Effects of Covering Behavior and Exposure to a Predatory Crab *Charybdis japonica* on Survival and *HSP70* Expression of Juvenile Sea Urchins *Strongylocentrotus intermedius*

Chong Zhao1,2, Nanjing Ji1, Binglong Zhang1, Ping Sun1, Wenping Feng1, Jing Wei1, Yaqing Chang1*

1 Key Laboratory of Mariculture & Stock Enhancement in North China’s Sea, Ministry of Agriculture, Dalian Ocean University, Dalian, China, 2 College of Marine Life Sciences, Ocean University of China, Qingdao, China

Abstract

Predation is a complex process among predator, prey and environment. Juvenile sea urchins are more susceptible to predators than adults, which affects community structure. Behavior is involved in anti-predator responses by changes in the expression of anti-predator responsive genes. Here, we investigated the effects of exposure to a predatory crab *Charybdis japonica* and covering behavior on survival and *HSP70* expression of juvenile sea urchins *Strongylocentrotus intermedius*. *C. japonica* consumed large numbers of juvenile *S. intermedius* in 12 hours with a mortality of 34.17%±11.43%. Covering behavior did not significantly reduce predation. Exposure to *C. japonica* did not significantly upregulate *HSP70* expression of juvenile *S. intermedius* in 12 hours. Covering behavior showed no significant regulatory effect on the gene expression of *HSP70* of juvenile *S. intermedius* exposed to *C. japonica* for 12 hours. The results indicate that the anti-predator function of covering behavior is limited and that *HSP70* expression does not appear to play an important role in the anti-predator process of *S. intermedius*.

Introduction

Sea urchins are ecologically important in structuring marine benthic communities, both as grazers and prey [1]. Predation is one of the most important biological factors affecting community structure of sea urchins [2,3]. Compared to adults, juvenile sea urchins are more susceptible to predators than adults [4]. Thus, predation on juveniles probably plays an important role in stabilizing the large variability in recruitment and fluctuations in population density of sea urchins [4,5,6]. The importance of predation on juveniles in determining recruitment to urchin populations has been well reported in *Strongylocentrotus purpuratus* [7] and *Strongylocentrotus droebachiensis* [8,9]. This highlights the ecological importance of the interaction between juvenile *Strongylocentrotus* urchins and their predators.

The sea urchin *Strongylocentrotus intermedius* is endemic to intertidal and subtidal bottoms in northern Pacific coastal waters of Hokkaido of Japan, Korea and Far East Russia [10]. It was introduced into China from Japan in 1989 for its commercial value [11]. In Japan, the mortality of juvenile *S. intermedius* transplanted to fishing grounds is high because of predation by several crab species (*Paugtilla quadridentis, Telmessus chiriquanus* and *Paralithodes brevipes*) [10]. This clearly indicates the prey-predator interaction between *S. intermedius* and crabs. The crab *Charybdis japonica*, distributed in intertidal zone and sallow water of western coast of the Pacific Ocean including China, Japan and Malaysia [12], obviously overlaps habitats with *S. intermedius* in both intertidal and subtidal zones. To our knowledge, the prey-predator relationship has never been reported between the two species, although *C. japonica* has been well documented of consuming various marine organisms, including bivalves, crustaceans and fish [13,14,15].

Since the risk of predation is ubiquitous, prey have evolved various behavioral strategies to minimize the impact on their survival and fitness traits [16]. Behavioral susceptibility to predator exposure has been reported in rainbow trout *Oncorhynchus mykiss* [17] and hermit crabs *Pogonoschema bernhardus* [18]. Covering behavior refers to sea urchins using their tube feet and spines to move objects, such as shells, stones and algae fragments, onto their dorsal surface in both shallow water [19] and the deep sea [20]. Six hypotheses have been proposed to explain the biological significance [21], including reflex action [22], ancillary feeding [23] and protection against predation [24,25], wave surge and floating debris [26,27], and over-exposure to light [28,29,30]. Among these hypotheses, protection against predation is of particular interest, because similar behaviors are involved in protective strategies of decreasing predation in terrestrial insects [31,32]. Agatsuma (2001) [23] reported that covering behavior significantly reduced predation of juvenile sea urchins *S. intermedius* in 2 hours of exposure to the crab *P. quadridentis*, but showed no
significant effect after 24 and 48 hours of exposure to the predator. This clearly indicates covering behavior of *S. intermedius* has a valid but limited protective function against *P. quadridens*. However, we do not know whether covering behavior of *S. intermedius* has a similar protective function against other potential predators (for example, *C. japonica*).

An arduous challenge of behavioral biology is to investigate the physiological and molecular mechanisms of biological functions of various behaviors. Predator-induced behaviors are regulated by the expression of stress-sensitive genes [33,34]. The family of heat shock proteins (HSP) includes a number of molecular chaperones of approximately 70kDa in size that serve indispensable roles in protein homeostasis [35]. Gene expression of *HSP70* is involved in physiological responses to predation in damselfly larvae [36], fish [34,37,38] and water flea [39], although the mechanism remains largely unclear. Whether *HSP70* expression may be involved in the predator-induced behavior of *C. auratus*. Thus, we were motivated to investigate whether covering behavior and exposure to a predator significantly affect *HSP70* expression in *S. intermedius*.

The main purposes of the present study were to investigate 1) whether the crab *C. japonica* significantly preys on *S. intermedius*; 2) whether covering is an important behavior that significantly reduces predation of *S. intermedius* exposed to *C. japonica*; 3) whether exposure to predators and covering behavior significantly affect the gene expression of *HSP70* of *S. intermedius* exposed to *C. japonica*.

**Results**

**Sea Urchins and Crabs**

The body weight of sea urchins was 1.99±0.025 g (N = 240, mean ± SE), with no significant difference among all experimental groups (P>0.05). Carapace width, height and the length of both chelipeds of *C. japonica* showed no significant difference between the groups with and without covering material (P>0.05, N = 3, Table 1). However, body weight of *C. japonica* in the covering material group was significantly higher than that in the group without covering material (P=0.019, N = 3, Table 1). Male and female *C. japonica* showed no significant difference in all measured traits (P>0.05).

**Predation**

There was no mortality of *S. intermedius* in the groups without predators. *C. japonica* consumed a large number of *S. intermedius* in 12 hours, with a mortality of 34.17±11.43% (P = 0.018, N = 3). Mortality of *S. intermedius* with covering material was obviously higher than that of individuals without covering material, although the p value was not significant (P>0.05, N = 3, Fig. 1). There was no significant interaction between covering material and predator on predation of *S. intermedius* (P>0.05, N = 3).

**Covering Behavior and Gene Expression of HSP70**

Exposure to predators showed no significant effect on either time to first covering (P>0.05, N = 3, Fig. 2) or number of shells covered per sea urchin (P>0.05, N = 3, Fig. 2). Neither covering material, predator exposure nor their interaction had a significant effect on the relative expression of *HSP70* (P>0.05, N = 3, Fig. 3).

**Discussion**

Identifying potential predators and assessing predation provide valuable ecological information about the organization of communities with implications to the conservation management of sea urchin populations [7]. To our knowledge, the relationship of prey-predator has not been documented between *S. intermedius* and *C. japonica*, although their habitats overlap in both intertidal and sublittoral zones. We observed that *C. japonica* readily consumes juvenile *S. intermedius*. This indicates that in addition to the crab *P. quadridens* [25], *C. japonica* is probably another important predator of juvenile *S. intermedius*, although further field studies should be performed to document this. Avoidance of predation by *C. japonica* would increase the survival of juvenile *S. intermedius* in regular repopulation programs for conservation and fishery in both China [11] and Japan [43].

Covering behavior appears to be a multiple-factor dependent phenomenon, although a number of hypotheses have been proposed to explain it [30]. Different from the biological significance of protection against UV light exposure, covering behavior is not well associated with benefits from protection against predation. This is because covering behavior by different sea urchin species exposed to different predators showed different effects [24,25]. In the present study, we found that availability of covering material failed to significantly increase the survival of juvenile *S. intermedius* after 12 hours of exposure to *C. japonica*. Our result is consistent with the finding of Agatsuma (2001) [25] that covering behavior did not have a significant protective effect on survival of juvenile *S. intermedius* after 24 and 48 hours of exposure to *P. quadridens*, but significantly reduced predation of juvenile *S. intermedius* after 2 hours of exposure to *P. quadridens*. It should be noted the *C. japonica* used in the present study were much larger than the *P. quadridens* used by Agatsuma (2001) [25]. These results suggest that covering behavior is an effective protective strategy against predators in short-term exposure to some special

### Table 1. Sex and size traits of the crab *Charybdis japonica* (N = 3, mean ± SE).

| Covering material | N | Y |
|-------------------|---|---|
| Sex ratio (♂ : ♀) | 2 : 1 | 2 : 1 |
| Carapace width (cm) | 7.67±0.17 | 8.00±0.00 |
| Carapace height (cm) | 6.00±0.00 | 6.00±0.00 |
| Length of left cheliped (cm) | 11.00±0.00 | 11.00±0.29 |
| Length of right cheliped (cm) | 11.17±0.17 | 11.00±0.29 |
| Body weight (g) | 98.03±3.56* | 117.97±3.81* |

Different letters refer to significant difference of body weight. doi:10.1371/journal.pone.0097840.0001

PLOS ONE | www.plosone.org | May 2014 | Volume 9 | Issue 5 | e97840
predators. This can be partly supported by the finding of Amsler et al. (1999) [24] that the survival rate of covered sea urchins Sterechinus neumayeri was significantly higher than uncovered individuals exposed to sea anemone Isotealia antarctica. There was a trend of higher mortality of sea urchins with covering material than that of individuals without covering material, although the p value was not significant. A possible explanation is that body weight of C. japonica in the covering group was significantly higher than those in the group without covering material. Another limitation of the present study is that only three crabs of both sexes (two males and one female) were used in each experimental group. Male and female C. japonica in the present study showed no significant difference in all measured traits, although sexual difference in claws was documented in another crab species.

Figure 1. Predation on Strongylocentrotus intermedius with and without covering material (N = 3, mean ± SE).
doi:10.1371/journal.pone.0097840.g001

Figure 2. Time to first covering of Strongylocentrotus intermedius and number of shells covered per S. intermedius with or without exposure to a predator (N = 3, mean ± SE). N refers to without predator exposure, Y refers to in predator exposure.
doi:10.1371/journal.pone.0097840.g002
Pachygrapsus crassipes [7]. In addition, the ratio of males:females was constant and would not affect overall predation. However, the limited number of C. japonica of both sexes probably reduces the robustness of the statistical analysis. Together with previous studies, the present study indicates that crabs probably play an important role in the complex predator-prey interaction of sea urchins and that the protective effect of covering material seems much weaker than habitat refuges [7].

We expected exposure to predators might significantly increase covering behavior of juvenile S. intermedius. However, we found that predation did not significantly increase covering behavior, either in time to first covering or number of shells covered per sea urchin. This result is consistent with the study of Dumont et al. (2007) [30], who found that predator stimulus showed no significant effect on covering behavior of the sea urchin S. droebachiensis. Therefore, covering behavior does not appear to play an important role in the anti-predator response of sea urchins in exposure of starved and voracious predators.

Upregulation of HSP70 has been linked to antipredator responses in both invertebrates [36,39] and vertebrates [34,37,38]. In the present study, although predation was high, neither exposure to predators, covering behavior nor their interaction showed significant difference in relative expression of HSP70. This result is consistent with Sørensen et al. (2011) [44], who reported that 96 hours of predator exposure did not induce significant response of HSP70 expression in tadpoles of the frog Rana temporaria. There are two possible explanations. One possibility is that an inducible response of HSP70 expression in juvenile S. intermedius would be missed after 12 hours of exposure to C. japonica because the quickness of HSP70 expression response to the exposure of predators is species dependent [44]. For example, HSP70 expression in goldfish (C. auratus) showed a significant rapid upregulation in 6 hours exposure of a predator fish [37]. However, Slos & Stoks (2000) [36] reported a slow significant response in HSP70 expression of 5 days in the damselfly Enallagma cyathigerum under a predation risk. Another possibility is other anti-predator sensitive genes besides HSP70 in juvenile S. intermedius responded to the 12h exposure of C. japonica. Further studies are required to investigate whether HSP70 expression is related to predation in other urchin species under various environmental and predation conditions.

In conclusion, the crab C. japonica preyed on juvenile S. intermedius. Covering behavior did not significantly reduce the mortality of juvenile S. intermedius in 12 hours of exposure to C. japonica. Exposure to C. japonica did not significantly upregulate HSP70 expression in 12 hours. Covering behavior showed no significant regulative effect on the gene expression of HSP70 of juvenile S. intermedius exposed to C. japonica for 12 hours. The present results indicate that the anti-predator function of covering behavior is limited in the exposure to starved and voracious predators and that HSP70 appears to not play an important role in the anti-predator process in juvenile S. intermedius. Compared to the experimental data in insects [31,32,36], the present study clearly suggests that anti-predator strategies are highly species dependent.

**Materials and Methods**

**Sea Urchins and Crabs**

Two hundred and forty juvenile S. intermedius approximately 10-20 mm in test diameter, from a batch produced in October, 2012, were transported from the Dalian Haibao Fisheries Company to the Key Laboratory of Mariculture & Stock Enhancement in the North China's Sea, Ministry of Agriculture at Dalian Ocean University on July 15, 2013. They were maintained at 22–24°C and under natural sunlight in 200L tanks and fed kelp Laminaria japonica ad libitum until the experiment began on August 6, 2013. The body weight of all sea urchins was measured before the
experiment. To avoid potential harms to juvenile urchins, we did not precisely measure their test diameter and height.

Six crabs *C. japonica* were locally caught at Heishijiao, Dalian (38°52' N, 121°34' E), where no specific permission for this activity is required. They were then separately maintained under the same culture condition of *S. intermedius* and fed clams (*Ruditapes philippinarum*) in the laboratory to ensure that they were capable of feeding and not molting. They were starved for one week before the experiment started. The size and sex of the six crabs were recorded before the experiment. The field studies did not involve endangered or protected species.

**Experimental Design**

The present study included three experiments. Experiments were carried out with sea urchins at 23.7–26.0°C, 31% salinity, and pH 8.12 and under natural sunlight. The experiment began on August 6, 2013. First, we investigated the mortality of *S. intermedius* exposed to *C. japonica* with and without covering material (presence of covering material and predator as the two factors). *C. japonica* were individually put into six experimental tanks (70L) with or without covering material. Two males and one female *C. japonica* were put into the experimental groups with and without covering material to ensure a constant ratio of males:females in each group. Six similar tanks without predators were set up as controls. According to our previous study [45], twenty shells of the small bivalve *Patinopecten yessoensis* (shell length: 20.31±0.99 mm, shell height: 21.54±1.10 mm, shell weight: 0.28±0.06 g) were used as potential covering material and distributed evenly on the bottoms of the experimental tanks. Twenty sea urchins were gently and evenly distributed onto the bottom of each experimental tank. Mortalities of *S. intermedius* in all experimental groups were recorded after 1h, 3h, 6h, 9h and 12h.

The second experiment was the behaviour tests with and without predators (presence of predator as the factor). We recorded the time to first covering (behavioral action time) and the number of shells covered per sea urchin 12 hours after the beginning of the experiment (behavioral ability) for each of the six experimental tanks. The third experiment was the relative expression of *HSP70* by the sea urchins with and without exposure to the predator and with and without the presence of covering material (presence of covering material and predator as the two factors). Twelve hours after the beginning of the experiment, coelomic fluid collected from each sea urchin using a 1mL syringe was centrifuged at 3000xrpm for 2 minutes (4°C) to harvest coelomocytes. All the samples were immediately frozen in liquid nitrogen and then stored at −80°C until used.

**Gene Expression of HSP70**

Total RNA was extracted with the total RNA kit (TIANGEN, China) following the manufacturer instructions. First strand cDNA was synthesized using the PrimeScript RT reagent Kit (TaKaRa, Japan) according to the manufacturer instructions.

To obtain the *HSP70* expression profile, cDNA samples were analyzed with quantitative RT-PCR. Gene specific primers 5’-ACACTCATCTCAGGAGGAG-3’ (*HSP70-F*) and 5’-CTTTCTATGCTTTCGTGTA-3’ (*HSP70-R*) were designed for the *HSP70* fragments by Primer Premier 5.0 software. The sea urchin 18S ribosomal RNA was used as the reference gene. It was amplified using gene-specific primers of 5’-GTTGGAAGGC-GATCAGATAC-3’ (18S-F) and 5’-CTGTCATTCTCCT-CACGTGTC-3’ (18S-R) [46]. The qRT-PCR was carried out in a 20 μL volume including 10 μL of 2×SYBR Green Master mix (TaKaRa, Japan), 0.4 μL ROX Reference Dye II, 1 μL of 1:5 diluted cDNA, 0.4 μM of each primer and 7 μL ddH2O using the Applied Biosystem 7500 Real-time system (Applied Biosystem, USA). The PCR condition was set as: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 32 s. Melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm amplification specificity. The comparative Ct method was used to calculate the relative expression levels of the *HSP70* [47].

**Statistical Analysis**

Mortalities of *S. intermedius* in the exposure to *C. japonica* with and without covering material were analyzed using two-way repeated measured ANOVA (presence of covering material and predator as the two factors with repeated measures on time). Time to first covering and the number of shells covered per sea urchin at 12h were analyzed by one-way ANOVA (presence of predator as the factor). Relative expression of *HSP70* in *S. intermedius* was analyzed by two-way ANOVA (presence of covering material and predator as the two factors). All analyses were performed with SPSS 13.0 statistical software. A probability level of *P*<0.05 was considered statistically significant.

**Acknowledgments**

We are grateful to Prof. John Lawrence for academic and editorial suggestions.

**Author Contributions**

Conceived and designed the experiments: CZ. Performed the experiments: CZ NJ PS WF. Analyzed the data: CZ. Contributed reagents/materials/analysis tools: BW. Wrote the paper: CZ NJ JW.

**References**

1. Pease JS (2006) Ecological role of purple sea urchins. Science 314: 940–941.
2. Duffy JE, Hay ME (2001) The ecology and evolution of marine consumer-prey interactions. In: Bertness MD, Gaines SD, Hay ME (eds) Marine community ecology. Sinauer Sunderland pp: 131–137.
3. Guidetti P, Bassotti S, Boero F (2005) Evaluating the effects of protection on fish predators and sea urchins in shallow artificial rocky reefs habitats: a case study in the northern Adriatic Sea. Mar Environ Res 59: 333–348.
4. Heren B, Zahala M, Linares C, Sala E (2005) The effects of predator abundance and habitat structural complexity on survival of juvenile urchins. Mar Biol 146: 293–299.
5. Sala E, Zahala M (1996) Fish predation and the structure of sea urchin *P. lividus* populations in the NW Mediterranean. Mar Ecol Prog Ser 140: 71–81.
6. Lopez S, Turon X, Montero E, Palacin G, Duarte CM, et al. (1998) Larval abundance, recruitment and early mortality in *P. lividus* (Echinodermata). Interannual variability and plankton-benthos coupling. Mar Ecol Prog Ser 172: 239–251.
7. Clemente S, Hernandez JC, Montaño-Moctezuma G, Russell MP, Ebert TA (2013). Predators of juvenile sea urchins and the effect of habitat refuges. Mar Biol 160: 579–580.
8. Scheibling RE, Robinson MC (2008) Settlement behaviour and early post-settlement predation of the sea urchin *Strongylocentrotus droebachiensis*. J Exp Mar Biol Ecol 365: 59–66.
9. Jennings LB, Hunt HL (2010) Settlement, recruitment and potential predators of juvenile echinoderms in the rocky subtidal zone. Mar Biol 157: 307–316.
10. Agatsuma Y (2013) *Strongylocentrotus intermedius*. In: Sea urchins: biology and ecology (ed. John M. Lawrence). Elsevier Amsterdam Press 437–447.
11. Chang Y, Ding J, Song J, Yang W (2004) Biology research and breeding of sea cucumber and sea urchin. Ocean Press, Beijing, China pp: 217–218. (In Chinese).
12. Smith PJ, Wehber WR, Mcveagh SM, Ingles GJ, Gust N (2003) DNA and morphological identification of an invasive swimming crab *Charybdis japonica* (A. Milne-Edwards 1863) in New Zealand waters. N Z J Mar Fresh 37: 753–762.
Covering Behavior and Anti-Predator in Sea Urchins

13. Yu ZH, Yang HS, Liu BZ, Xing K, Xu Q, et al. (2010) Predation of scallop Chlamys farreri by crab Charybdis japonica. Marine Sciences 34(12): 62–65 (in Chinese with an English abstract).
14. Sudo H, Kajihara N, Fujii T (2008) Predation by the swimming crab Charybdis japonica and piscivorous fishes: a major mortality factor in hatchery-reared juvenile Japanese flounder Paralichthys olivaceus released in Mano Bay, Sado Island, Japan. Fish Res 89(1): 49–56.
15. Jiang WM, Meng TX, Chen RS, Wei S (1998) Diet if Charybdis japonica (A.Miline-devarods) and Paracentrotus lividus (miers) in the Bohai Sea. Mar Fish Res 19(1): 54–58 (in Chinese with an English abstract).
16. Shang Y (2005) Ethology. Beijing: Peking University Press 118–188 (in Chinese).
17. Frost AJ, Jack S, Thomson JS, Smith C, Burton HC, et al. (2013) Environmental changes alters personality in the rainbow trout, Oncorhynchus mykiss. Anim Behav 85(6): 1199–1207.
18. Briffa M, Elwood RW (1999) Analysis of the finescale timing of repeated signals: does shell rapping in hermit crabs signal stamina? Anim Behav 59(1): 159–165.
19. Verling E, Crook A, Barnes D (2002) Covering behavior in Paracentrotus lividus: is light important. Mar Biol 140(2): 391–396.
20. Pauwels K, Stoks R, Meester LD (2005) Coping with predator stress: interclonal differences in induction of heat-shock proteins in the water flea Daphnia magna. J Evol Biol 18: 867–872.
21. Ardatova E, Waddell LS, Gurney R (2000) Individual variation in the covering behavior of the shallow water sea urchin Paracentrotus lividus. Mar Ecol 21(4): 275–287.
22. Lawrence JM (1976) Covering response on sea urchins. Nature 262: 490–491.
23. Douglas CA (1976) Availability of drift materials and the covering response of the seaurchin Strongylocentrotus purpuratus (Stimpson). Paci Sci 30: 83–89.
24. Amsler CD, McClintock JB, Baker BJ (1999) An antarctic feeding triangle: defensive interactions between macroalgal, sea urchin, and sea anemones. Mar Ecol Prog Ser 183: 105–114.
25. Agatsuma Y (2001) Effect of the covering behavior of the juvenile sea urchin Strongylocentrotus intermedius on predation by the spider crabs Pagurus bernhardus. Fisheries Sci 67: 1181–1183.
26. Miltown N (1975) The photosensitivity of echinoids. In: F. S. Russell & M. Yonge (Eds.), Advances in marine biology. Academic Press. New York: 1–52.
27. Richner H, Milinski M (2000) On the functional significance of masking behaviour in sea urchins an experiment with Paracentrotus lividus. Mar Ecol Prog Ser 205: 307–308.
28. Adams NL (2001) UV radiation evokes negative phototaxis and covering behavior in the sea urchin Strongylocentrotus droebachiensis. Mar Ecol Prog Ser 213: 87–95.
29. Sigg JE, Lloyd-Knight KM, Boal JG (2007) UV radiation influences covering behaviour in the urchin Lytechinus variegatus. J Mar Biol Ass UK 87: 1257–1261.
30. Dumont CP, Drolet D, Deschenes I, Himmelman JH (2007) Multiple factors explaining the covering behavior in the green sea urchin, Strongylocentrotus droebachiensis. Anim Behav 73: 979–986.
31. Bacher S, Luder S (2005) Picky predators and the function of the faecal shield of a cassidil larva. Funct Ecol 19: 263–272.
32. Williams JL, Moya-Larano J, Wise DH (2006) Burrow decorations as antipredatory devices. Behav Ecol 17: 586–590.
33. Slo S, De Meester L, Stoks R (2009) Behavioural activity levels and expression of stress proteins under predation risk in two damselfish species. Ecol Entomol 34(5): 297–303.
34. Kagawa N, Ryo K, Mugiy Y (1999) Enhanced expression of stress protein 70 in the brains of goldfish, Carassius auratus, reared with bluegills, Lepomis macrochirus. Fish Physiol Biochem 21: 103–110.
35. Murphy ME (2013) The HSP70 family and cancer. Carcinogenesis 34(4): 1181–1188.
36. Slos S, Stoks R (2008) Predator risk induces stress proteins and reduces antioxidant defense. J Exp Biol 22(4): 637–642.
37. Kagawa N, Mugiy Y (2002) Brain HSP70 mRNA expression is linked with plasma cortisol levels in goldfish (Carassius auratus) exposed to a potential predator. Zool Sci 19(7): 735–740.
38. Raman R, Weiland KL, Wagner P (2002) Effects of acute thermal stress on the survival, predator avoidance, and physiology of juvenile fall chinook salmon. Northwest Sci 76(2): 118–120.
39. Pauwels K, Stoks R, Meester LD (2005) Does shell rapping in hermit crabs signal stamina? Anim Behav 59(1): 159–165.
40. Bonaventura R, Poma V, Russo R, Zito F, Marinaga V (2006) Effects of UV-B radiation on development and hsp70 expression in sea urchin cleavage embryos. Mar Biol 149(1): 79–86.
41. Kim T, Agatsuma Y (1996) Predator on released seed of the sea urchin, Strongylocentrotus intermedius in Shiribeshi, Hokkaido, Japan. Fisheries Sci 62: 317–318.
42. Soerensen JG, Looeschke V, Merila J, Laurila A (2011) Effects of predator exposure on Hsp70 expression and survival in tadpoles of the common frog (Rana temporaria). Can J Zool 89: 1249–1255.
43. Zhao C, Feng W, Tian X, Zhou H, Chang Y (2013) Diel patterns of covering behavior by male and female Strongylocentrotus intermedius. Mar Freshw Behav Physiol 46(5): 337–343.
44. Zhou ZC, Bao ZM, Dong Y, Wang LM, Hao CB, et al. (2008) MIP gene expressions at transcription level in different stages of gonad of sea urchin Strongylocentrotus intermedius and hybrids. Heredity 100: 1453–1458 (in Chinese with an English abstract).
45. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using Real-Time quantitative PCR and the 2−ΔΔCT method. Methods 25: 402–408.