Hatching patterns and egg types of *Acartia erythraea* (Copepoda, Calanoida) in eutrophic bay

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부영양화된 만에서 요각류 *Acartia erythraea*의 부화 양상 및 난 형태

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Abstract

Acartia erythraea, a copepod species commonly found in the summer, was sampled weekly using a conical net, in Gamak Bay (southern Korea) from 19 July to 14 November 2013. Egg production rate (EPR) and hatching success rate (HS) were estimated weekly during the study period, for egg types with distinct surface morphologies, i.e. smooth or spiny. EPR ranged from 1.0 to 25.0 eggs female^{-1} day^{-1} with a mean of 12.0 eggs female^{-1} day^{-1}. Of these, all the eggs were smooth until August, whereas spiny eggs were produced from September, and the spiny eggs contributed 88% of the total eggs in November. Both egg types hatched within 12 to 48 h, and the average HS was 80% or higher. Contrary to previous studies, our findings suggest that it is impossible to determine whether an egg is subitaneous or diapause based on the egg surface structure. Spines on the surface of *A. erythraea* eggs in Gamak Bay could be an adaptation to seasonal variations and a rapidly changing coastal environment.

1. Introduction

In marine ecosystems, calanoid copepods play an important role as mediators in the food web, feeding on phytoplankton, which are the primary producers. Identifying the biological, physical and chemical factors that affect the reproduction, growth, survival and mortality of copepods, as well as the temporal and spatial variations in their distribution, is a basic objective of zooplankton ecosystem research [1].

In most calanoid copepods, no morphological differences are observed between their subitaneous and diapause eggs. However, species belonging to the family Acartiidae, predominantly occurring in coastal waters worldwide, show morphological differences between subitaneous and diapause eggs[2]. Generally, subitaneous eggs have a smooth surface, whereas diapause eggs have spines. The spines of diapause eggs have developed as an adaption to the environment by reducing mortality and increasing egg dispersion in refractory conditions. However, previous studies raised several questions regarding whether resting eggs can be distinguished morphologically, according to the presence or absence of spines on the egg surface [3].

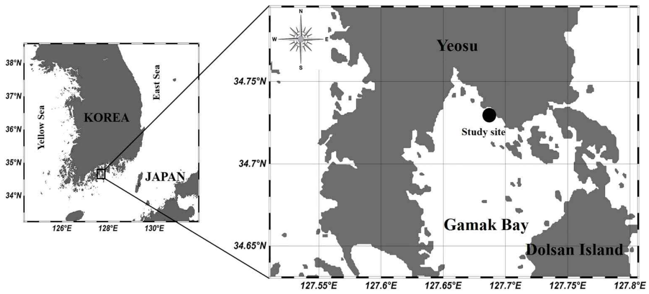
Acartia erythraea is predominant in summer and autumn in the coastal regions of Korea and produce two types of morphologically distinct eggs: subitaneous and diapause. According to a study by Kasahara et al. [4], *A. erythraea*, in the Inland Sea of Japan, lay diapause eggs with spines as a strategy for rest during winter.

The purpose of this study was to investigate the temporal changes in population abundance and egg production rate (EPR) and growth rate of *A. erythraea*. In addition, eggs laid by *A. erythraea* were examined for hatching patterns and egg surface morphology to determine whether they were subitaneous or diapause eggs.

2. Materials and methods

Zooplankton were sampled weekly at a depth of 10 m in Gamak Bay, Yeosu, South Korea during the daytime (between 3 and 5 p.m.). The sediment types have been reported as muddy in Gamak Bay. Zooplankton samples were taken from the bottom (B-1 m) to the surface layer using a conical net (mesh size 200 μm, mouth opening size 45 cm) at a speed of a 1 m·s^{-1} from 19

July to 14 November, 2013 during high tide (Fig. 1). Zooplankton was immediately fixed to a final concentration of 5% using neutralized formalin in the field. The water volume filtered was estimated using a flow meter (Hydro-Bios 438 115) attached to the net mouth. *A. erythrea* was sorted and counted under a stereomicroscope (Zeiss Stemi SV11, Jena, Germany) and sorted according to on copepodid stage. The copepodites (copepodids I–V) and adults were identified using morphological characteristics based on Huys and Boxshall [5]. The density of *A. erythraea* was converted into the number of individuals per cubic metre (indiv. m^{-3}).



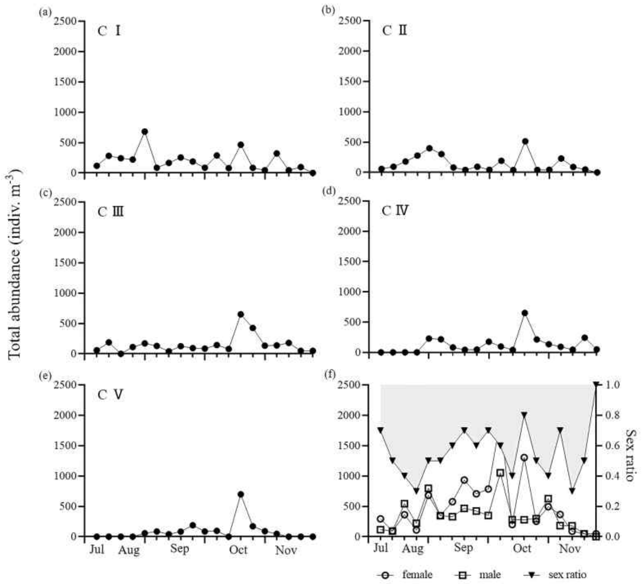
[Fig. 1] Location of the sampling site in Gamak Bay, Korea.

Live *A. erythraea* were collected in the field and transferred into a 4-L beaker containing surface water. Ten to thirty healthy females were immediately sorted using a pipette with a wide opening, and one female and one male were moved to each chamber of a 6-cell culture plate chamber (35 mm in diameter) filled with 15 mL of seawater, taking into account uncertainties as to when each female last mated. The low abundance of A. erythraea (one female, male) used minimized the effects of possible egg cannibalism. Empty eggs were included in the EPR.

Eggs with smooth and spiny surfaces were separated and counted using an inverted microscope (Zeiss Axioplan 2, Jena, Germany). To measure the hatching success (HS) rate according to egg morphology, smooth and spiny eggs were separately placed in a 6-cell culture plate filled with 10 mL of seawater pre-filtered through a GF/F glass fiber filter (Whatman Inc., Clifton, NJ, USA). Eggs were cultured using an incubator under the same conditions as adult females. HS was measured after 24 h and checked under the dissecting microscope at 12-h intervals for 48 h. The remaining eggs that did not hatch were observed for up to 30 days. The seawater was changed once every five days. Eggs that hatched within the time determined by the temperature function of Belehrádek's egg development time plus 24 h were defined as subitaneous eggs in this study. Eggs and females were stored for further size measurements and counting. Egg diameter and spine length were measured under an inverted microscope (Zeiss Axioplan 2, Jena, Germany)

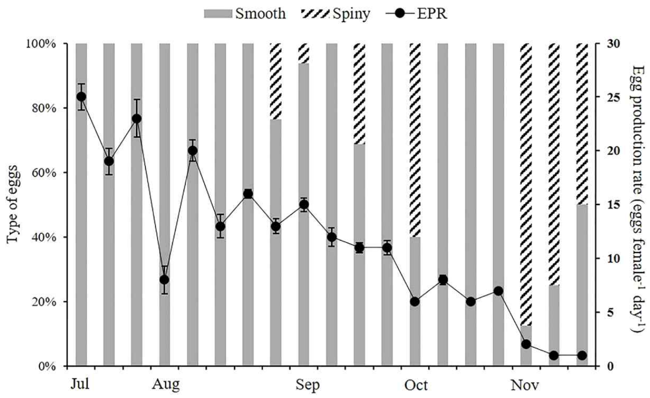
3. Results and Discussion

Copepodites and adult *A. erythraea* individuals were found in Gamak Bay throughout the sampling period. The total abundance ranged from 145 to 4,566 indiv. m^{-3} (mean: 1,562 indiv.m^{-3}), with the highest value observed on 7 October (Fig. 2). Copepodid I–II (CI and CII) stages gradually increased, with the highest numerical abundance in early August (Fig. 2a and b). However, the abundance remained low from August, with a second peak observed in late September to October. CIII–CV stages were maintained at a relatively low abundance until September, with the highest abundance observed in early October, following which the abundance gradually decreased (Fig. 2c-e). The abundance of males and females gradually increased from August and reached a peak in September and decreased to zero after late November (Fig. 2f). *A. erythraea* was collected at the sampling site until the end of December, but no adult females were identified.



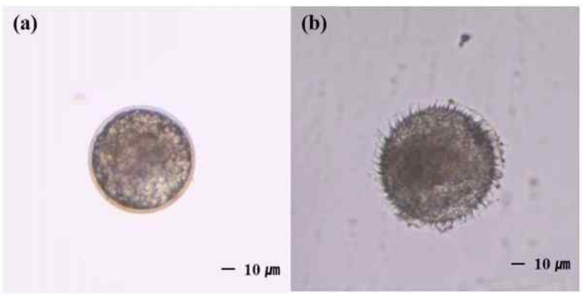
[Fig. 2] Weekly variations in stage-specific abundance and sex ratio (female to male ratio) in Gamak Bay, Korea during 19 July to 14 November, 2013.

The EPR of *A. erythraea* showed significant seasonal fluctuations and was characterized by three peaks. It fluctuated between 1.0 and 25.0 eggs $female^{-1}$ day^{-1} with a mean of 12.0 eggs $female^{-1}$ day^{-1}. EPR reached a maximum of 25.0 eggs $female^{-1}$ day^{-1} on 25 July, followed by a sharp decline on 13 August. The EPR increased sharply on 22 August and gradually decreased (Fig. 3). The number of smooth and spiny eggs produced by female *A. erythraea* is shown in Fig. 3. During the study period, *A. erythraea* continued to produce only smooth eggs until August. During the irradiation period, spiny eggs were observed seven times, starting on 3 September. However, spiny eggs were produced after 3 September, and 88% of the eggs were spiny by 5 November.



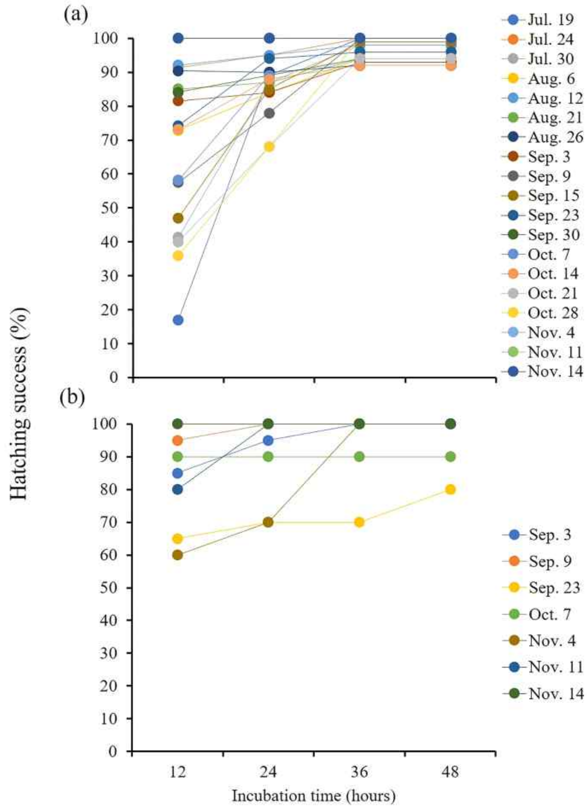
[Fig. 3] Weekly variations in egg production rate and egg type of *A. erythraea* in Gamak Bay, Korea from 19 July to 14 November 2013.

A. erythraea produced two types of eggs with smooth or spiny surfaces (Fig. 4). The diameters of smooth and spiny eggs, excluding spines, were significantly different, measuring 83.5 ± 0.5 μm (n = 20) and 92.4 ± 2.4 μm (n = 20), respectively (t-test, $p < 0.01$). The average length of spines was 5.8 ± 1.5 μm (n = 10). More than 80% of both smooth and spiny eggs hatched within 48 h (Fig. 5). On 19 July, the cumulative HS of smooth eggs showed extreme fluctuations (from 17 to 100%). All eggs that did not hatch within 48 h died within 30 days.



[Fig. 4] Light microscope images of eggs spawned by *A. erythraea* in Gamak Bay, Korea from 19 July to 14 November 2013. (a) Smooth egg and (b) spiny egg.

In the temperate coastal ecosystems, calanoid copepods produce different types of diapause or dormant eggs depending on the species, and the existence of dormant eggs varies depending on the habitat. In particular, calanoids of the genus *Acartia* occurring widely in coastal waters worldwide have displayed various spawning patterns [6]. *A. erythraea* produces two morphologically distinct eggs (subitaneous and diapause) [4]. In the Seto Inland sea, *A. erythraea* produced dormant eggs that were 90 μm in size with 6-μm long spines on the surface as a strategy for protection during winter [4].



[Fig. 5] Temporal variation of hatching success of eggs spawned by *A. erythraea* in Korea from 19 July to 14 November 2013. (a) Smooth egg and (b) spiny egg.

In the present study, *A. erythraea* in Gamak Bay produced two types of eggs with smooth or spiny surfaces. Both types of eggs were classified as subitaneous eggs because over 80% hatched within 12 to 48 h (total number of eggs: 1049; number of non-viable eggs: 83). The smooth and spiny eggs had diameters of 83.5 ± 0.5 μm (n=20) and 92.4 ± 2.4 μm (n=20), respectively, similar to the values reported in a previous study. In general, subitaneous and diapause eggs show distinct differences in morphological features, such as surface structure and spine length. However, there are many difficulties in distinguishing between subitaneous and diapause eggs depending on egg surface structure. Spiny eggs, spawned by *A.* cf. *erythraea* appearing in Tioman, Malaysia, were also classified as subitaneous eggs [7]. Smooth and spiny eggs produced by *Centropages hamatus* from the mouth of the Limfjord area in Denmark were also classified as subitaneous eggs [8]. Furthermore, *A. clausi* diapause eggs are smooth [4]. Our study shows that it is possible to incorrectly classify eggs as subitaneous or diapause based soley on the presence or absence of spines on the egg surface. Therefore, many researchers classify the eggs into subitaneous and diapause eggs based on the egg hatching time asserted that it was diffcult to distinguish the surface structure of eggs and therefore, they should be classified into subitaneous and diapause eggs depending on the length of their refractory phase. The female and egg forms of *A. erythraea* appearing in the Inland Sea of Japan are similar to those in Gamak Bay, but the hatching was different for the egg types. It needs to be established whether the two are the same species through genetic analysis of populations in the two regions, although the hatching patterns might differ by locality.

A. erythraea exists only in the water column in summer and autumn in Gamak Bay [9]. Therefore, low water temperatures are considered unfavorable, and *A. erythraea* have been recorded as absent in the water column in spring and winter [10]. Diapause eggs can be produced to avoid low water temperatures [11]. Diapause egg production is the key to linking the current population to subsequent generations. In the present study, two types of eggs hatched within 48 h, whereas eggs that did not hatch died within 30 days. Moreover, diapause eggs with a refractory phase were not identified, and the investigation was conducted until the end of December, at which time adults of *A. erythraea* were not found. In the future, it may be necessary to check for the presence of diapause eggs in the sediment at the bottom of Gamak Bay. It is quite possible that when the population disappears from the water column from winter to spring in Gamak Bay, connecting the temporarily separated populations each year can be achieved by producing diapause eggs with a long refractory phase.

In conclusion, water temperature significantly influences the seasonal fluctuation of the EPR of *A. erythraea*, and hatching patterns according to egg morphology differences in contradiction to the findings of previous studies. Physiologically, the simultaneous production of two different egg types by *A. erythraea* may be one of the most important strategies to promote the survival of their off-spring in coastal waters, where environmental conditions fluctuate markedly.

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