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Effects of Temperature and pH on the Egg Production and Hatching Success of a Common Korean Copepod

Eun Hye Lee [1], Seo Yeol Choi [1], Min Ho Seo [2], Seok Ju Lee [3] and Ho Young Soh [1,4,*]

1 Department of Environmental Oceanography, Chonnam National University, Daehak-ro 50, Yeosu 59626, Korea; eun30605817@gmail.com (E.H.L.); seoyeol84@gmail.com (S.Y.C.)
2 Marine Ecology Research Center, Manseong-ro 19, Yeosu 59697, Korea; copepod79@gmail.com
3 Marine Biological Resource Center, Manseong-ro 19, Yeosu 59697, Korea; mbrc-sj@naver.com
4 Department of Ocean Integrated Sciences, Chonnam National University, Daehak-ro 50, Yeosu 59626, Korea
* Correspondence: hysoh@chonnam.ac.kr; Tel.: +82-61-659-7147

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Abstract: The recent accelerated ocean acidification and global warming caused by increased atmospheric carbon dioxide may have an impact on the physiology and ecology of marine animals. This study was conducted to determine the egg production rate (EPR) and hatching success (EHS) of *Acartia ohtsukai* in response to the combined effects of an increase in temperature and a lower pH. *Acartia ohtsukai* with fresh surface seawater were collected in the northwestern Yeoja Bay of Korea in September 2017. The temperature and pH conditions applied included two different pH levels (representing the present: 7.9 and the future: 7.6) and three temperature values (26 °C, 28 °C, and 30 °C). In the pH 7.9, EPR significantly increased with increased temperature, but in pH 7.6, it significantly decreased as the temperature increased. EHS was lower in pH 7.6 than in pH 7.9. These results suggest that changes in the marine environment due to global warming and ocean acidification may affect *Acartia* populations and cause overall fluctuations in copepods of the genus *Acartia*.

Keywords: ocean acidification; global warming; egg production rate; egg hatching success; *Acartia ohtsukai*

1. Introduction

The atmospheric CO_2 concentration has increased rapidly, owing to human activities since the Industrial Revolution. To date, global warming has been a key topic of climate change. However, recently, there has been growing interest in a phenomenon known as ocean acidification (OA). The IPCC (Intergovernmental Panel on Climate Change) predicts that the average sea level temperature will rise by 0.6 °C (RCP 2.6 scenario) and 2.0 °C (RCP 8.5 scenario) during this century. Also, the atmospheric pCO_2 is expected to rise continuously to 670–936 µatm in 2100, reducing the pH of the seawater surface by 0.2–0.3 (RCP 6.0 and RCP 8.5) [1]. These changes may affect the behavior and physiology of marine animals [2,3].

It is predicted that the growth, survival, egg production, and hatching success rate of copepods (which play a pivotal role in marine planktonic food webs) will be affected by global warming and ocean acidification [4]. Additionally, decreased carbonate production is known to reduce the survival and growth rates of marine invertebrates such as shellfish and corals [5–7]. Fish are known to change metabolic enzymes, acid-base controls, and antioxidant enzyme activity in the body and exhibit decreased growth rates in lower pH conditions [8,9]. Copepod populations are regulated by biological factors (e.g., egg production, survival, growth, feeding, predation, etc.) in addition to physical environmental factors [10–13]. Among the biological factors that affect copepod populations, the egg production rate (EPR) is highly important and used for assessing the population inclusion rate in the ocean [14,15]. Another important factor is the egg hatching success (EHS). This parameter is used to identify the actual population because all eggs produced may not hatch [16]. However, the environmental factors that control these biological factors vary widely, making it very difficult to distinguish independent effects [17]. Therefore, egg production and hatching rate need to be evaluated concurrently if we are to assess the effects of various environmental factors on copepod populations.

In particular, the genus *Acartia* is composed of 65 species, with a predominant distribution in coastal and brackish waters worldwide [18]. *Acartia* species can differ in their physiological responses to environmental changes [4,19]. Many physiological studies have been conducted on *Acartia* species to understand the influence of individual factors such as temperature, salinity [19,20], pH [21], photoperiod [22], and feeding conditions [23]. However, recent studies have focused on the combined effects of environmental factors to obtain a greater understanding of their population dynamics. Vehmaa et al. [24] showed that the egg production of *Acartia* sp. increased when the water temperature was increased (with in situ pH levels), whereas it decreased when the water temperature was increased and the pH was decreased. Zervoudaki et al. [25] indicated that the egg production and hatching rate of *A. clausi* were not affected by increased water temperature under in situ pH conditions, but the increased water temperature combined with decreased pH had a negative effect on its egg production and hatching rate. Further studies are needed if we are to achieve a broader understanding of the physiological characteristics of the genus *Acartia*.

Acartia ohtsukai appears predominantly in high water temperatures in the coastal and estuary of Korea and is known to have high adaptability to a wide range of salinity changes [26–28]. Given the differences in physiological responses among *Acartia* species, the results of this study include the first information on the effect of changes in pH and water temperature on egg production and hatching rates. The purpose of this study was to understand the combined effects of pH and temperature on the EPR and EHS of the predominant copepod (*A. ohtsukai*) of the high temperature areas of Korean coastal waters.

2. Materials and Methods

Sampling was conducted at a station in the northwestern Yeoja Bay (34°48′55″ N 127°24′40″ E), located in the central area southern coast of Korea, in September 2017 (Figure 1). The depth of this station is about 3 m at high tide, and it receives suspended solids due to the inflow of nearby fresh water. The environmental factors (water temperature, salinity, pH) in the sampling site were measured at a depth of 1 m using a YSI multimeter (Model 600QS, YSI Inc., OH, USA). For the determination of chl-*a*, 0.5 L of surface water was filtered with a GF/F filter (Whatman, Maidstone, UK; pore size, 0.7 μm) and stored at −20 °C for further analysis in the laboratory. Later, the filters were extracted by 90% acetone and fluorometrically determined [29]. These results are shown in the following table (Table 1). *A. ohtsukai* were collected vertically from bottom to surface (3 m) using a conical plankton net (diameter, 45 cm; mesh size, 200 μm) and stored in an incubator set at the station temperature The samples were transported to the laboratory within 1 h. The seawater used for cultivation was filtered using a GF/C filter (Whatman; pore size, 1.2 μm) and then left to stabilize for 24 h before initiating the experiment.

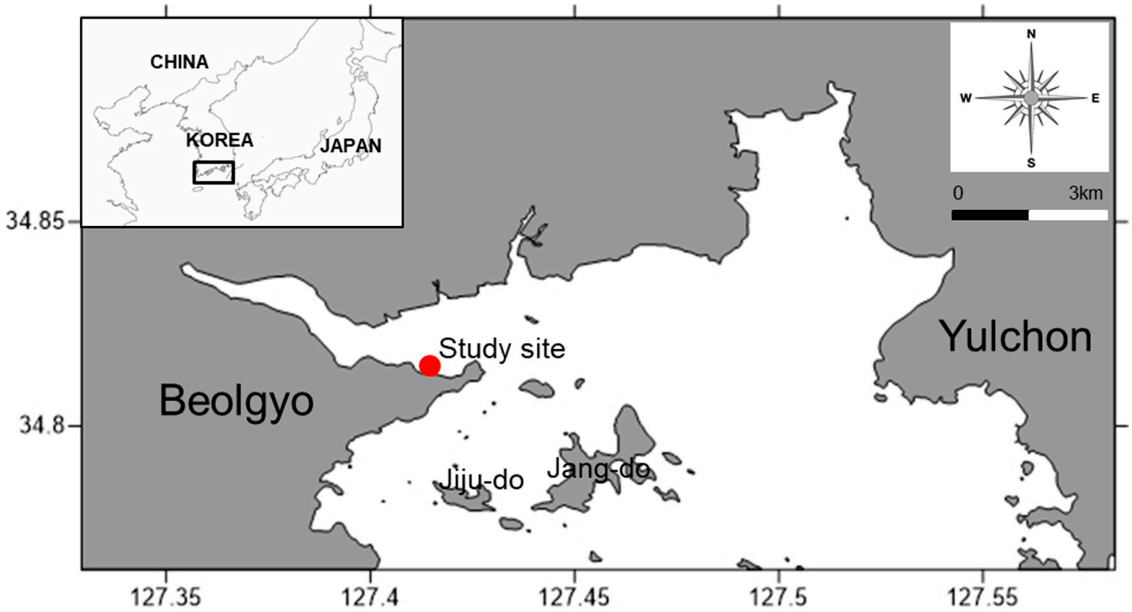

Figure 1. Map showing the study site located in the northwestern Yeoja Bay of Korea.

Table 1. The in situ conditions of this study area.

	Temperature (°C)	Salinity	pH	Chl-*a* (μgL^{-1})
In this study area	28.08	27.02	7.92	3.02

In our experiment, the copepods were exposed to different environments: 1) pH levels: 7.9 and 7.6; 2) temperatures: 26 °C, 28 °C, and 30 °C. The pH 7.9 remained consistent with that of the field-sampling site. For the pH 7.6, seawater filtered through a GF/C filter was placed in a 5-L glass bottle, and the CO_2 concentration of the seawater was controlled by bubbling the water with air containing CO_2. The flow rate of CO_2 were adjusted using a flow meter (M11.001, DAIHAN Scientific Co., Wonju, Korea). Filtered seawater was aerated with 0.5 mL min^{-1} CO_2. The CO_2 concentrations of the gas were 1000 ppm. After bubbling, the bottle was closed without head space to minimize CO_2 outgassing. After 1 h, pH was confirmed using a pH meter (WM 32EP, DDK TOA CO, Tokyo, Japan). Filtered seawater dissolved in CO_2 was carefully placed in a six-well cell culture plates.

Healthy adult females and males of *A. ohtsukai* were collected under a dissecting microscope (Nikon SMZ645, Nikon, Tokyo, Japan) and then acclimated in 2-L glass beakers filled with filtered seawater using GF/C filter for 24 h. To determine the EPR, pairs of healthy adult *A. ohtsukai* (a male and a female in each pair) were placed on six-well cell culture plates already adjusted to the pH treatment requirements. A total of 18 pairs of *Acartia* were used to measure egg production per treatment. After placing copepods, the evaporation and exchange of CO_2 were minimized with parafilm. The plates were then incubated in a Multi-Room incubator (WIM-RL4, DAIHAN Scientific Co., Wonju, Korea) according to the required temperature for each treatment. Three duplicates were undertaken for each treatment (Figure 2). During the experiment, the pH of the seawater was measured daily using a pH meter. The food provided to copepods was collected using a conical plankton net (diameter, 45 cm; mesh size, 20 μm) as natural food (2×10^3 cells mL^{-1}). The food was supplied once at the beginning of the experiment using a pipette. The EPR was measured after 24 h using a dissecting microscope (Nikon SMZ645). The eggs produced were transferred to the new six-well cell culture plates under the same pH, temperature, and food concentration conditions to minimize egg cannibalism by adult copepods during experiment. The EHS was measured counting nauplii every day for 5 days.

To understand the relationships among the EPR, EHS, and environmental factors (temperature and pH), we performed two-way ANOVA with SPSS 12.0 software (SPSS Inc., Chicago, IL, USA).

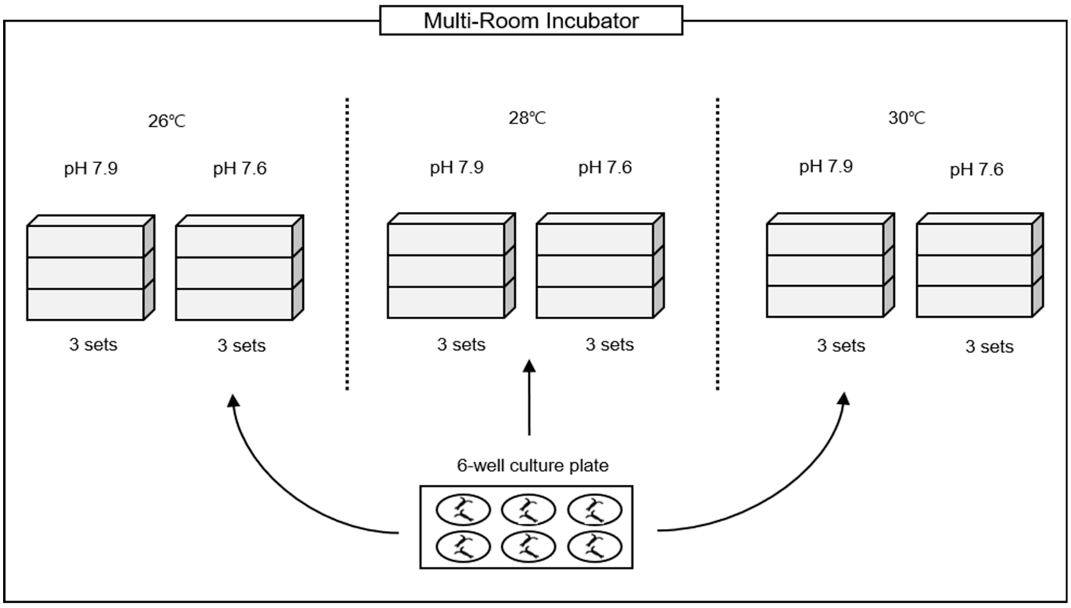

Figure 2. Illustration of the egg production rate and hatching success experiment.

3. Results

3.1. Egg Production Rate (EPR)

The mean EPR of the pH 7.9 and pH 7.6 groups was 9.60 and 5.60 eggs f^{-1} d^{-1}, respectively. In more detail, at 26 °C, the mean EPR was 8.94 ± 6.13 eggs f^{-1} d^{-1} for pH 7.9 and 6.44 ± 7.15 eggs f^{-1} d^{-1} for pH 7.6. At 28 °C, the mean EPR was 9.44 ± 8.10 eggs f^{-1} d^{-1} for pH 7.9 and 5.67 ± 5.46 eggs f^{-1} d^{-1} for pH 7.6. At 30 °C, the mean EPR was 10.28 ± 8.29 eggs f^{-1} d^{-1} for pH 7.9 and 4.56 ± 3.99 eggs f^{-1} d^{-1} for pH 7.6 (Figure 3, Table 2). EPR significantly differed between pH 7.9 and 7.6 ($F = 9.646$, $p = 0.0025$). However, EPR did not differ among each temperature ($F = 0.016$, $p = 0.9846$). There was no significant difference for the interaction of pH and temperature ($F = 0.5291$, $p = 0.5907$).

Table 2. Number of eggs per *Acartia ohtsukai* female in water temperature and pH conditions.

	pH 7.9			pH 7.6		
Cell Number	**26 °C**	**28 °C**	**30 °C**	**26 °C**	**28 °C**	**30 °C**
1–1	12	3	17	0	4	1
1–2	7	1	10	17	5	3
1–3	6	5	9	18	4	10
1–4	7	9	6	9	13	5
1–5	2	7	14	2	1	5
1–6	0	10	5	18	0	6
2–1	9	20	12	0	6	9
2–2	21	0	9	16	7	0
2–3	5	0	0	7	8	7
2–4	13	22	4	2	2	2
2–5	16	3	13	0	0	7
2–6	15	4	8	1	0	7
3–1	3	1	6	7	12	4
3–2	9	24	13	0	18	0
3–3	20	13	0	3	14	0
3–4	2	16	11	1	6	14
3–5	5	20	10	15	2	0
3–6	9	12	38	0	0	2
Total eggs	161	170	185	116	102	82
SD (±)	6.13	8.10	8.29	7.15	5.46	3.99
Average	8.94	9.44	10.28	6.44	5.67	4.56

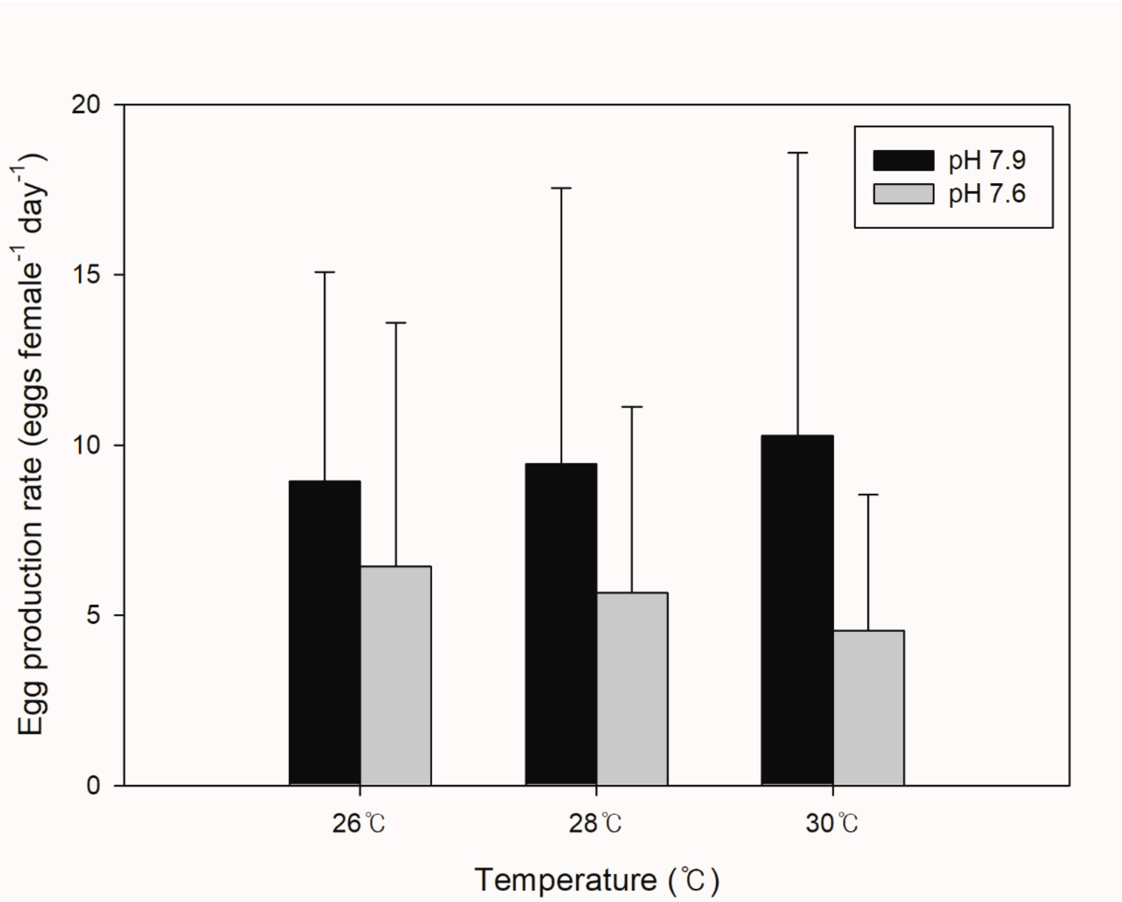

Figure 3. Egg production rate of the adult female copepod *Acartia ohtsukai* when maintained under two different pH and three different temperature conditions.

3.2. Egg Hatching Success (EHS)

The overall mean EHS was 74.4% for pH 7.9, whereas the pH 7.6 mean was 50.50%. When the temperature was maintained at 26 °C, the mean EHS was 70.50 ± 2.97% for pH 7.9 and 46.50 ± 2.89% for pH 7.6 (Figure 4). When the eggs were maintained at 28 °C, the mean EHS was 74.30 ± 5.35% for pH 7.9 and 51.20 ± 2.47% for pH 7.6. The mean EHS at 30 °C was 78.40 ± 4.95% for pH 7.9 and 54 ± 3.74% for the pH 7.6. EHS significantly differed between the pH 7.9 and 7.6 ($F = 122$, $p < 0.0001$). Also, EHS differed among the each temperature ($F = 4.203$, $p = 0.0318$). However, EHS did not differ for interaction of pH and temperature ($F = 0.332$, $p = 0.9674$). EHS by day was as follows (Figure 5): At pH 7.9, EHS increased until 3 days after the start of the hatching rate observation, and there was no variability after 3 days. At pH 7.6, EHS was more variable over time. There were statistically significant differences in EHS among different days ($F = 3.529$, $p = 0.0246$).

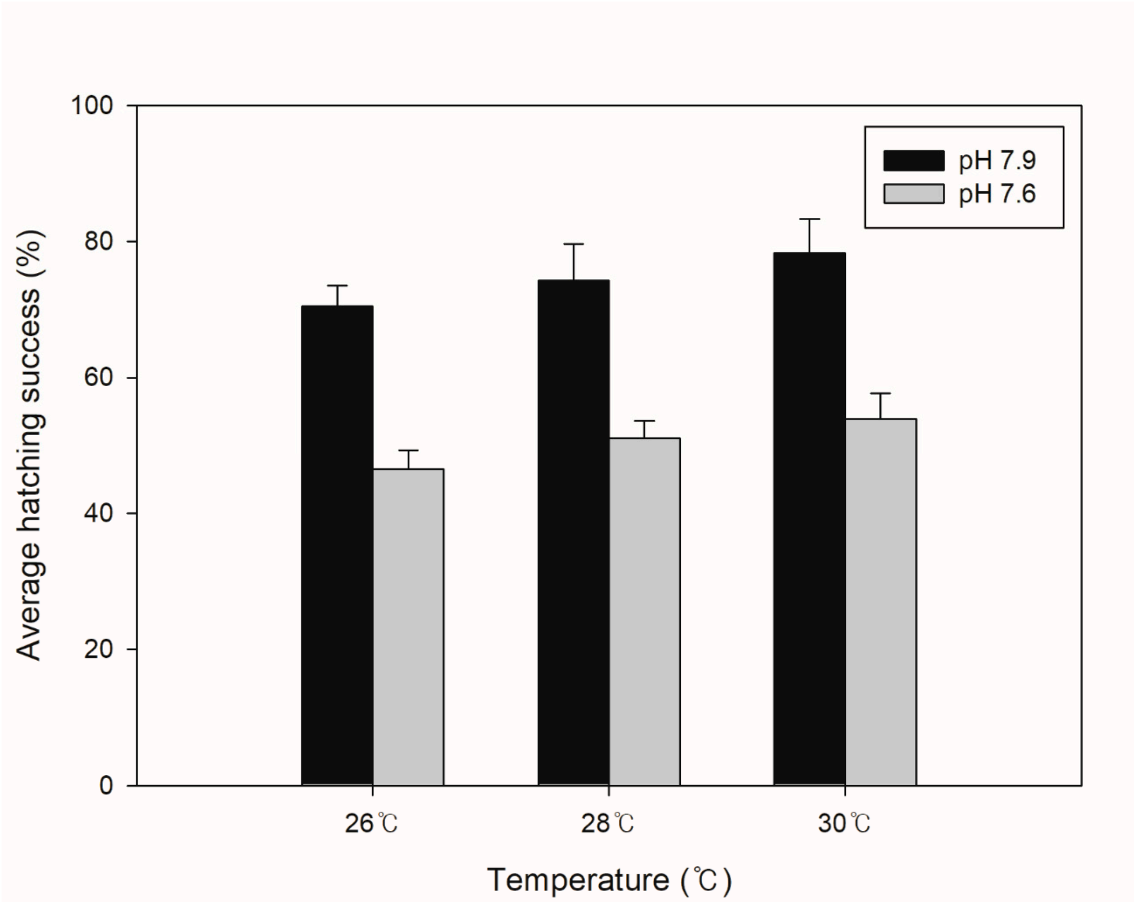

Figure 4. Hatching success of *Acartia ohtsukai* eggs when maintained under two different pH and three different temperature conditions.

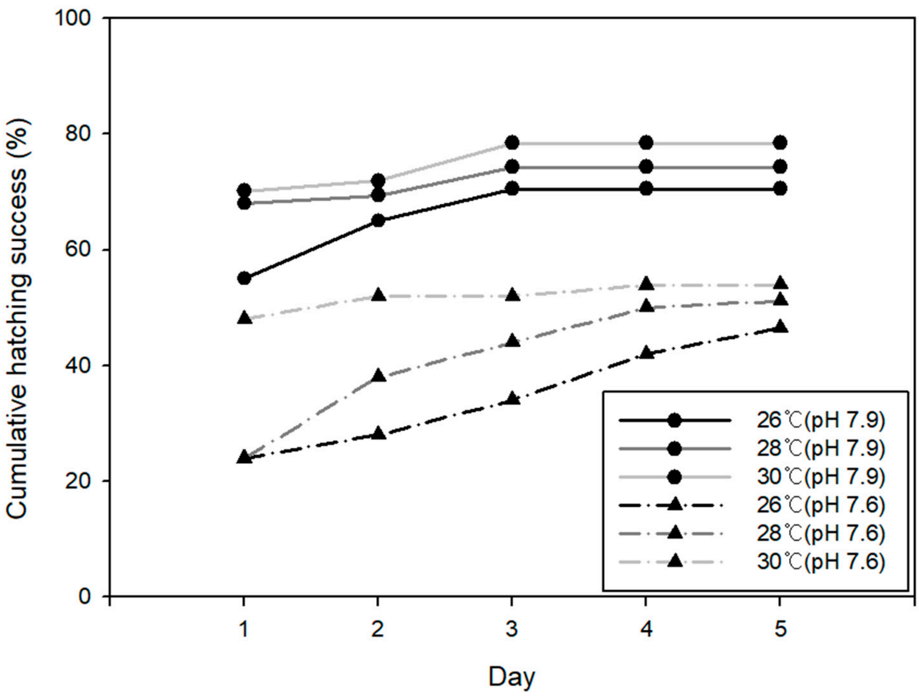

Figure 5. Hatching success of *Acartia ohtsukai* eggs over time when maintained under two different pH and three different temperature conditions.

4. Discussion

Female copepods show variations in reproductive capacity depending on environmental conditions, such as food quality and quantity, population density, etc. [12,30–32]. The above factors also determine the health and size of the produced eggs and nauplii [10,17]. In particular, water temperature and pH are important factors that change the egg production and hatching rate of copepods. The EPR and EHS of *Acartia clausi* significantly decreased with enhanced pCO_2 and temperature [25]. The EPR of *Calanus sinicus* was not affected by increasing temperature and pCO_2, but the EHS decreased significantly [33].

Acartia species are reported to produce 0.4–60 eggs f^{-1} d^{-1} [14,34–37]. The mean EPR of *A. ohtsukai* was 5.60 eggs f^{-1} d^{-1} in pH 7.6, which was approximately two-times lower than that in pH 7.9 (Figure 3). Kurihara et al. [4] also demonstrated that the EPR of *A. erythraea* was not affected by decreased pH, but the EPR of *A. steueri* decreased with decreased pH. The EPR of *A. pacifica* decreased at a pH of 6.90–8.17 [38]. The EPR of *A. bifilosa* increased with decreased pH with no effect on EHS [21]. It is evident that the EPR differs among *Acartia* species in response to combined temperature and pH variations. In addition, the physiological responses (egg production, hatching rate, survival, and mortality) of copepods are influenced by various factors, such as water temperature, salinity, food quantity and quality, and photoperiod [12,22,32,39–41]. It is difficult to estimate the effects of a single environmental factor on the EPR or EHS of copepods [17]. It is also not easy to directly compare the EPR between species because the environmental factors were applied differently in each study [12]. We may not be able to directly compare our results with those of previous studies, owing to the variations in the species and environmental factors, especially female stress responses. Our results show that the EPR of *A. ohtsukai* decreased approximately two-fold with the combined effects of increased temperature and lowered pH. This result is significant when considering the acidification (by increased carbon dioxide) of coastal areas due to global warming.

In this study, the EHS of *A. ohtsukai* in pH 7.6 was 23.90% lower than the EHS of those in pH 7.9. The lower EHS obtained in pH 7.6 is a similar result to that obtained for other copepods, especially within the *Acartia* species (*A. clausi*, *A. steueri*, and *A. erythraea*) [4,25,42,43]. In general, female copepods are affected by the physiological stress associated with changing environmental conditions. Adverse environmental conditions have a significant effect on egg health and viability, thereby affecting the population variation of copepods [10,17]. It has also been observed that copepods in temperate coasts produce resting eggs as a strategy to survive and maintain populations in unstable environmental conditions [44,45]. In general, dormant eggs of calanoid copepods are known to have spines on the surface of the eggs [46,47]. The genus *Acartia* shows morphological differentiation between the subitaneous and resting eggs. The subitaneous eggs are often smooth on the surface, whereas the diapause eggs have spines [47–50]. In this study, we found many variable types (smooth or spiny) in the eggs when viewed using a dissecting microscope. However, all egg types hatched. Recently, Nakajima et al. [51] suggested that eggs with spines are subitaneous eggs. In our experiment, it was difficult to consider the eggs that had not hatched as resting eggs because resting egg morphology has not been comprehensively assessed in *A. ohtsukai*.

There have been many studies on the reproduction of copepods in response to environmental conditions, with the methodology proving to be quite controversial. Breitburg et al. [52] suggested that to understand the response of marine organisms to global warming and ocean acidification, we need to be comprehensive and consider several factors rather than a single factor in isolation. Additionally, in many studies, the results of testing only a single adult female (and a single life cycle) grossly underestimated the effect of ocean acidification on copepods [53]. To survive in a changing environment, zooplankton are known to adapt through phenotypic plasticity, with the ability to change the physiological state or behavior of individuals and populations in response to environmental variation [54]. It has been suggested that copepods can survive, grow, and reproduce at low pH levels, owing to their high buffering capacity against the acidification of the sea, which is expected in 2100 [43,55–57]. Kurihara and Ishimatsu [55] showed significant differences in egg production after single-generation exposure and egg production after multiple-generation exposure. Cripps et al. [58] emphasized that the exposure of females and males before mating and the exposure of females and eggs, respectively, showed different results in egg production and hatch rate, and concluded that research should focus more on the exposure of the parent generation. Vehmaa et al. [59] deduced that exposure to the 2100-year pH level of the parent generation did not negatively affect egg production and hatching rate. Thus, it would not have a negative effect on the next generation. Vehmaa et al. [59] also inferred that the antioxidant defense capabilities of the female could protect the eggs from the oxidative stress caused by pH. Based on these findings, research on the EPR and EHS of *A. ohtsukai* has not been conducted, and a direct comparison with our research is impossible. Therefore, to more accurately identify the EPR and EHS of *A. ohtsukai*, it is necessary to understand the variability in the EPR and EHS in the ocean and to compare the EPR and EHS through multiple generations within the laboratory.

5. Conclusions

If ocean water temperatures rise owing to global warming and the current pH conditions are maintained, the EPR and EHS of *Acartia ohtsukai* might increase. However, if ocean acidification occurs in conjunction with global warming, this change might reduce *A. ohtsukai*'s EPR and EHS. These results suggest that ocean acidification caused by global warming may have a negative effect on the populations of *A. ohtsukai*.

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