Korean Journal of Malacology, Vol. 19(1): 25-32, June, 2003

Effects of the Red Tide and Toxic Dinoflagellates on the Survival and Growth of Larvae of the Mussel, *Mytilus galloprovincialis*

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ABSTRACT

To know the effects of the red tide and toxic dinoflagellates on survival and growth of larvae of the mussel, *Mytilus galloprovincialis*, laboratory experiments were conducted by incubating larvae with either unialgal culture of 4 dinoflagellate species (*Amphidinium carterae, Prorocentrum triestinum, Gymnodinium impudicum*, or *Akashiwo sanguinea*) or a standard food (*Isochrysis galbana*) for 10 days. The survival of larvae was higher than 80% when the food was *A. carterae, G. impudicum*, or *A. sanguinea*. The lowest survival (20%) was found when the food was *P. triestinum*. When the food was *P. triestinum*, the survival of larvae rapidly decreased from 87% at day 4 down to *ca.* 50% at day 6, and 20% at day 10. This implies that the larval population of *M. galloprovincialis* can seriously be affected if they are exposed to the red tide water dominated by *P. triestinum* for more than 4 days. Shell length of larvae either increased or decreased according to the food species. When the food was *A. carterae, G. impudicum*, or *A. sanguinea*, shell length of larvae increased. But, it decreased when the food was *P. triestinum*. Though shell length increased in 3 treatments, the daily increments (0.63 μm for *A. carterae*, 0.46 μm for *G. impudicum*, and 1.10 μm for *A. sanguinea*) were smaller than that of the standard food (3.79 μm for *I. galbana*). Correlation analyses showed that the change in shell length was not significant when the food was *A. carterae* or *G. impudicum*. Therefore, all of 4 dinoflagellates affected the growth of *M. galloprovincialis* larvae: growth was negative for *P. triestinum*, nil for *A. carterae* and *G. impudicum*, and positive but lower than standard food for *A. sanguinea*. These imply that the dinoflagellates are less valuable as foods for *M. galloprovincialis* larvae. So, decreased growth rate of larvae is expected during red tides, which will consequently cause delayed metamorphosis or failure to recruitment to the adult populations. In considering the harmful effects of red tides on the aquatic ecosystem, not only the effects on adult populations of fish and shellfish, but also the effects on larval populations should be included.

Keywords: *Mytilus galloprovincialis*, Larvae, Red tide, Dinoflagellates, Survival, Growth.

Received February 6, 2003; Accepted May 10, 2003
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1225-3480/19104

INTRODUCTION

Recently, the frequency and magnitude of phytoplankton blooms have been increasing along the coastal areas of Korea. The severity of algal blooms and the damages to coastal ecosystems also have been increasing. Most of marine harmful algal blooms (red tide) were dominated by dinoflagellates. Massive blooms of dinoflagellates have often caused large-scale mortalities of shellfishes in aquafarms (*e.g.* ECOHAB, 1995). Thus, there have been many studies on the effects of red tide dinoflagellates on shellfish populations, especially commercially important species (Widdows *et al.*, 1979; Nielsen and Strømgren, 1991; Lesser and Shumway, 1993; Luckenbach *et al.*, 1993; Matsuyama *et al.*, 1997; Li *et al.*, 2001). However, these studies mainly focused on the toxic effects of dinoflagellates on adult bivalves. Interactions between dinoflagellates and bivalve larvae have not been understood comprehensively yet. Instead, several studies were performed on the interactions between

microflagellates or diatoms and bivalve larvae in physiological viewpoints (Bayne, 1965; Riisgård *et al.*, 1980, 1981; Sprung, 1984a, b; Riisgård, 1988; Leonardos and Lucas, 2000). Here, the purpose of this study was established to know the effects of red tide dinoflagellates [*Amphidinium carterae, Prorocentrum triestinum, Gymnodinium impudicum* (formerly *Gyrodinium impudicum*), and *Akashiwo sanguinea* (formerly *Gymnodinium sanguineum*)] on the survival and growth of the larvae of the mussel, *Mytilus galloprovincialis*.

Mytilus galloprovincialis is a common bivalve in Europe (Moroño *et al.*, 1998; Tubaro *et al.*, 1998) and Asia (Matsuyama *et al.*, 1997; Choe *et al.*, 1999), and is spreading to other countries (McQuaid and Phillips, 2000). In Korea, this species (formerly misunderstood as *Mytilus edulis*, see Je *et al.*, 1990) is cultivated in aquafarms as an important food resource for human consumption. *Amphidinium carterae* is a benthic dinoflagellate and known to produce hemolytic compounds (Nayak *et al.*, 1997). Though not reported in Korean coastal waters, this species is found in Japan, New Zealand, Australia, Canada, UK, and USA (Anderson, *et al.*, 1995). Red tides dominated by A. carterae killed fishes (Yasumoto, 1990). The toxicity was reported as 1 MU/1.3 $\times$ 10^8 cells (Jeong *et al.*, 2001). *Prorocentrum triestinum* was commonly found in southern coast of Korea (Lee *et al.*, 2002). During the red tides, its maximum concentration was 4.8 $\times$ 10^4 cells/ml (Kim *et al.*, 1997). *Gymnodinium impudicum* was found especially during summer, in western coast (Yoo *et al.*, 2002) and southern coast of Korea with concentration up to 3.0 $\times$ 10^4 cells/ml (Jeong *et al.*, 2000). *Akashiwo sanguinea* was widely distributed in eastern coast (Lee *et al.*, 2002), southern coast (Lim *et al.*, 2002), and western coasts of Korea (Yoo *et al.*, 2002). Maximum concentration during the red tides was 3.8 $\times$ 10^3 cells/ml (Lim *et al.*, 2002). All 4 species of dinoflagellates are classified as potentially harmful (Anderson *et al.*, 1995).

To understand the effects of red tide dinoflagellates on the survival and growth of *Mytilus galloprovincialis* larvae, laboratory experiments were conducted by supplying either unialgal cultures of dinoflagellates or a standard food (*Isochrysis galbana*) to larvae for 10 days. In preliminary experiments with epifluorescence microscopy, it is proved that larvae could ingest all of the 4 dinoflagellates. The results from this study will provide basic information on the ecological interactions between red tide dinoflagellates and bivalve larvae.

MATERIALS AND METHODS

1. Preparation of the larvae

Adults of the mussel *Mytilus galloprovincialis* were collected from an aquafarm off Yeosu, Korea in March 2002. The temperature and salinity of ambient seawater during collection were 12℃ and 33.4 psu, respectively. Individuals with shell length from 45 to 65 mm were selected as the brood stock. The gonadal stage of this sized mussels were either late active or ripe stage. Mussels were transported to the laboratory within 6 hr after collection, then acclimated to the experimental temperature (15℃) for two months. During acclimation, mussels were reared in a 200 liter aquarium with 5-μm filtered seawater (30 psu). The microflagellate, *Isochrysis galbana* was provided at 1 $\times$ 10^5 cells/ml as food everyday. Mortality was checked everyday and dead individuals were removed immediately.

Induction of spawning was conducted in May, which is the natural spawning season in Korean coastal waters (Choe *et al.*, 1999). Ten individuals were used in spawning induction. The shell surface of the mussels was scraped to remove epibionts and rinsed thoroughly with freshly filtered seawater. To induce spawning, mussels were exposed to air for 1 hr, put back to a 10 liter aquarium filled with filtered seawater, and then water temperature was raised gradually up to 25℃. Most of mussels released sperms or eggs within 30 min after the water temperature reached 25℃. Mussels were allowed to spawn for 1 hr, then all mussels were removed from the aquarium. Ten-ml aliquots of water were taken to determine the fertilization rate. The fertilization rate was more than 95%. The diameter of fertilized eggs was ca. 60 μm. Egg suspension was passed through a 100-μm mesh screen to remove fecal materials and other large

Table 1. Design of experiments. Algal concentration and carbon content of each treatment for measurements of survival and growth of *Mytilus galloprovincialis* larvae incubated with red tide and toxic dinoflagellates. Carbon content was estimated from cell volume (Strathmann, 1967).

Treatment	Algal concentration (cells/ml)	Carbon content (μgC/ml)
No food (control)	-	-
Isochrysis galbana (Prymnesiophyceae)	4.63×10^4	1.02
Amphidinium carterae (Dinophyceae)	1.88×10^4	5.06
Prorocentrum triestinum (Dinophyceae)	2.97×10^3	0.64
Gymnodinium impudicum (Dinophyceae)	9.69×10^2	0.63
Akashiwo sanguinea (Dinophyceae)	5.05×10^2	1.13

particles and collected on a 35-μm mesh screen for smaller eggs and excessive sperms to pass through. Eggs were rinsed 3 times with filtered and autoclaved seawater, then were incubated in a 20 liter aquarium at 15℃ in darkness without aeration. Prior to incubation, the density of egg was adjusted to 20 eggs/ml. One day after fertilization, most eggs had developed to the trochophore larvae. From the first day, larvae were fed on 5×10^4 cells/ml of *Isochrysis galbana* everyday. During incubation, seawater was wholly renewed every other day.

2. Preparation of algal cultures

As foods for the *Mytilus galloprovincialis* larvae, unialgal cultures of a standard food (*Isochrysis galbana*) and 4 species of dinoflagellates (*Amphidinium carterae, Prorocentrum triestinum, Gymnodinium impudicum* and *Akashiwo sanguinea*) were prepared. They were grown at 20℃ with the f/2 medium (Guillard and Ryther, 1962) without silicate, with continuous illumination of 100 $\mu E/m^2/sec$ provided by cool-white fluorescent lights. Only cultures in exponential growth phase were used for experiments. Mean equivalent spherical diameter (ESD) of each alga was measured by a PAMAS-SVSS particle counter. The number of measured cells was more than 2000. Cell volume (V) was calculated as: $V=4/3\,\pi(ESD/2)^3$. The carbon content was estimated from cell volume according to Strathmann (1967).

3. Design of experiments

Experiments were designed to measure the survival and growth of *Mytilus galloprovincialis* larvae incubated with one of unialgal cultures of red tide and toxic dinoflagellates. Six sets of treatments, namely a control (no food), a standard (*Isochrysis galbana*) and 4 dinoflagellate species (*Amphidinium carterae, Prorocentrum triestinum, Gymnodinium impudicum*, and *Akashiwo sanguinea*) were established (Table 1).

The survival experiments were carried out using 12-well plates (polystyrene, Costar Co.) as test chambers. Experiments were triplicated for each treatment. Wells were filled with 4 ml of each algal culture of target concentration, then 20 individuals of 5 days old *Mytilus galloprovincialis* larvae were transferred into each well using a Pasteur pipette. The plates were placed on a incubator at 15℃ under dim light condition (5 $\mu E/m^2/sec$) for 10 days. Numbers of survivors were counted and survivors were transferred into new well plates with the same algal cultures everyday. Whether the larvae were dead or alive was determined by observing them under a compound microscope (× 100). Larvae showing no ciliary movements in the velum for 10 sec were regarded as dead.

The growth experiments were carried out using 270-ml centrifuge bottles (polycarbonate, Nalgene, Co.) as test chambers. Experimental bottles were filled with 250 ml of each algal cultures of target concentration (Table 1). Then, 5 days old larvae were injected to the final density of 10 larvae/ml. Bottles were placed on a wheel rotating at 1 rpm under conditions same as the survival experiments. To measure the shell length of the larvae, 10-ml aliquots of subsamples were taken from each treatment and fixed with 10% buffered formalin at 2 days interval. Shell length (the distance between both anterior- and posterior ends of the valve) of 10 larvae for each treatment was measured with a micrometer attached on the eyepiece of a compound microscope (× 100) to the nearest 1 μm.

4. Analyses of data

The shell length (SL) of Mytilus galloprovincialis larvae for each treatment was expressed as a linear function of incubation time (T) as follows: SL = a × T + b, where parameter a is the daily growth rate and b is the shell length at the beginning of experiment. To determine whether the increase or decrease in shell length from growth experiment was significant or not, the parametric correlation analyses between shell length and incubation time were conducted. Survival data with incubation time and shell length data at the end of incubation among treatments were compared by the one-way analysis of variance (ANOVA) on the SPSS program. Prior to ANOVA, data were tested for normality (Shapiro-Wilk's test) and homogeneity of variance (Bartlett's test). If at least one of the above requirements was not met, the data were $\log_{10}$ transformed, and then ANOVA was repeated. Multiple comparison was conducted using Tukey's HSD (Zar, 1984) to determine which means were significantly different from one another. For all analyses, a significance level of $\alpha = 0.05$ was used.

RESULTS AND DISCUSSION

1. Effects of dinoflagellates on the survival of *Mytilus galloprovincialis* larvae

The survivals of *Mytilus galloprovincialis* larvae in no food (control) and standard food (*Isochrysis galbana*) treatments after 10 days were 100% (Table 2). There was no effect of starvation on the survival of larvae. But, some of larvae died in treatments with red tide and toxic dinoflagellates. The survival of larvae was higher than 90% and showed no statistical significance when the food was either *Amphidinium carterae* ($F = 1.7$, $p = 0.153$) or *Akashiwo sanguinea* ($F = 0.8$, $p = 0.630$). The lowest survival (20%) was found when the food was *Prorocentrum triestinum* ($F = 119.6$, $p < 0.001$). In the treatment with *Gymnodinium impudicum*, mortality was less than 20%, however, the decrease in survival was statistically significant ($F = 2.5$, $p = 0.033$). The most drastic change was observed when the food was *P. triestinum* (Fig. 1). In the *P. triestinum* treatment, there was no significant change in survival from day 0 to day 4 ($p = 0.076$). But, after day 4, the survival rapidly decreased from 87% down to ca. 50% at day 6, and 20% at day 10. This implies that the larval population of *M. galloprovincialis* can seriously be affected if they are exposed to the red tide water dominated by *P. triestinum* for more than 4 days. Meanwhile, other dinoflagellate species, namely *A. carterae*, *G. impudicum*, and *A. sanguinea* seemed to affect little the survival of *M. galloprovincialis* larvae. It is noticeable that the toxic dinoflagellate

Table 2. Survival (%, mean ± SE) of the larvae of *Mytilus galloprovincialis* incubated with red tide and toxic dinoflagellates for 10 days.

Treatment	Survival (%)
No food (control)	100.0 ± 0.0
Isochrysis galbana (standard food)	100.0 ± 0.0
Amphidinium carterae	91.7 ± 3.3
Prorocentrum triestinum	20.0 ± 2.9
Gymnodinium impudicum	88.8 ± 6.0
Akashiwo sanguinea	98.3 ± 1.7

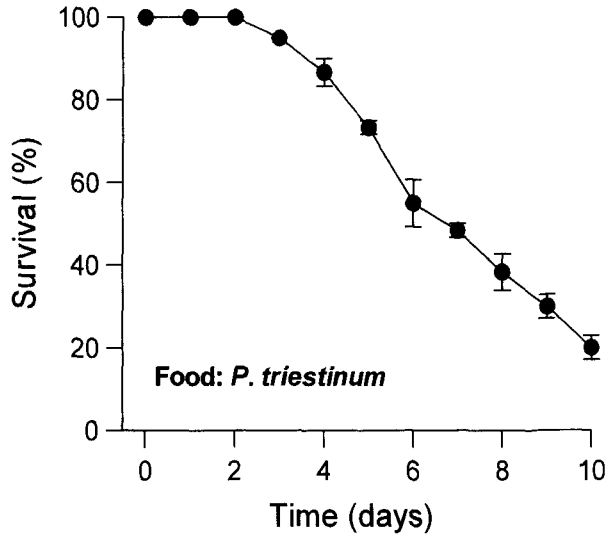

Fig. 1. Daily change in the survival (%) of *Mytilus galloprovincialis* larvae incubated with *Prorocentrum triestinum* for 10 days. Symbol represents the mean ± SE (n=3).

Amphidinium carterae has little effect on the survival of *M. galloprovincialis* larvae. Several explanations are possible for it. (1) *M. galloprovincialis* larvae did not ingest *A. carterae*; (2) The larvae ingested *A. carterae* but the ingestion rate was low; or (3) The toxicity of *A. carterae* was not so high as for the larvae to die. In epifluorescence microscopic observation, red color of ingested cells of *A. carterae* was distinctly found within the stomach of the larvae. Therefore, the first explanation cannot be applicable. Data from the growth experiment showed that the growth was positive (0.63 μm/day, see section below), which supports the second explanation. Jeong *et al.* (2001) reported that the heterotrophic dinoflagellate *Oxyrrhis*

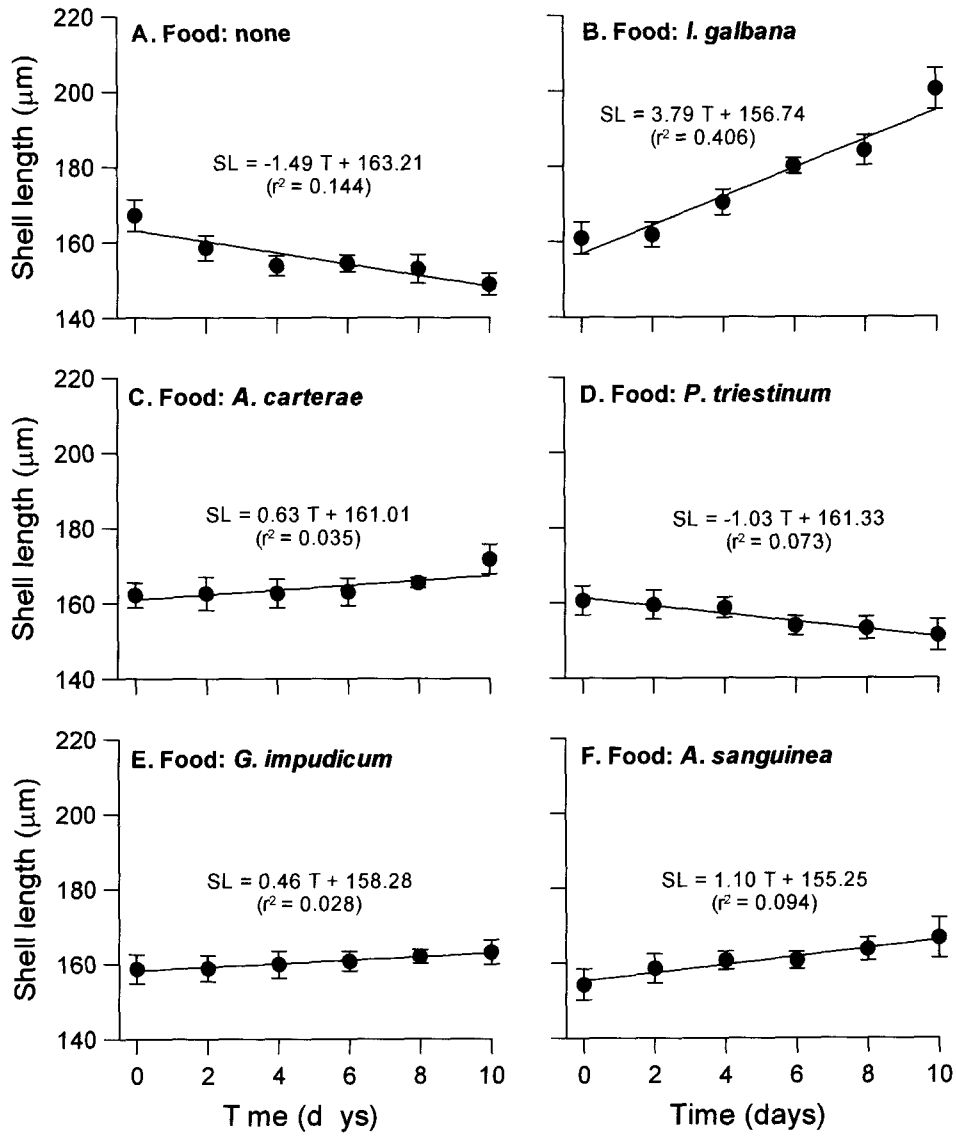

Fig. 2. Changes in the shell length of *Mytilus galloprovincialis* larvae incubated with red tide and toxic dinoflagellates for 10 days. Symbol represents the mean ± SE (n=10). Shell length (SL) data were fitted to a linear function of incubation time (T). A: no food (control), B: *Isochrysis galbana* (standard food), C: *Amphidinium carterae*, D: *Prorocentrum triestinum*, E: *Gymnodinium impudicum*, F: *Akashiwo sanguinea*.

marina was able to grow well on A. carterae whose toxicity was *ca.* 1 MU/1.3 × 10^8 cells. This supports the third explanation that the toxicity of *A. carterae* is not lethal to *M. galloprovincialis* larvae if the ingestion rate is not high.

More interesting is that the nontoxic *Prorocentrum triestinum* has strong effects on the survival of *Mytilus galloprovincialis* larvae. *P. triestinum* is known as potentially harmful (Anderson *et al.*, 1995), but there were no reports on its toxicity. The concentration of *P. triestinum* used in this study was lower than the maximum concentration that could be found in the fields (Kim *et al.*, 1997). Therefore, the high mortality in *P. triestinum* treatment is difficult to explain. To find the actual causes, detailed studies on the biochemical and physiological aspects of *P. triestinum* are necessary.

2. Effects of dinoflagellates on the growth of *Mytilus galloprovincialis* larvae

Shell length (mean ± SE) of *Mytilus galloprovincialis* larvae in no food (control) treatment decreased from 167.2 ± 4.2 to 148.9 ± 2.9 μm ($F = 3.3$, $p = 0.010$) during the incubation time (Fig. 2A), while that in standard food (*Isochrysis galbana*) treatment increased from 161.0 ± 4.2 to 200.4 ± 5.5 μm ($F = 11.8$, $p < 0.001$, Fig. 2B). The daily increment of shell growth of larvae in the *I. galbana* treatment was 3.79 μm. In treatments with red tide and toxic dinoflagellates, shell length either increased or decreased according to the food species (Fig. 2C-F). When the food was *Amphidinium carterae*, *Gymnodinium impudicum*, or *Akashiwo sanguinea*, shell length of larvae increased. But, shell length decreased when the food was *Prorocentrum triestinum*. Even though the shell lengths did increase in 3 treatments with dinoflagellates, the daily increments were much smaller than that of the standard food (*I. galbana*): 0.63 μm for *A. carterae*, 0.46 μm for *G. impudicum*, and 1.10 μm for *A. sanguinea*. After 10 days, shell length of larvae was significantly affected by 6 different treatments ($F = 18.8$, $p < 0.001$), and was higher in the order of treatments: *I. galbana* > *A. carterae* > *A. sanguinea* > *G. impudicum* > *P. triestinum* > no food. However, multiple comparison showed that there were no significant differences in shell lengths of larvae at day 10 among treatments with *A. sanguinea*, *G. impudicum*, *P. triestinum* and no food ($p = 0.136$).

Though there were either increase or decrease in shell length of *Mytilus galloprovincialis* larvae numerically after 10 days, not all the changes were statistically significant. Correlation analyses showed that change in shell length was not significant (Table 3) when the food was either *Amphidinium carterae* ($p = 0.184$) or *Gymnodinium impudicum* ($p = 0.225$). That is, there was no growth of larvae when they were fed on either one of these two species. Significant increase in the shell length of larvae was found in treatments with *Isochrysis galbana* ($p < 0.001$) and *Akashiwo sanguinea* ($p = 0.013$), while decrease found with *Prorocentrum triestinum* ($p = 0.021$) and no food ($p < 0.001$). Therefore, all of 4 dinoflagellate species used in this study affected growth of *M. galloprovincialis* larvae: growth was negative for *P. triestinum*, nil for *A. carterae* and *G.*

Table 3. Pearson's correlation coefficients (r) between shell length and incubation time for each treatment (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ns: not significant).

Treatment	r	p	Significance
No food (control)	-0.380	< 0.001	***
Isochrysis galbana	0.637	< 0.001	***
Amphidinium carterae	0.187	0.184	ns
Prorocentrum triestinum	-0.270	0.021	*
Gymnodinium impudicum	0.166	0.225	ns
Akashiwo sanguinea	0.306	0.013	*

impudicum, and positive but lower than standard food for *A. sanguinea*. These imply that these dinoflagellates are less valuable as foods for *M. galloprovincialis* larvae. So, decrease in growth rate of larvae is expected during red tides predominated by these dinoflagellates, which will consequently cause delayed metamorphosis or failure to recruitment to the adult populations. In considering the harmful effects of red tides on the aquatic ecosystem, not only the effects on adult populations of fish and shellfish, but also the effects on larval populations should be included.

ACKNOWLEDGEMENTS

This study was carried out as a part of the study entitled by "Eco-environmental studies for the restocking and enhancement of bivalve resources in the south coast of Korea (BSPG337-00-1447-3)" funded by the KORP (Korea Research Council of Public Science and Technology).

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