Korean J. Malacol. 32(1): 37-43 2016
http://dx.doi.org/10.9710/kjm.2016.32.1.37

Effect of water temperature on embryonic development and larval survival of an intertidal snail, *Nassarius festivus* (Powys, 1835)

Sin-Kil Kang, Chan-Gyoung Sung, Jiwoong Chung, Dong-Ho Park, Jong-Hyeon Lee and Chang-Hoon Lee

Environmental Health & Safety Research Institute, EH R&C Co. Ltd. Bucheon 14487, Republic of Korea

ABSTRACT

This study was carried out to determine the optimal water temperature for the embryonic development and laboratory culture of larvae of an intertidal mud snail, *Nassarius festivus*. The embryos and hatched veliger larvae of *N. festivus* were incubated at six different temperatures (5, 10, 15, 20, 25 and 30℃). Developmental time for each stage decreased as water temperature increased. The elapsed time to develop to the veliger larva at 15, 20, 25 and 30℃ was 559, 155, 131 and 103 hrs, respectively. At 5 and 10℃, embryo developed to veliger larvae but failed to hatch out of the egg capsule. In contrast, all embryos successfully hatched in the temperature range from 15 to 30℃. The biological minimum temperature during the embryonic development of *N. festivus* was estimated to be 9.5 ± 0.4℃. The cumulative water temperatures for blastula, gastrula and veliger stages were calculated as 111 ± 84, 486 ± 185, 1,164 ± 72℃, respectively. Temperature also affected the larval survival. Five days after hatching, more than 84% of larvae survived at all experimental temperatures. However, survival began to decrease after 6 days. It was 0% at 30℃. Survival of larvae incubated for 8 days was higher at 15 and 20℃ than other experimental temperatures. We therefore suggest that the optimal range of temperature for embryonic development and larval survival of *N. festivus* is 15-20℃.

Key words: *Nassarius festivus*, temperature, embryonic development, larval survival

Introduction

Nassarius festivus (Powys 1835) (Class Gastropoda; Family Nassariidae) is a small gastropod and is an important scavenger of benthic ecosystem that inhabits sandy and muddy intertidal and subtidal areas of western and southern coast of Korea (Shin *et al*., 1989; An and Koh, 1992; Jang and Kim, 1992; Lim and Choi, 2001; Kim and Hwang, 2003). Recently, several ecological and basic physiological studies on this species were carried out on the reproduction (Cheung and Lam, 1999; Chan and Morton, 2005), population dynamics (Morton and Chan, 2004), and feeding behavior (Morton and Yuen, 2000; Cheung *et al*., 2006). Other studies have dealt with the response of this species to the change in environmental factors such as temperature (Cheung and Lam, 1995), salinity (Cheung and Lam, 1995; Cheung, 1997) and dissolved oxygen (Chan *et al*., 2008; Cheung *et al*., 2008). Cheung and Lam (1995) studied the effects of thermal stress by high temperature on the survival of adult *N. festivus* from Hong Kong waters. They found that this species is highly tolerant to the thermal stress and the threshold temperature for survival is as high as 32℃.

However, the physiological characteristics during the embryonic development and early larval stages of this species are still lacking. Information on the optimal ranges of temperature on the development and larval survival of *N. festivus* could be a crucial factor for controlling the recruitment to the adult population.

Received: March 7, 2016; Revised: March 23, 2016; Accepted: March 31, 2016
Corresponding author : Chang-Hoon Lee
Tel: +82 (70) 5102-4501, e-mail: c.lee@ehrnc.com
1225-3480/24608

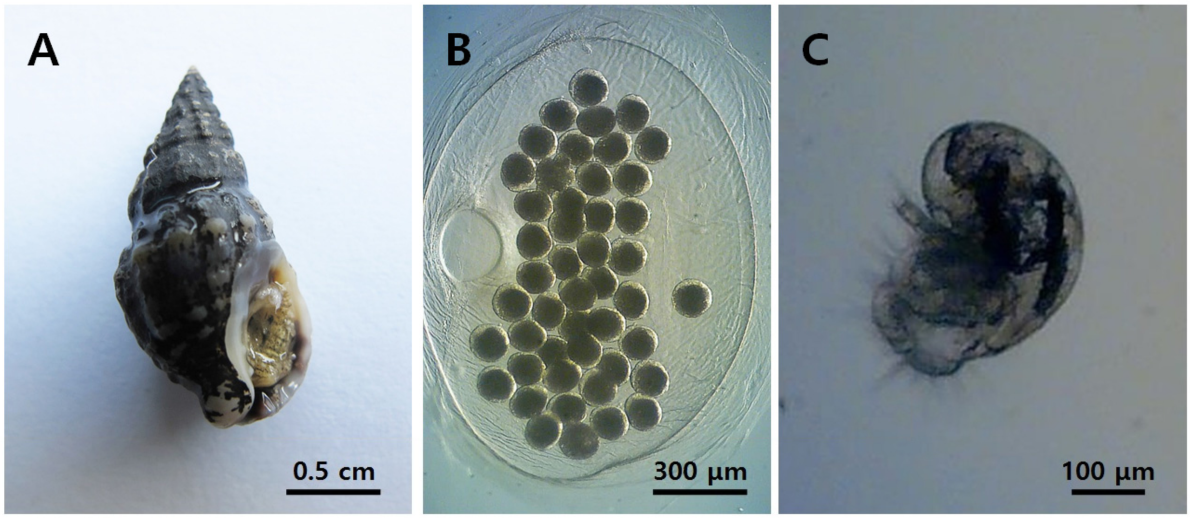

Fig. 1. The adult (**A**), egg capsule (**B**), and veliger larva (**C**) of *Nassarius festivus*.

Here, we set the purpose of this study to determine the optimal temperature ranges for embryonic development and larval survival of *N. festivus*.

To this end, we performed laboratory experiments to determine the developmental time to reach morula, blastula, gastrula, veliger, and free swimming larva stages under different temperature conditions, and then calculated biological minimum temperature (BMT) and cumulative water temperature (CWT). Experiments for determining the optimal range of temperature for the hatched larvae to survive were also performed. The basic information on the early developmental stage of *N. festivus* provided here may be useful to the needs of laboratory culture and the assessment of marine environmental quality.

MATERIALS AND METHODS

Approximately 40 individuals of *Nassarius festivus* (shell height: 11.35-16.69 mm, Fig. 1A) were collected at Shinduk beach (34° 49′ 22″ N, 127° 46′ 09″ E), Yeosu, southern coast of Korea in April, 2015. The surface water temperature during collection was 18℃. The snails were transported to the laboratory and maintained in a 4 L glass aquarium with well-aerated 1 μm filtered seawater (FSW, salinity: 30 ± 2 psu, temperature: 20 ± 2℃). They were kept and conditioned in the aquaria for spawning, fed daily with freshly excised carrion and commercial fish food, Tetra Bits®. FSW was exchanged every 2 days to prevent accumulation of metabolic wastes.

According to previous study, the reproductive season of *N. festivus* (Hong Kong population) is reported as from September to May (Chan and Morton, 2005). But, those in this study began to spawn in late April. They lay eggs in capsules. The shape of egg capsule is ellipsoidal, with 20-54 eggs per capsule (Fig. 1B). Immediately after spawning, egg capsules were removed carefully from the walls of glass aquaria. Six egg capsules were transported into each of six 20 mL scintillation vial with 10 mL of FSW. Each vial was then placed in a temperature-controlled incubator. Experimental temperatures were set to 5, 10, 15, 20, 25, and 30℃. To examine the developmental stages of the embryo, egg capsule was observed under a stereo-zoom microscope (Olympus, 20 ×) for 1 day interval during 25 days. For each sample, the numbers of embryos at each developmental stage (< 8 cells, morula, blastula, gastrula, veliger, and free swimming larva) were counted.

To know the effect of temperature on larval survival, 10 newly hatched (< 24 hrs) veliger larvae (Fig. 1C) per vial and 5 replicates per temperature condition were prepared. Experimental temperatures were the same as the experiments for embryonic development. Survival was checked under a microscope for 1 day interval during 8 days. Larvae with no apparent external- and internal movement were regarded as dead. During observation, dead individuals were removed immediately from the vial.

The biological minimum temperature (BMT) was estimated by linear regression between water temperature and the inverse of developmental time. The cumulative water temperature (CWT) for each developmental stage was calculated as:

CWT = (T - BMT) × DT,

where T is water temperature, BMT is the biological minimum temperature, and DT is the developmental time (Jun *et al.*, 2002).

Larval survival among different experimental temperatures was compared by one-way analysis of variance (ANOVA) using a significance level of α = 0.05. To determine which means were significantly different from one another, multiple comparisons were conducted using Tukey's HSD (Zar, 1984).

RESULTS

1. Effects of temperature on embryonic development

There were large differences in the developmental time of *Nassarius festivus* among different temperatures (Fig. 2). At 5℃, average elapsed time to develop morula, blastula and gastrula stage was 103, 175, and 595 hrs after fertilization, respectively. After 595 hrs, all embryos stopped further development and died before the end of experiment. At 10℃, average elapsed time to develop morula, blastula, gastrula, and veliger stage was 55, 103, 343, and 595 hrs after fertilization, respectively. After veliger stage, all embryos did not show further development and failed to hatch out of the egg capsule, then gradually died before the end of experiment. Temperature range of 5 to 10℃ seems not appropriate for the *N. festivus* embryo to develop normally into free swimming larva.

At 15℃, average elapsed time to develop morula, blastula, gastrula, veliger, and free swimming larva was 19, 31, 103, 223, and 559 hrs after fertilization, respectively. Time to reach free swimming larva was

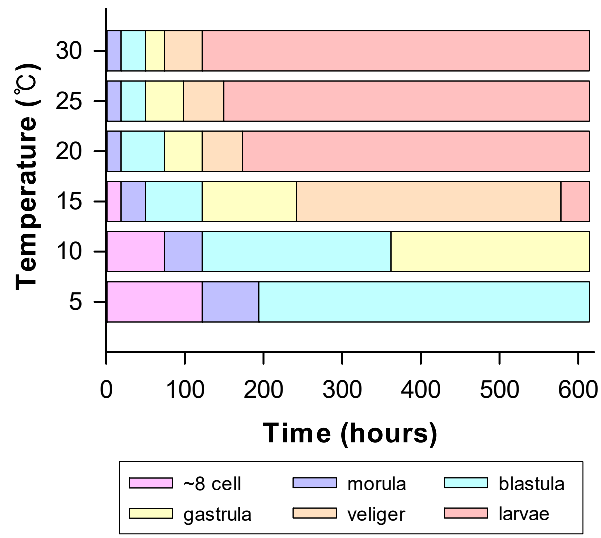

Fig. 2. Effect of water temperature on the developmental time of each stage of *Nassarius festivus*.

approximately 23 days. As temperature increased over 20℃, the developmental time was shortened greatly. The average elapsed time to reach free swimming larvae at 20, 25, and 30℃ was as short as 6, 5, and 4 days after fertilization, respectively. At these temperature conditions, the early embryonic development was so fast that we could not observe morula stage even at only 1 day after the beginning of experiments.

There were quite good linear relationships between water temperature and inverse of elapsed time (Fig. 3). The linear regression equations between temperature (T) and inverse of elapsed time (hr^{-1}) for each developmental stage are as follows;

1/hr = 0.0096 × T - 0.0943 (r^2 = 0.915) for blastula
1/hr = 0.0019 × T - 0.0180 (r^2 = 0.971) for gastrula
1/hr = 0.0009 × T - 0.0086 (r^2 = 0.988) for veliger
1/hr = 0.0005 × T - 0.0048 (r^2 = 0.921) for free swimming larva

From these regression equations, the average BMT was estimated to be 9.5 ± 0.4℃. The CWTs (mean ± SD) required for morula, blastula, gastrula, veliger and free swimming larvae of *N. festivus* were calculated as 27 ± 0, 111 ± 84, 486 ± 185, 1,164 ± 72 and 1,918 ±

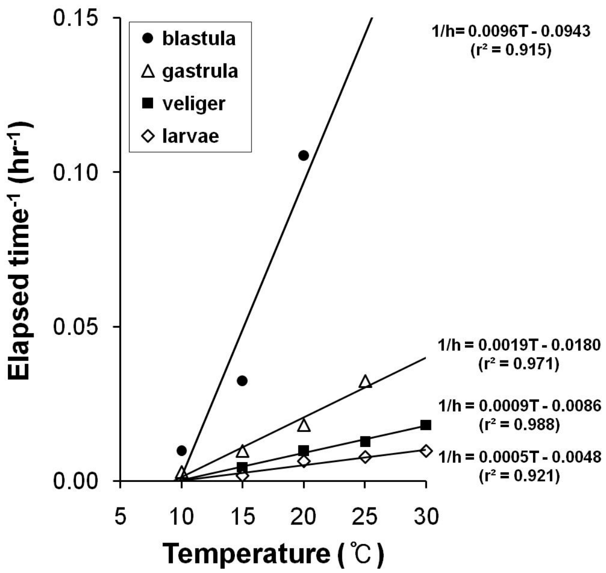

Fig. 3. Relationship between water temperature (T) and inverse of elapsed time (hr^{-1}) for each developmental stage of *Nassarius festivus*.

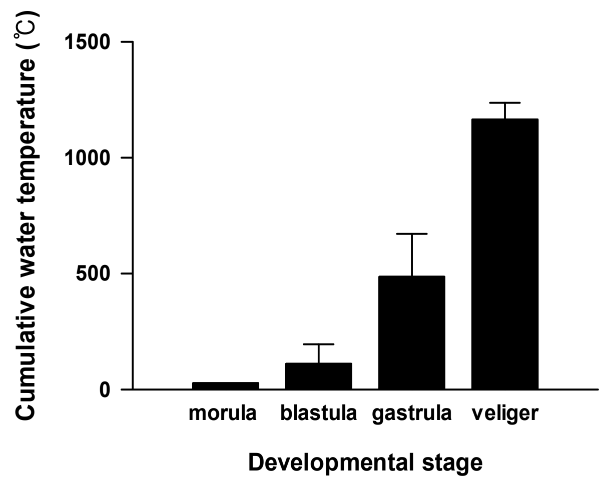

Fig. 4. Cumulative water temperature for each developmental stage of *Nassarius festivus*.

261℃, respectively (Fig. 4).

2. Effects of temperature on larval survival

Temperature also affected the larval survival of *Nassarius festivus*. Until day 2, larval survival at all experimental temperature was 100% (Table 1). At day 3, dead larvae were observed at 5, 10, 20, and 30℃. Among them the larval survival at only 30℃ was significantly lower than any other temperature conditions. Until day 5, larval survival decreased gradually but slightly at each temperature showing only significant difference at 30℃. At day 6, all the larvae at 30℃ were dead. Still the larval survival at other temperatures was over 80% showing no statistical differences ($p = 0.065$). At day 8, larval survival (78 ± 4%) at 15℃ was significantly higher than any other temperatures. As temperature increased or decreased from 15℃, larval survival decreased. There were no significant differences in larval survival among 5, 10, 20, and 25℃. ($p = 0.082$)

DISCUSSION

There were strong effects of water temperature on the embryonic development of *Nassarius festivus*, especially at lower temperature range (5-10℃). Embryo under these temperature conditions failed to hatch;

Table 1. Daily changes in survival (%, mean ± SD, n = 5) of veliger larvae of *Nassarius festivus* under different temperature conditions. Values with different superscripts in the same row are statistically different ($p < 0.05$, one-way ANOVA)

Time	Temperature					
	5℃	10℃	15℃	20℃	25℃	30℃
Day 1	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
Day 2	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
Day 3	96 ± 5^{ab}	96 ± 5^{ab}	100 ± 0^{a}	98 ± 4^{ab}	100 ± 0^{a}	92 ± 4^{b}
Day 4	94 ± 5^{ab}	94 ± 5^{ab}	100 ± 0^{a}	98 ± 5^{a}	100 ± 0^{a}	88 ± 4^{b}
Day 5	88 ± 8^{ab}	94 ± 5^{ab}	98 ± 4^{a}	96 ± 5^{ab}	98 ± 4^{a}	84 ± 9^{b}
Day 6	82 ± 4^{a}	92 ± 4^{a}	84 ± 5^{a}	84 ± 5^{a}	82 ± 8^{a}	0 ± 0^{b}
Day 7	70 ± 7^{b}	86 ± 5^{a}	78 ± 4^{ab}	70 ± 7^{b}	72 ± 8^{b}	0 ± 0^{c}
Day 8	52 ± 8^{b}	54 ± 11^{b}	78 ± 4^{a}	62 ± 8^{b}	48 ± 8^{b}	0 ± 0^{c}

eventually died. This range is near or below the BMT calculated in this study (9.5℃). Therefore, we can conclude that embryo cannot develop normally below BMT. In contrast, embryo of *N. festivus* seems quite well-adapted to higher range of water temperature as high as 30℃. Almost all of embryo successfully hatched under temperature from 15 to 30℃. The embryo developed to free swimming larvae 3.6 times faster when temperature increased from 15 to 20℃. In contrast, only 1.2 and 1.3 times differences in developmental time were observed when temperature increased from 20 to 25℃ and from 25 to 30℃, respectively. There exist some great changes in factors controlling embryonic development of *Nassarius festivus* affected by temperature between 15 and 20℃. It is recommended that temperature of at least 20℃ is required for satisfactorily developing embryo of *N. festivus* under the laboratory condition.

Since there is no reported BMT data for embryonic development of other gastropods, only comparisons with other taxonomic groups are possible (Table 2). The BMT for embryonic development of *N. festivus* is higher than marbled sole (*Limanda yokohamae*), stone flounder (*Kareius bicoloratus*), brown sole (*Limanda herzensteini*), surf clam (*Spisula sachalinensis*), sea urchin (*Strongylocentrotus intermedius*), or starfish (*Asterias amurensis*), and is only comparable to oyster (*Crassostrea rivularis*). From Table 2, we can find that there is a strong relationship between BMT and the spawning season, irrespective of taxonomic group. Species spawning during summer (*N. festivus* and *C. rivularis*) have higher BMT than those spawning during spring (*L. herzensteini* and *S. sachalinensis*), autumn (*S. intermedius*) or winter (*L. yokohamae*, *K. bicoloratus*, and *A. amurensis*). This relationship indicates that the embryonic development of marine organisms in temperate regions is optimized to the water temperature during their spawning seasons.

Chan and Morton (2005) reported that *N. festivus* is a winter spawner; the spawning season being September to May. But their population inhabits sub-tropical region (Hong Kong). The temperature range of Hong Kong water during their spawning season is 13.7-28.0℃, which is quite higher temperature than the same period in Korean waters. During this study, we could observe spawning in the laboratory from late April to August during which the water temperature of their natural habitat is quite similar to that during spawning season of Hong Kong population. Therefore, Korean population of *N. festivus* has adapted to the optimal temperature range during summer so that this species should regarded as a summer spawner, not a winter spawner.

From the results of larval survival experiments, temperature over 30℃ does not seem suitable for the rearing larvae of *N. festivus*. The tolerance threshold of adult of sub-tropical population of this species against thermal stress was reported as 32℃ (Cheung and Lam, 1995). In this point of view, we can infer either that larva is less tolerant to high temperature than adult or that population in the temperate region has adapted to lower range of temperature than that in sub-tropical region.

The larval survival is highest at 15-20℃. The

Table 2. Comparisons of the biological minimum temperature (BMT) and major spawning season among species in various taxonomic groups.

Taxonomic Group	Species name	BMT (℃)	Spawning season	Reference
Fishes	*Limanda yokohamae*	0.0	Winter	Yasunaga (1975)
	Kareius bicoloratus	1.3	Winter	Jun *et al.* (2002)
	Limanda herzensteini	2.6	Spring	Lee *et al.* (1997)
Bivalves	*Spisula sachalinensis*	5.5	Spring	Lee *et al.* (2002)
	Crassostrea rivularis	10.4	Summer	Yoo and Kang (1995)
Echinoid	*Strongylocentrotus intermedius*	1.6	Autumn	Lee *et al.* (2003)
Asteroid	*Asterias amurensis*	1.0	Winter	Lee *et al.* (2004)
Gastropod	*Nassarius festivus*	9.5	Summer	Present study

optimal temperature conditions for embryonic development (> 20℃) and larval survival (15-20℃) from this study does not overlap. Embryo seems to have preference to slightly higher temperature than larva. Therefore, developing embryo at 20℃ and larval rearing at 15-20℃ is recommended.

In this study we focused only on the effects of temperature on the early life stage of *N. festivus*. For a better understanding of larval ecology and conditions for larval culture, more detailed studies on the effects of other physic-chemical factors, such as salinity, pH, dissolved oxygen, etc., and on the biological processes such as feeding and respiration will be needed.

ACKNOWLEDGEMENTS

This research was a part of the project titled "Development of techniques for assessment and management of hazardous chemicals in the marine environment", funded by the Ministry of Oceans and Fisheries, Korea.

REFERENCES

An, S. and Koh, C.-H. (1992) Environments and distribution of benthic animals on the Maygyung-Dongjin tidal flat, west coast of Korea. *The Journal of the Oceanological Society of Korea*, **27**(1): 78-90. (in Korean)

Chan, K. and Morton, B. (2005). The reproductive biology of *Nassarius festivus* (Powys, 1835) (Gastropoda: Nassariidae) in relation to seasonal changes in temperature and salinity in subtropical Hong Kong. *Aquatic Ecology*, **39**(2): 213-228.

Chan, H.Y., Xu, W.Z., Shin, P.K.S. and Cheung S.G. (2008) Prolonged exposure to low dissolved oxygen affects early development and swimming behaviour in the gastropod *Nassarius festivus* (Nassariidae). *Marine Biology*, **153**(4): 735-743.

Cheung, S. G. (1997) Physiological and behavioural responses of the intertidal scavenging gastropod *Nassarius festivus* to salinity changes. *Marine Biology*, **129**(2): 301-307.

Cheung, S.G. and Lam, S.W. (1995) Effect of salinity, temperature and acclimation on oxygen consumption of *Nassarius festivus* (Powys, 1835) (Gastropoda: Nassariidae). *Comparative Biochemistry and Physiology*, **111A**(4), 625-631.

Cheung, S.G. and Lam, S. (1999) Effect of food availability on egg production and packaging in the intertidal scavenging gastropod *Nassarius festivus*. *Marine Biology*, **135**(2): 281-287.

Cheung, S.G., Gao, Q.F. and Shin, P.K.S. (2006) Energy maximization by selective feeding on tissues of the venerid clam *Marcia hiantina* in the marine scavenger *Nassarius festivus* (Gastropoda: Nassariidae). *Marine Biology*, **149**: 247-255.

Cheung, S.G., Chan, H.Y., Liu, C.C. and Shin, P.K.S. (2008) Effect of prolonged hypoxia on food consumption, respiration, growth and reproduction in marine scavenging gastropod *Nassarius festivus*. *Marine Pollution Bulletin*, **57**: 280-286.

Jang, I.-K. and Kim, C.-H. (1992) A study on the changes of the molluscan and crustacean fauna after the construction of the Naktong estuary barrage. *Bulletin of Korean Fisheries Society*, **25**(4): 265-281. (in Korean)

Jun, J.C., Kim, C.H., Chung, E.Y., Lee, C.H. and Kim, B.G. (2002) Influence of water temperature and salinity on early development of the stone flounder, *Kareius bicoloratus* from west sea of Korea. *Korean Journal of Ichthyology*, **14**: 190-197.

Kim, J.-S. and Hwang, S.-D. (2003) Biomass of shellfish in the Saemangeum tidal flat on the west coast of Korea. *Journal of Korean Fisheries Society*, **36**(6): 757-761. (in Korean)

Lee, C.-H., Ryu, T.-K. and Choi, J.-W. (2004) Effects of water temperature on embryonic development in the northern Pacific asteroid, *Asterias amurensis*, from the southern coast of Korea. *Invertebrate Reproduction and Development*, **45**(2): 109-116.

Lee, C.-S., Kim, W.-K., Kim, D.-H., Jeong, S.-H. and Park, K.-Y. (2003) Effect of temperature on the development of egg and larvae of sea urchin (*Strongylocentrotus intermedius*). *Journal of Aquaculture*, **16**: 94-98. (in Korean)

Lee, J.Y., Kim, W.K. and Chang, Y.J. (1997) Influence of water temperature and salinity on egg development of flatfish, *Limanda herzensteini*. *Journal of Aquaculture*, **10**: 357-362. (in Korean)

Lee, J.-Y., Chang, Y.-J., Kim, Y.-S., Choi, Y.-H. and Chang, Y.-J. (2002) Influence of water temperature on egg development of surf clam, *Spisula sachalinensis*. *Journal of Aquaculture*, **15**: 39-42. (in Korean)

Lim, H.-S. and Choi, J.-W. (2001) Community structure of subtidal macrobenthos in Hampyung Bay during autumn in 1997, southwest coast of Korea. *Journal of Korean Fisheries Society*, **34**(4): 327-339 (in Korean)

Morton, B. and Chan, K. (2004) The population dynamics of *Nassarius festivus* (Gastropoda: Nassariidae) on three environmentally different beaches in Hong Kong. *Journal of Molluscan Studies*, **70**(4): 329-339.

Morton, B. and Yuen, W.Y. (2000) The feeding behaviour and competition for carrion between two sympatric scavengers on a sandy shore in Hong Kong: the gastropod, *Nassarius festivus* (Powys) and the hermit crab, *Diogenes edwardsii* (De Haan). *Journal of*

Experimental Marine Biology and Ecology, **246**(1): 1-29.

Shin, H.C., Choi, J.W. and Koh, C.H. (1989) Faunal assemblages of benthic macrofauna in the inter- and subtidal region of the inner Kyeonggi Bay, west coast of Korea. *The Journal of the Oceanological Society of Korea*, **24**(4): 184-193. (in Korean)

Yasunaga, Y. (1975) Environmental factors or marine fish eggs and larvae, with respect to water temperature, salinity, dissolved oxygen and hydrogen-ion concentration. *Bulletin of Tokai Regional Fisheries Research Laboratory*, **81**: 171-183.

Yoo, S.K. and Kang, K.H. (1995) Influence of water temperature and salinity on egg development and larvae rearing of oyster, *Crassostrea rivularis*. *Korean Journal of Malacology*, **11**: 165-170. (in Korean)

Zar, J.H. (1984) Biostatistical Analysis, 2nd ed., Prentice-Hall international, Englewood Cliffs, NJ.