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Investigating the Link between Microalgal Nutrition and the Environment in Hen Clam (*Mactra chinensis*) Larvae Growth and Survival

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Abstract: Historically, various types of shellfish inhabited the Jeju area to the extent that large mounds of shells were found. However, the most endangered of such species, *Mactra chinensis,* currently inhabits only a few villages on Jeju Island. This pioneering study aimed to explore the effect of the environment on *M. chinensis* larval growth on Jeju Island. First, we assume that the findings of this study are based on the specific environmental situations and conditions of Jeju Island. This study mostly explored the effects of environment and food diversity (microalgal species) on the growth and survival of *M. chinensis* (hen clam) larvae. The average seawater temperature ranges between 19 and 22 °C during the summer season. We tested analogous seawater temperature conditions specific to the Jeju coastal environment: 17, 22, 27 and 32 °C. *M. chinensis* larvae reared at higher temperatures experienced higher growth but lower survival rates, reaching shell lengths of >220 µm at 15 days after hatching. The larvae exhibited the lowest growth (149.3 µm shell length) at 17 °C. Moreover, the *M. chinensis* larvae had the highest daily growth and survival rate of 10.3 µm and 75.5%, respectively, at a salinity of 30 practical salinity units (psu). For breeding Jeju hen clams, we found that the optimal water temperature and salinity for larval rearing were 22 °C and 30–35 psu, respectively. Larvae fed only *I. galbana* or *Isochrysis* sp. also exhibited high growth and survival rates. However, larvae restricted to a diet containing only *P. lutheri* exhibited low growth and survival rates. Therefore, the optimal diet of *M. chinensis* larvae should include more than one species of microalga, particularly *I. galbana* and *Isochrysis* sp., to increase their growth and survival rates, and hence the productivity of this clam species.

Keywords: hen clam; *Mactra chinensis*; larva; growth; microalga; survival rate

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1. Introduction

Hen clam (*Mactra chinensis*) is a clam species widely found in the soft sandy bottom of the upper subtidal and intertidal zones of the coastal areas of Russia, Korea, Taiwan and Japan [1]. According to the results of a cytochrome coxidase I (COI) DNA investigation, hen clams living in the Asia-Pacific region can be categorized into three types of species [2]. In Korea, they are abundant in the low and mid-intertidal zones of the west and south coasts, within a depth range of approximately 10 m; around Jeju Island, they are only known to be found off the coast of Gwakjiri, a village in the town of Aewol (Eup).

The hen clam is the main source of income for the villagers in Gwakjiri, and more than 300 kg of hen clams is produced annually. This shellfish only inhabits the Gwakjiri village, but the catch has recently been decreasing since the average water temperature in the coastal waters of Jeju Island has risen by 1.5 °C over the past 40 years due to climate change [3]. However, there have been few studies on their habitats.

Hen clam (*M. chinensis*) is distributed across Korea, Taiwan, and Japan.

Shellfish species are generally referred to as bivalves, whose fertilized eggs develop through the stages of blastula, gastrula, and trochophore to form facets and shells, eventually becoming D-stage (veliger) larvae. Larvae of both adherent and non-adherent bivalve species develop through the free-floating stage until they either attach themselves to a substrate or settle into the sediment.

As the free-floating larvae are greatly affected by their surrounding environment, it is essential to monitor their breeding environment and diet. In particular, *M. chinensis* is a non-adherent bivalve with larvae that undergo a free-floating veliger stage; thus, it is necessary to carefully manage the surrounding environment during their early fertilization and larval stages for the efficient mass production of this clam species.

The key factors affecting the growth of bivalve larvae include water temperature, salinity, illuminance, and the supply of microalgae. Several studies have been conducted on the factors that affect the growth and survival of bivalve larvae, including the effects of water temperature and salinity [4,5], diet [6], quality of fertilized eggs [7], amount of feed [8], feeding behavior of larvae [9], and food selectivity [10]. In Korea, some research on the feeding and growth rates during the larval stage of certain bivalves [11] has been reported, and studies on clams [12], sea scallops [13], oysters [14], and silk scallops [15] have been recently conducted.

Studies have addressed the ecology [16] and reproduction [17] of *M. chinensis*; however, the environmental properties contributing to their survival and growth have not been systematically researched despite their contribution to the local economy. Therefore, we designed this study to investigate the effects of environmental conditions such as water temperature, salinity, and microalgal supply on the growth and survival rate of hen clam (*M. chinensis*) larvae.

2. Materials and Methods

2.1. Shellfish

Non-adhesive bivalves, such as *M. chinensis* and *Meretrix lusoria*, have a similar habitat environment; these bivalves live in sand or pearls, while exhibiting a floating behavior. In this study, the samples of *M. chinensis* were used because of their significance to the local economy of Jeju Island society. First, 100 hen clams with an average length of 82.2 ± 3.1 mm were purchased and used in the fishing village community in Gwakjiri, Jeju-si, Jeju Province, South Korea (Figure 1a,b). Before the main experiment, to prevent the natural spawning of the mother clams, they were placed in a four-ton flowing water system indoor water tank maintained at a water temperature of 17 ± 0.3 °C and a salinity of 31.0 ± 0.5 practical salinity units (psu) for 2 days. A sufficient amount of oxygen was supplied.

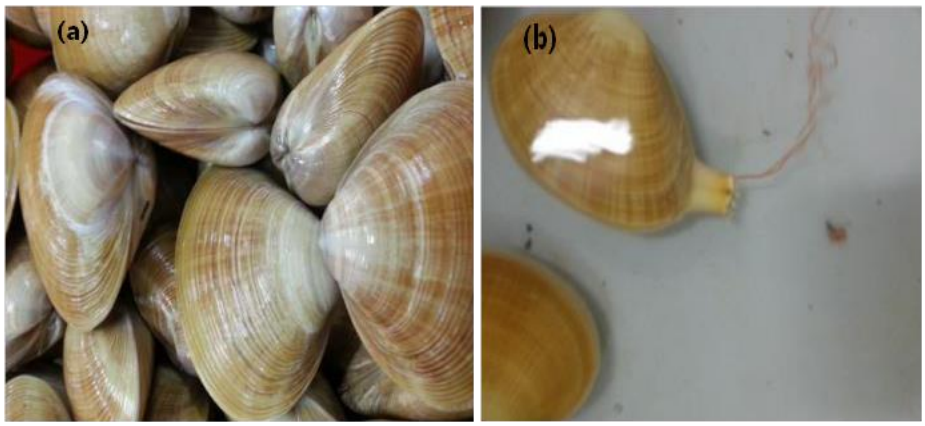

Figure 1. Female and male *Mactra chinensis* used in the experiment (**a**); and *M. chinensis* spawning (**b**).

All microalgae used in this study were purchased from the Korean Maritime Microalgae Culture Center (KMMCC). Female and male *M. chinensis* with a shell length of 82.2 ± 3.1 mm (n = 100) were used in the experiment (Figure 1a) and for spawning

(Figure 1b). On Jeju Island, the peak season for *M. chinensis* spawning is during May–September, with the main spawning occurring during June–July. When the gonads turn red in females and pale yellow in males, the clams reach maturity and can reproduce embryo clams after one year.

The microalgal species *Isochrysis galbana* (KMMCC H-2), *Pavlova lutheri* (KMMCC H-3) and *Isochrysis* sp. (KMMCC H-13), which were used as feed for the adult clams, were cultured in Conwy medium and maintained at 10–30 $\times$ 10^4 cells per 1 mL of breeding water.

2.2. Larval Production

To induce the production of eggs, female and male *M. chinensis* were stimulated by exposure to air (25 °C for 1 h) and heat shock (a 5 °C increase in the breeding water temperature). Male clams cannot be visually distinguished from females. Males were made to donate sperm first in the same tank, and then females spawned and fertilized them. Fertilized eggs were collected using a 30 µm filter and washed three or four times in filtered seawater. For the larval production experiment, the hatched D-type larvae were selected after accommodating the fertilized eggs in a 10 L circular plastic container for 24 h.

2.3. Analysis of Water Temperature, Larval Survival, and Growth Rate

To determine the optimal water temperature for survival and growth, D-stage larvae (75.2 $\pm$ 2.5 µm in size) were housed in 1 L beakers at a density of 20 individuals mL^{-1} at water temperatures of 17, 22, 27 and 32 °C. Each experimental group was housed in three beakers. Therefore, a total of 12 beakers (3 beakers $\times$ 4 experimental groups) were prepared for this water temperature experiment. The water temperature was adjusted using a heater and a cooler.

A feed mixture of *I. galbana*, *P. lutheri* and *Isochrysis* sp. at a density of 5000–15,000 cells per larva was added to the beakers twice a day. The ratio of food supply was 30% for *I. galbana*, 30% for *P. lutheri*, and 40% for *Isochrysis* sp. When culturing the organisms used as the hen clam food source, the temperature of the culture chamber was maintained at 20 °C, and the intensity of illumination in the lab was maintained at 3000 lux.

To maintain the water quality during the experiment, breeding water was prepared in advance, and the water in the beakers was changed every two days. *M. chinensis* larvae settled on the underwater floor after swimming in water for 15 days. Additionally, a substrate that sinks into the underwater floor was provided. Therefore, the larvae that submerged in the underwater floor were removed, and the survival rate was measured three times by counting the larvae floating in the water.

The clam survival and growth rates were recorded at the time of water change. The growth rate was measured using a light microscope (Nikon Profile Projector V-12B; Nikon, Minato City, Tokyo, Japan), and the survival rate was determined by counting the number of live individuals.

2.4. Analysis of Salinity, Larval Survival, and Growth Rate

To define the optimum salinity for clam larval survival and growth, D-stage larvae (76.1 $\pm$ 2.1 µm in size) were housed in 1 L beakers at a density of 20 individuals mL^{-1} at salinities of 25, 30, 35 and 40 psu. The control was set to 30 psu since the salinity of water in the Jeju area is approximately 30~33 psu. The salinity of the water used to rear the larvae was adjusted by mixing seawater, distilled water, and sea salt. Water temperature was maintained at 23 °C. Survival and growth rates were determined as described in Section 2.3.

2.5. Analysis of Feed Type, Larval Survival, and Growth Rate

To evaluate the optimal microalgal feed species, the D-stage larvae (80.6 $\pm$ 2.3 µm in size) were fed *I. galbana, P. lutheri or Isochrysis* sp. alone or a mixture of these three microalgal species. The survival and growth rates were tested and determined as described in Section 2.3.

2.6. Statistical Analysis

All experiments were conducted three times, and the results were statistically analyzed by one-way ANOVA using IBM® SPSS® Statistics 28 (SPSS Inc., Chicago, IL, USA). The mean significant difference in the data values was calculated using Duncan's multiple range test. Significant differences between experimental groups were determined at $p < 0.05$.

3. Results

3.1. Effect of Water Temperature on Survival and Growth Rates of M. chinensis Larvae

After three independent experiments with 30 larvae in each beaker, their sizes were noted. The growth rate of the larvae increased as the water temperature increased from 17 to 32 °C (Table 1). At water temperatures >22 °C, the larvae had grown by 220 μm on day 15 of rearing, but at 17 °C, the average shell length was only 149.3 ± 6.29 μm by day 15. On day 15 of the experiment, significant differences in shell size were found between larvae kept at 32 °C (312.7 ± 18.23 μm in size) and 27 °C (265.5 ± 14.55 μm in size), and between the larvae kept at 22 °C (222.6 ± 8.61 μm) and 17 °C (149.3 ± 6.29 μm) ($p < 0.05$). The daily growth rate at 32 °C (19.7 ± 2.31 μm) was significantly faster than that at 27 °C (12.6 ± 2.25 μm) and 17 °C (4.8 ± 0.23) ($p < 0.05$).

Table 1. The initial and final shell lengths, daily increment of shell length (SL), and the survival rate of *Mactra chinensis* reared at different water temperatures for 15 days.

		Shell Length (μm)		Daily Increment of SL (μm)	Survival (%)
		Initial	Final		
Temperature (°C)	17	75.2 ± 2.5	149.3 ± 6.29 ^{a}	4.8 ± 0.23 ^{a}	82.5 ^{d}
	22	75.2 ± 2.5	222.6 ± 8.61 ^{b}	9.8 ± 1.52 ^{b}	65.4 ^{c}
	27	75.2 ± 2.5	265.5 ± 14.55 ^{c}	12.6 ± 2.25 ^{b}	50.5 ^{b}
	32	75.2 ± 2.5	312.7 ± 18.23 ^{d}	19.7 ± 2.31 ^{c}	23.6 ^{a}

We intend a < b < c < d in shell length. Values in the same column with different superscript letters are significantly different ($p < 0.05$).

The survival rate of the hen clam larvae decreased as the water temperature increased (Table 1), and an 82.5% survival rate was recorded at 17 °C, which was significantly higher than that recorded at 22 °C (65.4%). The lowest survival rate (23.6%) was observed at 32 °C (Table 1). Figure 2 shows the different larval developmental stages.

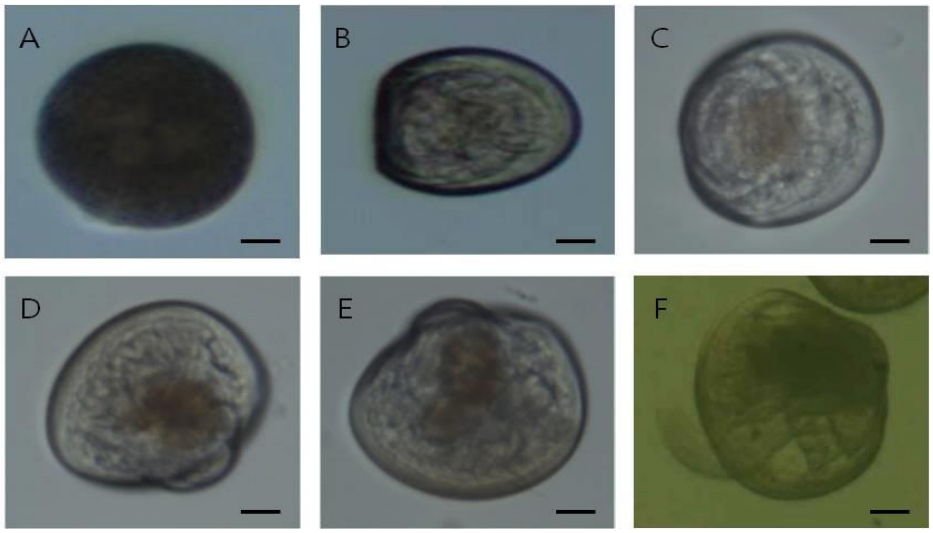

Figure 2. Developmental stages of *Mactra chinensis*—(**A**): fertilized egg (50–60 μm); (**B**): D-stage larva (70–100 μm shell length (SL)); (**C**): early umbonal stage (110–140 μm SL); (**D**): umbonal stage (150–170 μm SL); (**E**): late umbonal stage (180–220 μm SL); and (**F**): settled (metamorphosing) stage (300–350 μm SL). The scale bar indicates 50 μm.

3.2. Effect of Salinity on Survival and Growth Rates of M. chinensis Larvae

After three independent experiments with 30 larvae in each beaker, the growth rate in response to salinity was observed. Among all treatments, the growth rate of the clam larvae was the highest at a salinity of 30 psu (Table 2). On day 15, the larval growth was the fastest at 30 psu with a shell length (SL) of 231.6 ± 10.63 µm. Growth was slower at 25 psu with an SL of 162.4 ± 8.75 µm. Notably, the growth rate at 40 psu was the lowest, with an SL of 145.9 ± 6.65 µm. Daily growth was remarkably the fastest at 30 psu (10.3 ± 1.32 µm in SL) and slowest at 40 psu (4.6 ± 0.84 µm).

Table 2. The growth rate, daily increment of shell length SL and the survival rate of *Mactra chinensis* reared at different salinities for 15 days.

		Shell Length (µm)		Daily Increment of SL (µm)	Survival (%)
		Initial	Final		
Salinity (psu)	25	76.1 ± 2.1	162.4 ± 8.75 ^{b}	5.7 ± 0.65 ^{a}	45.5 ^{a}
	30	76.1 ± 2.1	231.6 ± 10.63 ^{d}	10.3 ± 1.32 ^{b}	75.5 ^{d}
	35	76.1 ± 2.1	224.3 ± 9.28 ^{c}	9.8 ± 1.06 ^{b}	70.4 ^{c}
	40	76.1 ± 2.1	145.9 ± 6.65 ^{a}	4.6 ± 0.84 ^{a}	52.5 ^{b}

We intend a < b < c < d in shell length. Values in the same column with different superscript letters are significantly different ($p < 0.05$).

The survival rate was 75.5% at 30 psu, which was significantly higher than 70.4% at 35 psu. The group at 25 psu had the lowest survival rate (45.5%), whereas the survival rate was 52.5% at 40 psu ($p < 0.05$; Table 2).

3.3. Effect of Diet on Survival and Growth Rates of M. chinensis Larvae

On day 15, the growth of *M. chinensis* larvae was the fastest (247.5 ± 13.2 µm) in the experimental group fed the mixture of microalgae (Table 3). The groups fed the single microalgal species *I. galbana* or *Isochrysis* sp. grew >220 µm in SL, but the group fed *P. lutheri* showed poor growth (<200 µm). The control group without feed supply showed the slowest growth rate (116.6 ± 1.4 µm) ($p < 0.05$). The daily growth was the fastest in the experimental group fed the microalgal mixture (11.1 ± 1.10 µm), but no significant difference in daily growth was found between this group and the group fed only *I. galbana*. The survival rate of the clam larvae was the highest (70.5%) in the group fed the microalgal mixture and was significantly higher than that of the groups supplied with *I. galbana* (62.4%) or *Isochrysis* sp. alone (60.6%). The group supplied with only *P. lutheri* had a survival rate of 45.8%, which was significantly lower than that of the other three groups. The control group that was not fed at all displayed the lowest survival rate of 4.0% ($p < 0.05$; Table 3).

Table 3. The growth rate, daily increment of shell length (SL), and the survival rate of *Mactra chinensis* provided with a different microalgal supply for 15 days.

	Shell Length (µm)		Daily Increment of SL (µm)	Survival (%)
	Initial	Final		
Isochrysis galbana	80.6 ± 2.3	234.1 ± 10.6 ^{cd}	10.2 ± 0.85 ^{d}	62.4 ^{c}
Pavlova lutheri	80.6 ± 2.3	192.8 ± 8.3 ^{b}	7.4 ± 0.51 ^{b}	45.8 ^{b}
Isochrysis sp.	80.6 ± 2.3	226.4 ± 11.3 ^{c}	9.7 ± 0.66 ^{c}	60.6 ^{c}
Mixture	80.6 ± 2.3	247.5 ± 13.2 ^{d}	11.1 ± 1.10 ^{d}	70.5 ^{d}
Not fed	80.6 ± 2.3	116.6 ± 1.4 ^{a}	2.4 ± 0.15 ^{a}	4.0 ^{a}

We intend a < b < c < d in shell length. Values in the same column with different superscript letters are significantly different ($p < 0.05$).

4. Discussion

This study showed that water temperature and diet were significant factors affecting the growth rate of *M. chinensis* during the larval stage. As there have been no studies on the growth or survival of *M. chinensis* larvae to date, the results of this study were compared to those of previous studies on *Meretrix lusoria* [18].

Accumulating evidence suggests that a suitable water temperature range for the optimal growth of *M. lusoria* larvae is 28–32 °C. In a previous study using the D-stage larvae of *M. lusoria* with an initial SL of 109.5 μm, the larvae grew to 209.0 μm over 20 days when reared at 23–28 °C [19]. Another study showed growth in the SL of D-stage larvae of 112–209.0 μm at a water temperature of 27 °C by day 20 of the experiment, whereas the daily growth rate was recorded to be 6.0 μm [20]. In the present study, the SL of *M. chinensis* larvae was 222.6 ± 8.61 μm at 22 °C on day 15 and gradually increased as the water temperature increased. However, at a water temperature above 27 °C, the survival rate dropped below 30%. Therefore, these findings indicate that the suitable range of water temperature to encourage the growth of *M. chinensis* larvae is 17–27 °C, with an optimal temperature of 22 °C. Therefore, the optimal temperature for *M. chinensis* growth is lower than that for *M. lusoria* growth. These results demonstrate that the optimal water temperature for breeding varies in a species-specific manner.

The *M. lusoria* larvae showed a high survival rate (80–90%) at a salinity of 10–15 psu and slower growth at lower salinities or those with >25 psu. In this study, the growth rate of *M. chinensis* larvae showed a tendency to decrease at salinities that deviated from 30 psu. In particular, the survival rate was <50% at 25 psu, suggesting that *M. chinensis* is less resilient at low salinity compared to *M. lusoria*. Therefore, the optimal salinity level for *M. chinensis* larvae is 30–35 psu.

Another important environmental factor for the successful breeding of bivalves is the type of microalgae in the feed. It is important to understand how food affects bivalve growth [21]. Microalgae suitable for bivalves mainly include *I. galbana*, *P. lutheri*, *Isochrysis* sp., *Chaetoceros gracilis* and *Chaetoceros calcitrans* [22,23]. In this study, *I. galbana*, *P. lutheri* and *Isochrysis* sp. were supplied either individually or as a mixture of all three to *M. chinensis* larvae. The highest growth and survival rates of *M. chinensis* larvae were achieved in the experimental group fed the combination of the three microalgae, followed by the experimental groups only fed *I. galbana* or *Isochrysis* sp.

A previous study indicated that mixed feeding of either *I. galbana* and *P. lutheri* or *Isochrysis* sp. and *Chaetoceros* sp. yields the best results for the growth of bivalve larvae [24]. Feeding with only a single species of microalgae may result in nutritional deficiency, leading to decreased growth and survival rates [24]. In this study, on day 15 of the experiment, the *M. chinensis* larvae showed the highest growth rate when fed a mixture of three types of microalgae, while the groups fed either *I. galbana* or *Isochrysis* sp. alone also showed growth in SL of >220 μm. The group fed *P. lutheri* alone showed poor viability and a growth rate of <200 μm. These findings are consistent with those of previous studies; however, our data also suggest that feeding the larvae *Isochrysis* sp. rather than *P. lutheri* yielded slightly better results in terms of the growth and viability of *M. chinensis*.

5. Conclusions

In recent years, there has been a decline in the population of *M. chinensis* in the Jeju area to the extent that they currently inhabit only a few villages on Jeju Island. However, research on the habitat and growth of shellfish found in Jeju is limited or not well known in the local society. This study aimed to provide important information on the effect of the environment on the growth and survival of *M. chinensis* on Jeju Island.

Overall, the findings of this study indicate that the optimal conditions for *Mactra chinensis* larval growth and survival are a water temperature of 22 °C, salinity of 30–35 psu, and a feed supply comprising a mixture of microalgal species, including *I. galbana* and *Isochrysis* sp., rather than a single nutrient source. In the future, more in-depth studies (e.g., to determine the macroalgal nutritional requirements of larvae) should be conducted to improve the productivity of *M. chinensis*.

Author Contributions: M.-S.J. provided direction to the research work and participated in the research. M.-S.J. conducted the literature review and collected the relevant data and M.-S.J. and C.-Y.H. wrote the manuscript. In addition, M.-S.J. searched and collected data on entire experiments; he searched and collected literature and evidence. Both M.-S.J. and C.-Y.H. revised the paper. All

authors collected and analyzed the literature referenced in this manuscript. All authors have read and agreed to the published version of the manuscript.

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