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Water temperature and salinity tolerance of embryos and spat of the mussel, *Musculista senhousia*

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ABSTRACT

The effects of water temperature and salinity on embryonic development and spat survival of mussel *Musculista senhousia* were investigated. Embryos were incubated in water ranging from 0 to 35℃ and with salinity from 5‰ to 40‰. Mussel spat were tested in water from 0 to 40℃ and with salinity from 0‰ to 100‰. The optimal conditions for mussel embryos were 20-25℃ and salinity from 25‰ to 35‰, based on Within this temperature range, higher temperatures correponded to a shorter duration of the embryonic period. Optimisation of mussel spat survival was at 25-35℃ and salinity from 30‰ to 40‰; both values are higher than those for embryo, which hinted *M. senhousia* embryos are more vulnerable than spat. Temperatures below 15℃ were lethal for embryos, making temperature a feasible method with which to control the large population of *M. senhousia* in ark-shell farm during its spawning period.

Keywords: water temperature; salinity; *Musculista senhousia*; embryo; spat

Introduction

As a bivalve that originates from northeast Asian seas, *Musculista senhousia* (Mytilidae family) is widely distributed in Australa, the western United States, and the Mediterranean Sea (Sgro, 2002). In recent years, *M. senhousia* has become a dominant harmful organism in the Korean aquaculture industry, especially in relation to ark-shell breeding.

Autecological studies of bivalves have clearly demonstrated that development, growth and survival are affected by physical parameters. In particular, temperature and salinity have been described as "master factors" for many marine organisms (Kinne, 1964). because adult bivalves possess protective shell valves that can isolate their bodies from the external environment by closing the shell after withdrawing the exposed mantle, adult individuals have relatively higher tolerance to temperature and salinity.

Generally, ontogenesis process can be devided into two important phases in a mussel life cycle (Conn *et al*., 1993): from fertilization until larval settlement, when individuals are pelagic and only protected by a soft shell, and after settlement, when individuals become benthic and develop a hard mytiliform shell. The earliest life stages are the most sensitive in bivalve, and as larvae develop into a benthic juveniles, their tolerance toward various environmental conditions increases (Bayne, 1976). Claudi (1993) also suggested that larval phase is the most vulnerable and may be susceptible to changes in the external environment. Therefore, one must determine the tolerance of mussel embryo at different developmental stages and of spat to two main environmental factors: water temperature and salinity. Our results could be used to control the invasion of mussels in the future.

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Materials and methods

Sexually mature *M. senhousia* broodstock (n=300; shell length: 20.4-25.3 mm body weight: 0.54-1.04 g)

were collected from ark-shell farm in Seonso, Yeosu city, during September 2008, before the start of the spawning season. Mussels were thoroughly scrubbed and rinsed to remove epifaunal organisms, and then maintained in a flow-through broodstock tanks in water colder than that measured in the field. Mussels were fed four times each week with live *Isochrysis galbana* at a concentration 3×10^5 cells/ml. Seawater was changed every 2 days and tanks were cleaned with freshwater once a week to remove possible algae and tubeworms. Dead individuals were removed from the tanks daily.

Active mussels were placed together in one spawning tank and induced to spawn using photochemical stimuli (Madrones-Ladja, 1997). Fertilized eggs were washed using UV light-irradiated seawater, collected in a 20 μm mesh sieve, transferred to a 2-L beaker filled UV light-irradiated seawater, and stirred. Using a 5 ml pipette, 1 ml subsamples were taken and counted via a hemacytometer. A portion of the fertilized eggs was used in the subsequent embryonic development experiment, while the rest were incubated in ambient filtered seawater (33‰) at a room temperature of approximate 20℃. These eggs were used to monitor the normal developmental process.

Experiment 1: Embryo water temperature and salinity tolerance

The experiment was carried out in triplicate at eight different constant water temperatures (0, 5, 10, 15, 20, 25, 30, or 35℃) and salinities (5, 10, 15, 20, 25, 30, 35, or 40‰). In the temperature test groups, salinity was kept at 33‰, that of fresh seawater; The water in the salinity test groups was 23 ± 1℃. Temperature and salinity were monitored daily throughout the experiment to ensure that they did not vary outside the prescribed ranges.

The constant water temperature was maintained using electric heating rod device, adjustable illumination incubator and ice bag. The adjustable illumination incubator can supply the temperature ranging from 4 to 30℃ and water temperature at 0℃ could be maintained by mixed with ice bags. Salinity of water was prepared by the addition of freshwater or brine solution to ambient seawater until the desired level was reached. Salinity was determined with a handle salinity meter precalibrated with distilled water. Fresh and seawater used in the experiment all were UV light-treated and changed half per day. Thirty 1-h-old embryos were added at random to each test solution at 1 embryo/5 ml in a 250 ml flask. The developmental process was examined every 15 min during the initial 24 hours. As to the remainder of the experiment, observations were conducted every 2 h until individuals reached the Umbo larval stage. The duration of and survival rate at each developmental stages (4-cell, 16-cell, Morula, D-shaped larvae, and Umbo larvae) were recorded. When Half of surviving embryos displayed the stage characteristics, all were assumed to have reached this stage.

Experiment 2: Spat water temperature and salinity tolerance

Musculista senhousia spat with a mean shell length of 15.4 ± 0.7 mm and total weight of 0.4 ± 0.1 g were used in these experiments. Spat were collected from an Samples were immediately transported to the laboratory and cultured in 50-L polythene containers. Until the experiment commenced, the surface water in those experimental containers. Until the Experiment commenced, the surface water in those experimental containers was aerated with an air pump and kept a constant temperature that was the same as the sampling site.

Mussel spat were tested under nine temperatures (0, 5, 10, 15, 20, 25, 30, 35, or 40℃) and 11 salinity levels (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100‰) in triplicate. Each 5-L polythene container with a fixed heating rod was inoculated with 30 spats and the test solution was provied. The duration of acute tests of temperature and salinity effects was 24 and 72 h, respectively.

Survival rates during the two experiments were recorded. Since recovery time reflects the degree of damage that individuals suffered during the stimulation (Laing I., 2002), mussels exposed to a temperature or salinity treatment were tranferred into fresh seawater from the collecting site and the

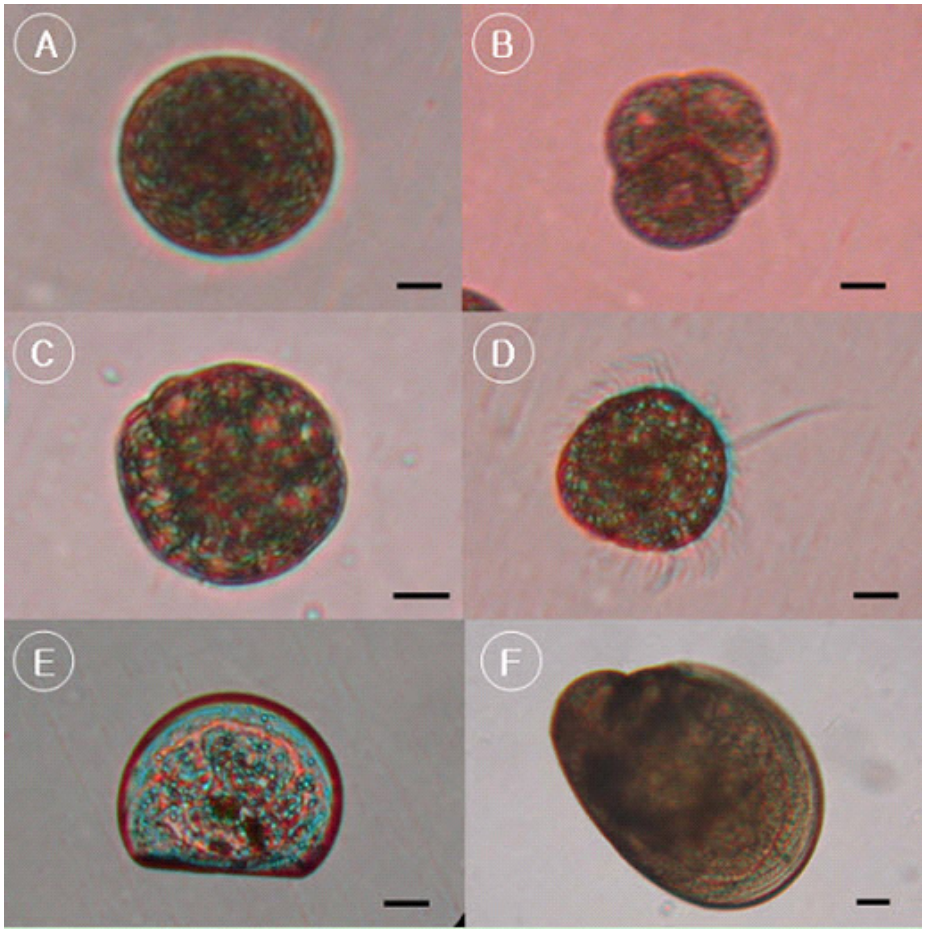

Fig. 1 Primary developmental stages of the mussel *Musculista senhousia*. A, Fertilized egg; B, 4-cell stage; C, Morula stage; D, trochophore stage; E, D-shape stage; F, Umbo stage. Scale bar: 10 μm.

recovery time was noted. The recovery status was defined as a small gap appeared between shells of the mussels as normal survival under laboratory conditions. Mussels were deemed to have died when they failed to gentle probing of the mantle and gills by either closing their valves or withdrawing the mantle.

Statistical analysis

The duration and survival data on embryo were analysed using two-way analysis of variance (ANOVAs); the survival rate of spat, were analyzed using one-way analyses and Tukey test. Because the data had a wide range, all results are presented as the mean±standard deviation.

Results

Normal fertilized eggs of *M. senhousia* were 51 ± 5 μm in diameter. The first cleavage occurred after about 1 hour under room temperature. within 3 h, embryos devided into four different-sizes cells 50 ± 5 μm in diameter. The development of viable fertilized eggs followed the normal spiral pattern of cleavage. According to our monitoring, embryos reached morula stage in nearly 11h; 4 or 5 h later, embryos reached the trochophore stage. Production of normal D-shape larvae from fertilized eggs was completed within 35 h. Umbo larvae (85 ± 10 μm) were found after nearly 10 days of incubation (Fig. 1).

The Experiment on *M. senhousia* embryos

Table. 1 The relationship between water temperature and time required to reach each developmental stages after egg fertilization

Developmental stages	Elapsed time after fertilization (hour)									
	Water temperature (℃)									
	0	5	10	15	20	25	30	35		
4-cell	–	9^{b}	6^{ab}	4^{a}	3^{a}	2.5^{a}	2.5^{a}	1^{a}	1	
16-cell	–	20^{bc}	13^{b}	10.5^{b}	7^{b}	5^{ab}	3.5^{a}	3^{a}	2	
Morula	–	35^{c}	24^{bc}	17^{bc}	12^{b}	8.5^{b}	8^{b}	7^{ab}	5	
D-shape larva	–	–	50^{d}	25^{cd}	16^{c}	14^{bc}	11^{bc}	9^{bc}	7	
Umbo larva	–	–	–	300^{e}	160^{e}	140^{e}	128^{e}	–	–	

Different superscripts on the bars within a figure are significantly different ($P < 0.05$).

The Relationship between water temperature and the duration of embryonic development from fertilization to umbo larval stage is shown in Table. 1.

The fertilized eggs incubated at 0℃, 5℃, 10℃, and 35℃ did not successfully develop into Umbo larvae by the end of the experiment. All embryos tested at 0℃ died within 30 min, which indicated that this water temperature is extremely deleterious to embryo development in this mussel. Fertilized eggs cultured at 5℃ ceased develapment at the morula stage; the egg cultured under 10℃ and 35℃ ceased development at the D-shape larvae stage. At water temperature ranging from 15℃ to 30℃, the duration of developmental stages was inversely related to water temperature. No significant differences were detected in the relationship between water temperature (15℃, 20℃, 25℃, or 30℃) and developmental duration ($P > 0.05$). At the early developmental stage, from fetilized egg to the 16-cell stage, stage duration was significantly shorter at 30℃ than at 15℃ and 20℃ ($P < 0.05$).

Embryonic survival was negatively related with incubation time at all water temperatures tested (Fig. 2). Within the survivable temperatures, embryos that were incubated at 15℃ and 30℃ had survival rates of 46.33 ± 5.7 and 41.50 ± 4.5 %, respectively. Survival rates at 20℃ and 25℃ were 66.33 ± 4.2 and 66.67 ± 5.5% respectively, which was higher than the mean survival rate. No significant difference among survival rates was found at the four survivalable water temperatures ($P > 0.05$).

Only embryos cultured at salinity from 15‰ to 35‰ were able to complete the Umbo larval stage successfully (Table. 2). Embryos incubated at a salinity 30‰ had the shortest developmental duration was found from salinity 15‰. No significantly difference in developmental duration was found from salinity levels of 15-35‰ ($P > 0.05$). However, the duration to the D-shape larva stage at a salinity of 15‰ was significantly shorter than 30‰ and 35‰ salinity levels ($P < 0.05$). Moreover, eggs cultured at a salinity 5‰ died quickly and did not reach 4-cell stage. When the salinity was 10‰ and 40‰, development ceased at the 16-cell and D-shape stage, respectively.

The salinity tolerance of mussel embryos was relatively extensive (Fig. 3). Mussel embryos were susceptible only to extreme salinity 5‰, 10‰, and 40‰. All embryos died at a salinity 15‰ and the survival rate was below 50% at a salinity of 20‰. The survival of eggs cultured under salinity levels of 25‰, 30‰, and 35‰ ranged from 60.00 ± 4.4% to

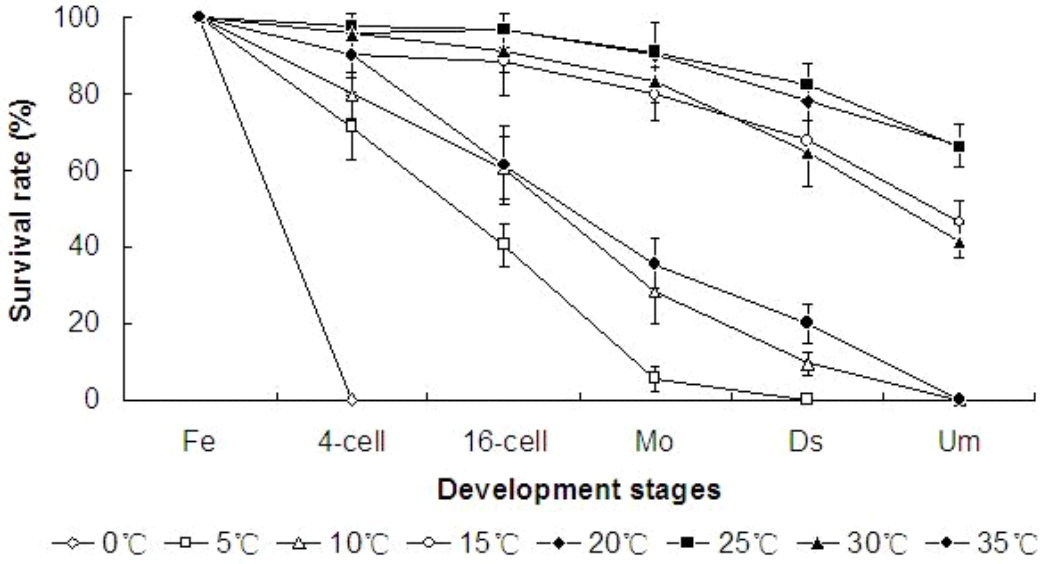

Fig. 2 The relationship between water temperature and survival of *Musculista senhousia* during the various early developmental stages. Fe: Fertilized eggs; Mo: Morula; Ds: D-shaped larvae; Um: Umbo larvae.

Table. 2 The relationship between salinity and time required to reach each developmental stages after egg fertilization

Developmental stages	Elapsed time after fertilization (hour)							
	Salinity							
	5	10	15	20	25	30	35	40
4-cell	-	6 a^{b}	4 ^{a}	3 $.2^{a}$	3 ^{a}	2 $.5^{a}$	2 $.5^{a}$	3 $.5^{a}$
16-cell	-	1 3^{bc}	1 0^{b}	7 $.8^{b}$	7 $.5^{ab}$	5 ^{ab}	5 ^{ab}	8 ^{ab}
Morula	-	-	1 4^{bc}	1 1.5^{bc}	1 1^{bc}	9 ^{b}	9 ^{b}	1 2^{bc}
D-shape larva	-	-	4 0^{d}	3 3^{cd}	2 8^{cd}	2 5^{c}	2 5^{c}	3 0^{cd}
Umbo larva	-	-	3 15^{e}	2 94^{e}	2 78^{e}	2 45^{e}	2 46^{e}	-

Different superscripts on the bars within a figure are significantly different ($P < 0.05$).

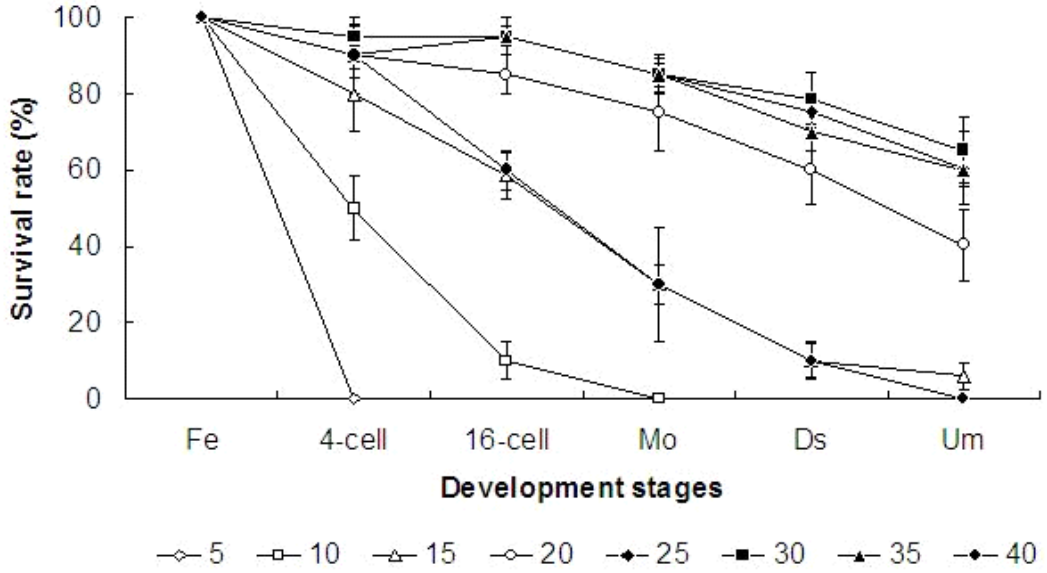

Fig. 3 The relationship between salinity and survival of *Musculista senhousia* in the different early developmental stages. Fe: Fertilized eggs; Mo: Morula; Ds: D-shaped larvae; Um: Umbo larvae.

Table. 3 Survival of *Musculista senhousia* spat at various water temperature

Temp. (℃)	Amount of spat	Exposure time (hr.)	Recovery time (min.)	Survival rate (%)
0	30	24	90	60
5	30	24	80	90
10	30	24	50	100
15	30	24	20	100
20	30	24	5	100
25	30	24	0	100
30	30	24	0	100
35	30	24	90	50
40	30	5	-	0

65.25 ± 8.6%. In addition, survival was significantly lower at a salinity 15‰ compared to 25‰, 30‰, or 3 5‰, corresponding to a survival rate of 5.8% ($P < 0.05$).

The experiment on *M. senhousia* spat

The one-way ANOVAs showed a signicant difference in survival of mussel spat at different water temperatures and salinity levels ($P < 0.05$, Table. 3).

In the water temperature test, no spat survived at 40℃, indicating that this is a fatal temperature for spat. At 0℃, 5℃, and 35℃, spat survival ranged from 50% to 90%, exhibiting relevant resistance to abrupt changes in ambient environment. The higgest survival rate (100%) occurred from 10℃ to 30℃. However, from 10℃ to 20℃, spat needed 5-50min to recover compared with no need recovery time at 25℃ and 3 0℃, which might have been attributed to stimulation

Table. 4 Survival of *Musculista senhousia* spat at various salinity levels

Salinity	Amount of spat	Exposure time (hr.)	Recovery time (min.)	Survival rate (%)
0	30	72	-	0
10	30	72	150	60
20	30	72	30	100
30	30	72	0	100
40	30	72	0	100
50	30	72	90	30
60	30	72	-	0

by an inappropriate external environment. In contrast, spat were more obviously injured by lower temperature than by higher temperature based on recovery time.

Musculista senhousia spat were susceptible only to extreme salinity: all spat died at 0‰ salinity and within the range from 60‰ to 100‰. Surval was 100% at salinity levels from 20‰ to 40‰ (Table. 4). At salinity levels of 10‰ and 50‰, survival was 30 % and 60%, respectively. According to our experimental results, mussel spat can survive in seawater with salinity ranging from 20‰ to 40‰ for 72 h, and should be considered resistant to changes in salinity. Recover time data indicated that spat were more tolerant at higher salinity (40‰) than lower salinity (20‰). Under the salinity levels of 30‰ and 40‰, no recovery time was needed, which might indicate that this is the optimal salinity range for the mussel.

Discussion

Both water temperature and salinity affected the speed and completion of early development in the mussel, *M. senhousia*. Embryos had little tolerance to reduced salinity (< 15‰) and did not develop at the lower extremes of water temperature (< 15℃). Moreover, at high water temperature (35℃) and salinity (40‰), embryos also could not develop to the Umbo larval stage. Our results indicate that water ranged from 15℃ to 30℃ and salinity ranging from 15‰ to 35‰ are required for embryo survival. Within this temperature range, those embryos incubated under higher temperatures developed more quickly, indicating that temperature is positively related to mussel developmental speed. This phenomenon also has been observed in previous studies on bivalves embryo development (Dix and Sjardin, 1975; Robert *et al*., 1988; Doroudi, 1999;). Heasman (1996) pointed out that with increasing temperature and the concomitant increase in metabolic rate, bivalve larvae must acquire more energy through increased algal consumption to maintain a positive energy balance. No such relationship has been found for survivable salinity levels, and mussel embryo seem to have a critical lower threshold.

During the early development of most bivalves, water temperature is the main restricting environmental variable. Lemos (1994) and Robert (1988), reported that discounting nutrition, temperature was clearly the dominant factor influencing the development of oyster, *Ostrea edulis* (L.), larvae. Doroudi (1999) also found that within a suitable salinity range, the growth of *Pinctada margaritifera* larvae depends primarily on temperature.

Using 50% survival as the threshold for the optimal condition criteria, the optimal temperature for *M. senhousia* embryonic development was from 20℃ to 25℃ and 25-35‰ for salinity. According to our records, the salinity of the mussel habitat in Seonso varied from 28.5‰ to 31.4‰. Thus, salinity is optimal for mussel spawning all year-round. However the water temperature varied from 4.5℃ to 25.9℃ during the year, which limit mussel reproduction. De Vooys (1999) also found that temperature is an important

species-specific factor for spawning initiation.

Musculista senhousia embryos are much more vulnerable than spat. All spat survived in water ranging from 10℃ to 30℃, showing survival in a relatively wide range of temperature, similar to those experienced by adult. The favourable temperatures for the bivalve *Pinctada fucata* are 7-29℃ (Wada, 1991). The mussel *Mytilus edulis* can survive in water below 29℃. (Fabioux *et al*., 2005). Adult *Dreissena polymorpha* mussels can survive in water ranging from 0 to 29℃ (Karatayev, 1995). However, only spat reared at 25-30℃ required no recover time, possibly indicating that this is the optimum temperature range. This temperature range was slightly higer than that for embryos (20-25℃), indicating that spat have a higher water temperature tolerance.

In this study, the mussel spat were generally tolerant of salinity levels ranging from 20‰ to 40‰, with low survival (< 60%) when salinity was less than 10‰ or more than 50‰. Usually, cultivation sites are chosen at which salinity varies within this range and where these salinity conditions prevail, temperature is generally considered to be the most important factor affecting the performance of the spat (Wilson, 1987; Chauvaud *et al*., 1998; Laing, 2000). Note, however, that the cultivation sites may occasionally have lower salinity conditions due to increased freshwater input from rivers and land runoff following heavy rainfall. Limited data are available on the effect of relatively low salinity on the spat of other common economically important bivalves, but generally, they are sensitive to varying degrees. In *Argopecten purpuratus,* a salinity less than 27‰ negatively affects growth (Navarro and Gonzalez, 1998). A salinity of 16‰ or less is lethal to sea scallop (*Placopecten magellanicus*) and severe catatonic shock can be observed at salinity of 18‰ and 21‰ (Bergman *et al*., 1996). In comparison, *M. senhousia* is a typical euryhaline species, with spat being able to survive across a larger salinity range.

All spat survived in water from 10℃ to 30℃ and salinity 20-40‰, although they did not complete development to adulthood. This information help explain the spread of the species and its advantage in interspecific competition, which usually results in this species being dominant in benthic sea communities. Meanwhile, the hardiness of mussel spat implies that controlling the *M. senhousia* population is easier at the beginning of early development than at the spat stage. A previous study monitoring the reproductive cycle of *M. senhousia* in Korea found that they reproduce once per year, between the end of September and November, consistent with Sgro (2002). During this period, mussel embryos need a certain external environment, especially in terms of water temperature. Based on current results, temperature below 15℃ will induce death and suspend the early development process in embryos. Thus, water temperature may be a feasible way to control the large population of *M. senhousia* in ark-shell farms.

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