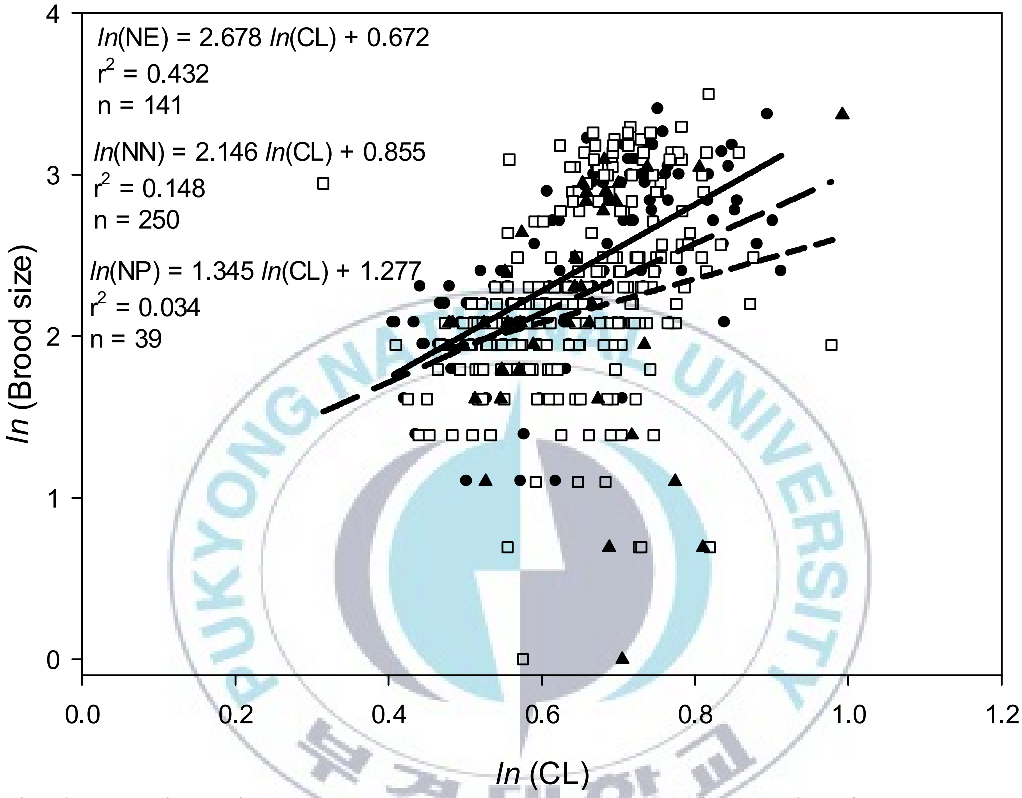

Fig. 9. Relationships between the carapace length of brooding females and the number of eggs and larvae (NE: Number of embryonic stage, NN: Number of nauplioid stage, NP: Number of post-nauplioid stage). Solid line: Embryonic stage (●), Long-dashed line: Nauplioid stage (□), Short-dashed line: Post-nauplioid stage (▲).

IV. Discussion

Total length (TL) and carapace length (CL) are measurements; they are used by many authors in study of crustacean such as growth rate. However, TL is shrunk in individuals preserved in ethanol or formalin. As a result of rigor mortis aspect of individuals in which the abdomen is often curve, CL is easier and more accurate to measure (Delgado *et al*., 2013). However, CL is also affected by chemical and physical damage which is broking and contraction of carapace (Fanelli *et al*., 2009). Most mysids have two statolithes which are special sense organs in the endopods of the uropods (Mauchline, 1980). Statolith consists of a protein matrix surrounded by a calcareous shell that is rich in calcium fluoride (Enbysk & Linger, 1966). Statolith is relatively harder than TL or CL, and the error of measurement will reduces due to the simple structure. Statolith diameter increases with body size during ontogenetic development (Schlacher *et al*., 1992). The size of statolith is used by Morgan and Beeton (1978) in analysing the occurrence of individual instars within a population of *Mysis relicta*. Therefore, we suggests that statolith diameter of mysids can be an indicator of body lengths in mysids.

The growth and size of mysids are related to water temperature. Juveniles grow almost linearly with temperature (Toda *et al*., 1984). However, adult size is correlated inversely to temperature which measured at that time. In temperate regions, the small sizes adult mysids are collected at high water temperature in summer and the large size adult mysids are collected at low temperature in winter

(Toda *et al*., 1987; Lejeusne & Chevaldonné, 2005; Sudo *et al*., 2011; Delgado *et al*., 2013). Similar results were reported in *N. americana*, *N. integer*, *N. japonica* and *N. awatschensis* populations (Ishikawa & Oshima, 1951; Pezzack & Corey, 1979; Toda *et al*., 1982; Toda *et al*., 1984; Mees *et al*., 1994; Yamada *et al*., 2007). The collected adult individuals during the period of warm temperatures grew up with a low growth rate during the cold period. For the same life span, the collected individuals of cold season are a smaller mean size than those of warmer season (Lejeusne & Chevaldonné, 2005). Hence, considering the average life-span of *N. awatschensis* is 6-7 months (Toda *et al*., 1982), this time lag between the effects of water temperature on growth and the carapace length of adult individuals can explain the variation of the mean CL of adult *N. awatschensis* in the Ojo-ri embayment.

The temperature has an influence on the fluctuations in mysid populations (Mauchline, 1980). The change of temperature induces the movement of mysids for escaping from mass mortality or temperature shock (Kotta & Kotta, 2001). *Hemimysis lamornae mediterranea* disappeared during July-August (26.3°C) in Ebro delta, followed by a subsequent increase in the number of individuals in autumn (Delgado *et al*., 2013). *Neomysis integer* also disappeared in the Gulf of Riga, due to high temperature in summer (Kotta & Kotta, 1999). Although *Neomysis awatschensis* is considered the eurythermal mysid (Tattersall & Tattersall, 1951), the upper lethal temperature range of this species is from 24.2 to 25.4°C (Hair, 1971). *N. awatschensis* can migrate other water mass (Yamada *et al.*, 2007). Probably, *N. awatschensis* may disappear to the surface or shallow water due to high temperatures of Ojo-ri embayment in summer.

All species of mysids lay their eggs into a marsupium (Mauchline, 1980). The strong positive correlation between the length of brooding females and their brood size is a general mysid characteristic (Mauchline, 1980; Toda *et al*., 1984). This relationship corresponds to the water temperature. Large females resulted from an overwintering generation of mysids while smaller females constituted a summer generation (Toda *et al*., 1982). *N. mirabilis* and *N. integer* are reported to the relationship (Bremer & Vijverberg 1982; Yamada *et al*., 2007). *N. awatschensis* also has that a relationship of between brooding female size and brood size is a linear positive relation (Yamada *et al*., 2007). Thus, the brood size of *N. awatschensis* changes with seasonal variations of brooding female size that correspond to the seasonal water temperature variations.

The water temperature is considered one of the main factors conditioning the occurrence and efficiency of recruitment and reproduction of mysids (Mauchline, 1980; Wittmann, 1984). Several mysids stop breeding during winter due to the strong seasonal variation of water temperatures in some areas of the mid-latitudes (Wittmann, 1984). The reproductive season of *N. awatschensis* was continuous from March through November (Toda *et al*., 1982). This reproductive trend is reported in *N. awatschensis* of the North-western Pacific Ocean (Yan 1982; Yamada *et al.*, 2007). On the other hands, the continuous reproduction is a biological phenomenon in neritic mysids between 25 and 50°N (Pezzack & Corey, 1979). The appearance of brooding females and juveniles all year round indicates that the species reproduces year round (Pinheiro & Fransozo, 2002).

The fluctuations of the sex ratio may be the result of migration, differences in the minimum size at which secondary sexual characteristics appear (Mauchline, 1980), different life duration or different rates of mortality among males and females (Hakala, 1978). In addition, the sex ratio of mysids is related to salinity. For example, *Schistomysis kervillei* population tends to have a decreasing proportion of females with increasing salinity (Rappé *et al*., 2011). Although the sex ratios within mysid populations are variable, the number of females frequently is higher than that of males (Mauchline, 1980). Higher proportion of females than that of males has been reported in mysids, such as *Hemimysis lamornae mediterranea*, *Mesopodopsis orientalis* and *Siriella clausii* (Hanamura *et al*., 2008; Barberá *et al*., 2013; Delgado *et al*., 2013). The proportion of females in *N. awatschensis* is also higher than that of males (Toda *et al*., 1982; Yan, 1982).

The size at sexual maturity is a critical indicator of the reproductive capacity of population. It may sign a variation in the growth rate of many crustacean species (Street, 1969). Female mysids grow larger than males (Mauchline, 1980). It is due to more frequent moults in males combined with a slower growth rate. This difference growth indicates that male sexual characteristics are distinguished at smaller sizes than females (Toda *et al*., 1987). The CL in females is positively related to a brood size, and large CL leads to high brood size (Toda *et al*., 1984). Smaller size of males than that of females serves fast mobility for getting females and reducing the risk for predators (Correa & Thiel, 2003). Males for mysids reached sexual maturity at a smaller size than females (Sudo, 2003; Fockedey *et al*., 2005), and our data agree with this general pattern.

Although reproduction occurs every month, the relative proportion of juveniles and brooding females make some spawning groups. In continuous reproduction, the different proportion of brooding females is possible to recognize some peak in some seasons (Pinheiro & Fransozo, 2002). The occurrence of this spawning group in a species reproducing all year round indicates either the presence of more intensive periods of reproduction followed by intensive periods of breeding or more intensive periods of recruitment (Lejeusne & Chevaldonné, 2005). In case of *N. awatschensis*, the relative proportion of juveniles is more than 50% from June to December (Yamada *et al.*, 2007), and that of brooding females has several peaks from March and September (Toda *et al.*, 1982). The time lag between the period to mature and the relative proportion of juveniles or brooding females is used to estimate the number of generation of *N. awatschensis* by Yan (1982) and Yamada *et al.* (2007). *N. awatschensis* matures within 6 weeks in April-May and appears to mature in 3 weeks in June (Toda *et al.*, 1982), and they grow to adult in 15–20 days in 20–25°C and 20–30 days in 15–20°C (Toda *et al.*, 1984; Toda *et al.*, 1987). However, *N. awatschensis* have short generation time, and their brood interval decreases with increasing temperature (Murano, 1964; Toda *et al.*, 1984; Toda *et al.*, 1987). These short generation time indicate that field studies should consider short time scales for the estimation of generation number of warm-water species. Nine-teen overlapping cohorts of *Orientomysis robusta* were recognized over an annual cycle (Sudo *et al.*, 2011). Their findings are based on weekly samplings and growth rate which studied in the laboratory. As a result, our monthly samples were not enough to explain generation number of *N. awatschensis*.

In conclusion, statolith diameter can be a substitution for measurement of length of mysid. *N. awatschensis* was characterized by small size at sexual maturity of males, high proportion of females, continuous reproduction and four major spawning groups year round. Population structure and life history of *N. awatschensis* were mainly influenced by water temperature. However, the precise number of generations of *N. awatschensis* remains unknown. To clarify this problem, weekly sampling and laboratory rearing experiment should be conducted.

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