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Effect of temperature on egg development time and productivity of *Acartia steueri* and population variations of family Acartiidae in Dadaepo Beach, Busan, Korea

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Abstract

Aim: This research aimed to study the effect of temperature on egg production rate, egg development time, hatching success rate and egg size of *Acartia steueri* and seasonal variation of species composition of family Acartiidae.

Methodology: Population variations of Acartiidae were investigated based on weekly collected samples from Dadaepo beach, Busan, Korea. Live copepods were reared in laboratory at five temperature regimes (10, 14, 18, 22 and 26 ℃) to estimate temperature effects on the egg development time and productivity of *A. steueri*.

Results: Total five acartiid species were found in the study area: *Acartia omorii*, *A. steueri*, *A. pacifica*, *A. erythraea*, and *A. sinjiensis*. *A. steueri* and *A. omorii* were dominated in colder months from January to April, while *A. pacifica*, *A. erythraea* and *A. sinjiensis* were abundant during warmer months from August to December. Egg production rates were significantly correlated with water temperature. The embryonic development time of *A. steueri* was longest at 10 ºC and shortest at 22 ºC. The egg size decreased with increase in water temperature.

Interpretation: This study indicated that temperature influenced embryonic development rate, generation time and population variations of acartiid copepods.

Key words: *Acartia steueri*, Dadaepo beach, Egg production rate, Population variation

To study temperature effects on the egg production rate and development time, and hatching success of *A. steueri* and to investigate the seasonal variation of species composition of family Acartiidae

Laboratory Experiment	Field Experiment
- Five temperature regimes (10, 14, 18, 22 and 26ºC) - Egg production rate; - Development time - Hatching success rate; - Egg size	- Weekly collected samples - Population variations

Laboratory Experiment	Field Experiment
- Egg production rates correlated with temperature. - Egg size decreased when temperature increased - Egg development time and hatching success of *A. steueri* was longest and lowest at lower temperature.	- Total five Acartiid species : Acartia omorii, A. steueri, A. pacifica, A. erythraea, A. sinjinensis - Colder months (From January to April) : A. steueri, A. omorii dominented - Warmer months (From August to December) : *A. pacifica, A. erythraea, A. sinjinesis*

This study indicated that temperature influenced embryonic development rate, generation time and population variation of acartiid copepods.

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Introduction

Copepod metabolism can be controlled by exterior environmental factors such as temperature, salinity and food concentrations (Landry, 1975; Miller *et al.,* 1977). In particular, temperature is an important factor for the population variations (Uye, 1982; Liang and Uye, 1996), life histories such as reproductive rate and growth rate (Lee *et al.,* 2003), egg production rate and hatching success (Ara, 2001; Begum *et al.,* 2012). Increasing water temperature accelerates egg production rate and hatching success, and shortens egg development time (Landry, 1975). Water temperature changes driven by climate changes can adjust strategically their egg production to maintain populations (Uye, 1982; Hays *et al.,* 2005).

Copepods of family Acartiidae are common inhabitants of coastal water in all oceans of the world (Uye, 1980, 1981; Kang and Kang, 1998; Yoo *et al.,* 1991; Mauchline, 1998). The abundance of members belonging to family varies with seasons. *Acartia longiremis* is dominant in colder season while *A. steueri* is dominant in the warmer season at Okkirai Bay in Japan (Yamada *et al.,* 2012). *A. longiremis*, *A. omorii* and *A. hudsonica* appears from early spring to summer. The abundance of *A. steueri* increases during summer, decreases until January, and then disappears for two or three months from April at Okkirai Bay in Japan (Yamada *et al.,* 2012).

Dadaepo beach is located towards Tsushima Warm Current and flows from Southern Sea of Korea. Tsushima Warm Current may transport multi species of family Acartiidae and result in seasonal community changes of acartiid species in the area. Dadapo beach displays a gentle slope within 100 m with high nutrients, due to tidal mixing (Na *et al.,* 1990). Primary production in Dadaepo beach is high, resulting in high abundance of copepods (Na *et al.,* 1990).

This research was carried out to study temperature effects on egg production rate and development time, and hatching success of *A. steueri*, and also to investigate the relationship between water temperature and species composition of family Acartiidae in the study area.

Materials and Methods

Sampling and maintenance of live specimens : Zooplankton was horizontally sampled using a conical zooplankton net (45 cm in mouth diameter and 330 μm in mesh size) at Dadapo beach, Busan, Korea during two weeks in March 2016. Live copepods were transported within hours to laboratory. Adult *Acartia steueri* were separated under a microscope and were placed in acrylic water tanks, maintained at similar temperature of the sampling site. *Isochrysis galbana* and *Tetraselmis suecica* obtained from Gyeongsangnam-Do Fisheries Research Institute were fed to adult *A. steueri*. During the experiment, sea water was changed two times per day.

Egg production rates and sizes : Live adult copepods were separated under microscope using a 0.5 ml pipette. After two days of acclimation, live individuals were maintained in 2.5 l acryl container with full filled sea water of five temperature regimes (10ºC, 14 ºC, 18 ºC, 22 ºC and 26 ºC). After two days, each individual was put into the modified 60 ml acryl cylinder containing filtered seawater and mixture of *I. galbana* and *T. suecica* for one week. Phytoplankton concentration at each cylinder was over the 10,000 cell ml^{-1}. Ten replicates were made for same experimental condition. Photoperiod was 12 hr light and 12 hr dark.

The modified acrylic cylinder was designed to minimize the stress of copepods during the experiment. The upper part of acryl cylinder was 10 cm while lower part was 3 cm high. Considering the diameter of eggs (about 80 μm), the mesh size of upper part was 200 μm and that of lower part was 30 μm.

Copepod eggs were collected, counted and fixed in 5% neutral formalin to determine the effects of water temperature on the egg size. The size of copepod eggs were measured under the microscope (Stereo discovery V12).

Hatching success rate and embryonic development time: Adult copepods were placed under the microscope using 0.5 ml pipette. Ten copepods were cultured in a modified acryl container with sea water at five temperature regimes (10ºC, 14ºC, 18ºC, 22ºC and 26ºC). After 4 hrs, 1 to 3 scattered eggs on the bottom of the container were placed in a 24 well plate container of 0.3 ml of filtered sea water at five temperature regimes. Naupliar hatching was observed under the microscope every 4 hrs hrs and hatched nauplii were removed from the well-plate. After 144 hrs from the eggs to the last hatching time, unhatched eggs were determined as resting or failed hatching eggs.

The relationship between temperature and embryogenesis time was calculated by using Bělehrádek's equation (Corkett and McLaren, 1970).

$$D_E = a(T - \alpha)^b$$

Where, D_E is the egg incubation time (day), T is the water temperature (ºC), and a, b, and α are optimization constants. To obtain optimization constant, the equation was converted to log. α was arbitrarily substituted to obtain α having highest regression relationship, and a and b were obtained.

The equation converted to log is as follows:

$$\text{Log } D_E = \log_a + b\log (T-a)$$

Zooplankton sampling for population variations : Zooplankton was collected using a conical net based on weekly collected samples from Dadaepo beach, Busan, Korea from August 2015 to April 2016. The collected specimens were fixed in 70% alcohol, and then transported to laboratory for analysis.

Water temperature and salinity were measured by thermometer and refraction salino meter.

Statistical analyse : One-way ANOVA was performed using SPSS program (Statistical Package for the Social Science, Version 12.0) to determine the difference by water temperature regimes between egg production rates, egg size and embryonic development time. The significance ($p<0.05$) between the average was investigated by Tukey-test.

Results and Discussion

Environmental factors : The surface water temperature during the research period ranged from 9.8 to 25.4ºC. The surface temperature was lowest in January 2016 (9.8ºC) and the highest in August 2015 (25ºC). The salinity ranged from 30.7 to 34 psu (Fig. 1).

The surface water temperature and salinity in the southern coast of Korea comprise a large seasonal pattern with temperature ranging from 8.4 – 26.7ºC and salinity 15.0 – 34.3 psu (Yun and Paik, 2001). The water temperature in the southern coast of Korea is influenced by warm current influx from the south, surface cooling and vertical mixing in winter and by rainfall and evaporation. Seasonal variation of sea water salinity and temperature in the study area may influence changes in egg development and production of zooplankton in the area.

Egg production rates : The egg production rates of *A. steueri* were estimated by averaging the counts of *A. steueri* eggs for one week in five temperature regimes (Table 1). The production range of *A. steueri* was 8.7 – 35.0 per female, lowest at 10ºC, and highest at 22ºC. The egg production rates increased with elevating water temperature, but decreased sharply at 26ºC ($p<0.05$). The mortality rate of adult *A. steueri* was lowest at 26ºC (Fig. 2).

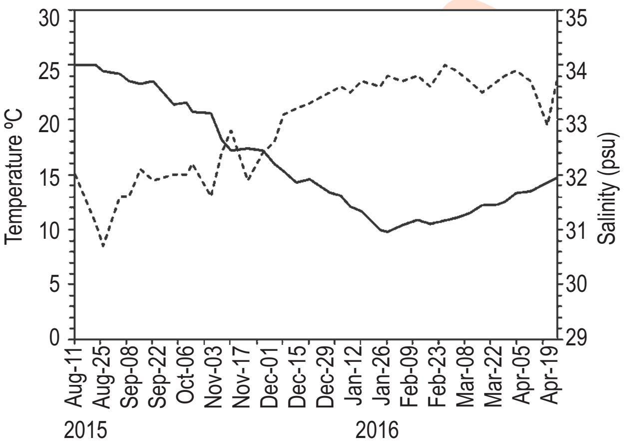

Fig. 1 : Weekly variation of surface temperature (line) and salinity (dotted line) in the Dadaepo beach of Korea from August 2015 to April 2016.

The difference in egg production rate by temperature was analyzed through one way ANOVA test. There was a significant relationship between egg production rates and water temperatures (Table 2). There was no significant difference at 10ºC and 14ºC, but there was a significant difference at other temperature regimes (Fig. 2).

Egg production rate is affected by water temperature, salinity and the quantity and quality of food (Koski and Kuosa, 1999; Castro-Longoria, 2003; Holste and Peck, 2006; Santhanam *et al.,* 2013). In particular, the egg production rate of copepods increases with elevating temperatures (Ara, 2001). The highest productivity of *Acartia tonsa* was found at 23ºC (Holste and Peck, 2006), while *A. clausi* and *A. steueri* in Onagawa Bay produced the highest number of eggs at 15ºC and 20ºC (Uye, 1981). The egg production rate of *A. tonsa* in Chesapeake Bay increased up to 27ºC, but was reduced at higher temperature (White and Roman, 1992). The egg production rate of *A. bifilosa* was also positively correlated with temperature. The number of eggs were highest at 18ºC and the lowest at 24ºC (Koski an Kuosa, 1999). Daily egg production rate of *A. lilljeborgi* ranged from 13.8 to 66.8 eggs per female per day. The highest egg production rate of *A. clausi* was observed in spring month at 8.6 – 18.5ºC whereas the lowest values were found in fall months with the ranges of 16.94 – 23ºC (Üstün and Bat, 2014). The egg production rate of *A. lilljeborgi* increased as temperature increased from 19.5 to 25.2ºC, after then decreased between 28.4ºC and 29.1ºC in Cananéia Lagoon estuarine (Ara, 2001). The egg production rate tended to decrease sharply above the optimum water temperature (Uye, 1981).

In this study, egg production rates also increased with increasing temperature up to 22ºC. When water temperature was over 22ºC, the egg production rate decreased rapidly. The optimal water temperature in each copepod sampling site varied from 18ºC to 27ºC. This research indicates that copepods may acclimatize to the water temperature of their respective regions, which may affect the egg production rate, further leading to variation in the populations.

Egg size : The average egg size of *A. steueri* in five temperature regimes ranged from 72.51 to 82.74 µm. The egg size decreased when the water temperature increased, the mean egg sizes were 82.74, 77.29, 75.92, 73.65, and 72.5 µm, respectively (Fig. 3).

One way ANOVA was used to test the variations of egg sizes by water temperature. There was a significant relationship between egg sizes and water temperatures ($p<0.05$).

The egg size is affected by temperature and quantity of food (Zamora-Terol and Saiz, 2013; Woodward and White, 1981). The egg size increases with increasing food concentration due to change in the amount of protein, carbohydrate and lipid concentration (Guisande and Harris, 1995). In particular, the egg

Table 1: Mean egg production of *Acartia steueri* at five different temperatures

Temperature (ºC)	No. of total females	No. of experimental females	Average egg production (eggs/female/day)	S.D.
10	10	7	8.7	0.75
14	10	8	13.4	1.36
18	10	7	28.4	1.75
22	10	7	35.0	2.89
26	10	9	20.7	2.28

Table 2: Analysis of variance (ANOVA test) of egg production of *Acartia steueri* at five different temperatures

Source of variation	d.f	Sum of squares	Mean squares	F
Temperature	4	3267.7	816.9	23.20

$P < 0.05$

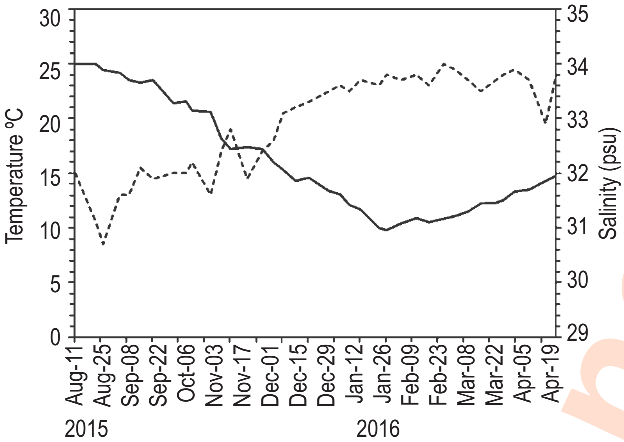

Fig. 2 : Egg production rates of *Acartia steueri* at five different temperatures. Values are mean ± SE of each treatment. One-way ANOVA was performed between the treatments followed by Tukey-Karmar multiple comparison test (P < 0.05). Letters (a, ab, bc, cd, and d) represent statistical significance between each treatment.

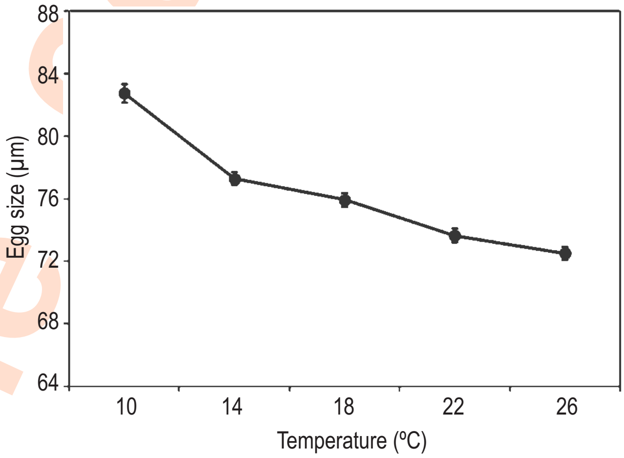

Fig. 3 : Egg size of *Acartia steueri* at five different temperatures.

size of copepods decreases with increasing temperature. The eggs of *Acartia tonsa* produced at 6ºC was 85 µm in diameter, but decreased to 80 µm at 24ºC (Hansen *et al.,* 2010). The egg diameter of *Acartia tonsa* was 82.2 µm at low temperature (6.5ºC) and 78.4 µm at 17.5ºC (Uye and Fleminger, 1976). The egg size of *Sinodiaptomus* (*Rhinediaptomus*) *indicus* also decreased with increasing temperature, but above optimum temperature (≥ 30ºC), the egg size changed at the low temperature (Begum *et al.,* 2012). In this study, the egg size of *A. steueri* showed similiar pattern with *A. tonsa*.

The egg size is affected by female body size. The body size is positively correlated with egg size (Dvoresky and Dvoretsky, 2014), but inversely with temperature (Kobari *et al.,* 2003). In other words, the egg size increased with growing body size at low temperatures. The eggs produced from relatively small female is small because the space for mature oocyte is small (Niehoff, 2007). This research indicated that egg sizes are concerned with the number of egg and the arrangement of oocytes in the gonad.

Hatching success and embryonic development time : Hatching success rate of eggs were lowest at 10ºC (2.1%) and increased sharply to 98% at 18ºC, followed by 94% and 77% at 22ºC and 26ºC, respectively (Fig. 4). The relationship between embryonic development time and temperature of *A. steueri* was as follows

$$D_E = 40.4\ (T-5.9)^{-1.23} \qquad (r^2 = 0.9737)$$

The duration of embryonic development of *A. steueri* at five temperature regimes varied from 1.2 to 7.5 days. The embryonic development time was lowest at 7.5 days for 10ºC and 1.2 days at 22ºC. The embryonic development at 26ºC, the highest temperature, was lower than 22ºC.

Table 3: Analysis of variance (ANOVA test) of embryonic development of *Acartia steueri* at five different temperatures

Source of variation	d.f	Sum of squares	Mean squares	F
Temperature	4	361956.9	90489.226	724.2

P < 0.05

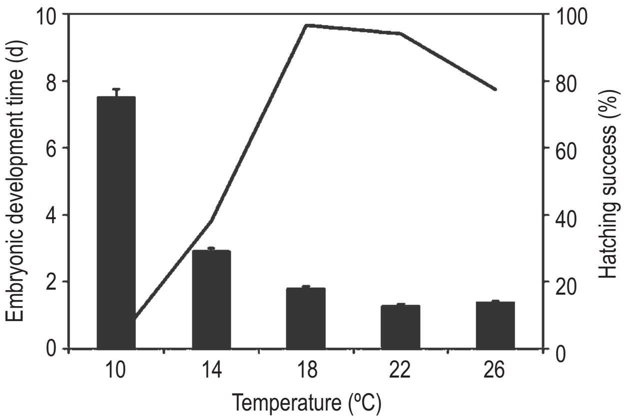

Fig. 4 : Embryonic development time (bar) and hatching success rates (line) of *Acartia steueri* at five different temperatures.

There was a significant relationship between duration and temperature of embryonic development (Table 3). Significant difference was observed at 10ºC and 14ºC than other temperature regimes (p<0.05), while no significant difference was noted between 26ºC and 22ºC (p>0.05, Tukey test).

Temperature is affected by embryonic development time (Yoshida *et al.,* 2012). The embryonic development time of *Thermocyclops neglectus* increased when water temperature increased, and the embryonic development time was longer than the optimal temperature (32.5ºC) (Burgis, 1970). The egg development times of *Acartia erythraea*, *A. pacifica* and *A. spinicauda* were shortest at high temperature (Yoshida *et al.,* 2012). Hatching time of *Eurytemora affinis* was 1.4 days at 5ºC, 1.6 days at 22ºC and 0.5 day at 25ºC (Andersen and Nielsen, 1997). The egg development times of *Eurytemora affinis* and *Psudodiaptomus forbesi* reduced with increasing temperature (Sullivan and Kimmerer, 2013). The egg development of cold water species (*Cyclops vicinus* and *Diacyclops bicuspidatus*) was faster than *Mesocyclops leukarti* and *Thermocyclops crassus* at 5 - 15ºC (Maier, 1989). The result showed that temperature affect embryonic development times.

Hatching rate increased when temperature elevated, but decreased above optimum temperature. Hatching success rates were highest at 27 ºC, 22 ºC (or 27 ºC) and 31 ºC for *A. erythraea*, *A. pacifica* and *A. spinicauda*, respectively and lowest at 10ºC for all three species (Yoshida *et al.,* 2012). Hatching rates of *A. tonsa* was highest at 23ºC (92.2%), but there was no hatching 12ºC (Holste and Peck, 2006). The hatching success of *Calanus glacialis* and *C. finmarchicus* from Disko Bay, Western Greenland was highest at low temperature (0ºC) and the egg development time of two species decreased with increasing temperature between 0ºC and 10ºC (Jung-Madsen and Nielsen, 2015). Because the optimum temperature of two species in Arctic was lower than experiment temperature regimes.

The hatching rate also showed pattern with the developmental time. Copepods have their own optimal temperature and salinity for the hatching success rate and developmental time. Thus, the hatching rate decreases and embryonic development time becomes longer when the temperature exceeds the optimal temperature.

Population variations of acartiidae : Total five Acartiid species appeared in the study area. *A. steueri* occurred continuously during the study period. The monthly mean densities ranged from 8.0 to 152.7 inds. m^{-3}, which was highest in March, and lowest in August 2015 and January 2016, respectively. *A. omorii* was present continuously during the study period except October. Monthly mean densities ranged between 0 – 229.3 inds. m^{-3}, which was highest in February. *A. sinjinesis* appeared from August to January. *A. pacifica* occurred until December. The monthly mean density ranged between 0 – 108.5 inds. m^{-3}, 0 – 17.0 ind. m^{-3}, which was higher in October and September than other months. The monthly mean densities of *A. erythraea* were lowest from August to October, ranging between 1.8 – 5.5 inds. m^{-3} (Fig. 5).

Population variations in genus *Acartia* are influenced by environmental factors such as temperature, salinity and Chl *a* concentration (Soh, 2003). *A. omorii* are found between 6.19 – 26.32ºC. *A. pacifica* and *A. erythraea* occur with a range between 18.07 – 27.28ºC and 14.62 – 26.48ºC, respectively (Kang, 2011). The optimum water temperature for *A. sinjinesis* are 25.03 – 27.5ºC and *A. steueri* are found as 11.26 – 14.24ºC. *A. omorii* in the southern part of Korea was dominant in spring and early summer, and salinity (>33.0 psu) and Chl *a* concentration were high. However, due to increase in water temperature (>20ºC), the number of *A. omorii* decreased, and the number of *A. erythraea* and *A. pacifica* increased. In Okkirai Bay, Japan, *A. omorii* appears in winter and spring when water temperature is low, while *A. steueri* appears year-round except when the temperature is lowest (Yamada *et al.,* 2012). In this study, *A. steueri* occurred continuously during the study period with peaks at lower temperature season. There was differences of *A. steueri* occurrence because different monthly water temperatures

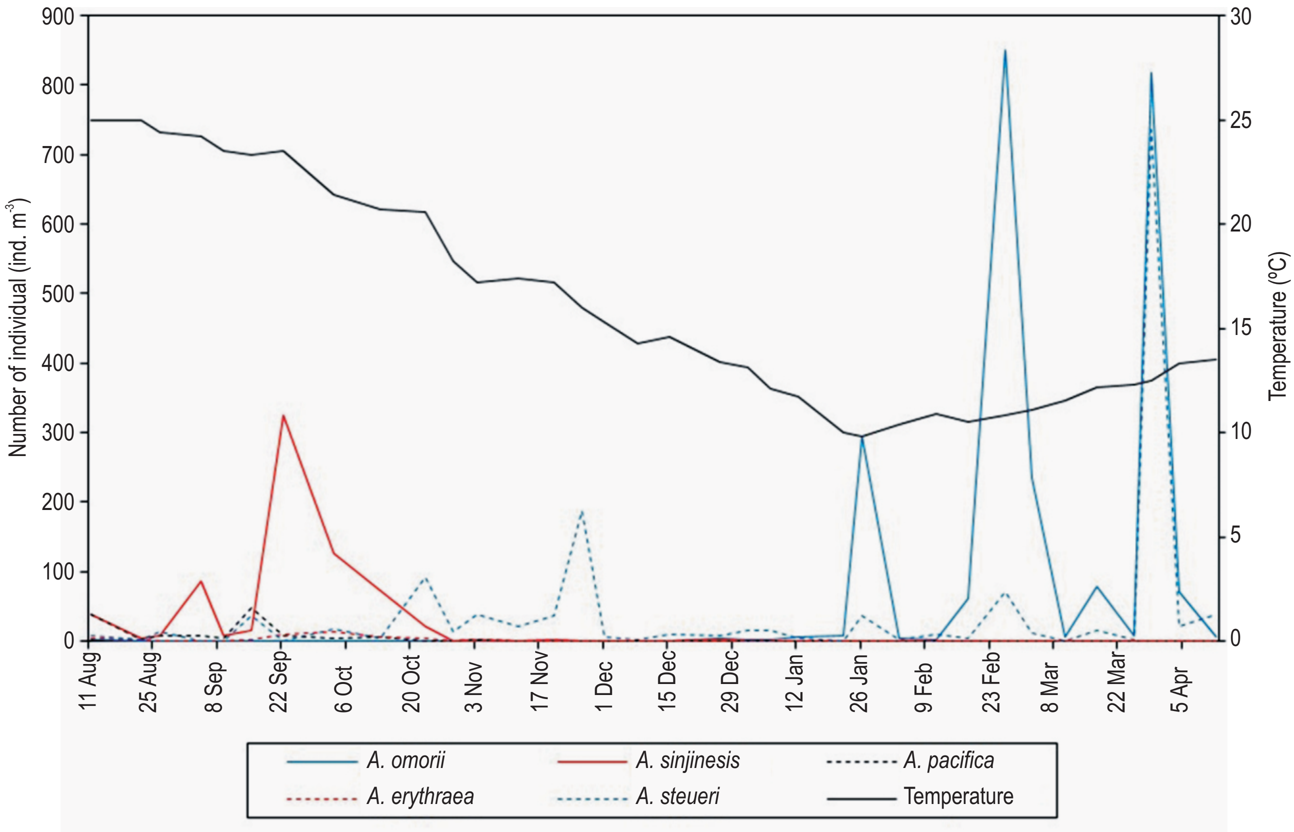

Fig. 5 : Population variations of Acartiid copepod in Dadaepo beach from August 2015 to April 2016.

between Okkirai Bay, Japan and the present study area. Okkirai Bay, Japan is located at higher latitude than the study area so that temperature of Okkirai Bay is lower than that of study areas. The highest temperature of Okkirai Bay, Japan all around the year is approximately 20ºC, while that of the study area was 25ºC. This difference in temperature change between two locations resulted different pattern of *A. steueri* occurrence. *A. steueri* at both areas occurred at similar water temperatures. Similar to previous studies, *A. pacifica*, *A. erythraea* and *A. sinjiensis* occupied the highest proportion of individuals when water temperature was high during the study period. As the temperature decreased, the proportion of individuals also decreased with decreasing water temperature. The optimum water temperature of *A. omorii* and *A. steueri* was lower than that of other species. Thus, population of acartiid species changes seasonally because the optimal habitat temperature differs from species to species.

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