



Physiological stress response and gene expression of the Hsp70 and Hsp90 in abalone *Haliotis squamata* under thermal shock

^{1,2}Ngurah S. Yasa, ¹Murwantoko, ¹Alim Isnansetyo,
³Niken S. Nur Handayani, ²Gemi Triastutik, ²Lutfi Anshory

¹ School Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Jl. Flora, Bulaksumur, Yogyakarta, 55281, Indonesia; ² National Broodstock Centre for Shrimp and Mollusk, Karangasem, Bali, Indonesia; ³ Department of Genetics, Faculty of Biology, Gadjah Mada University, Jl. Flora, Bulaksumur, Yogyakarta, 55281, Indonesia.
Corresponding author: N. S. Yasa, ngurahsyasa@gmail.com

Abstract. An increase in temperature can be a significant stressor for aquatic organisms. Abalones, a type of single-shelled gastropod, are the most important commercial species in shellfish cultivation in Indonesia. To evaluate the potential ecological risks posed by temperature stress, we measured biological responses such as survival rates, mucus production, muscle hardness and histologically foot muscle abnormalities in *Haliotis squamata*. In addition, biochemical responses and gene expression profile were also evaluated in the exposed experiment to various temperature gradients. Thermal shock was carried out in 4 rectangular glasses aquarium (100 L). Abalones were divided into four groups for temperature treatment, in triplicates. The temperature treatments were adjusted to 28, 30, 32 and 34°C in advance. Every aquarium contained 3 pcs of 20 cm long 3" PVC pipe for rearing 30 abalones 32.97 ± 1.83 mm per PVC. Following 4 days of temperature exposure, the dead abalones were collected from each aquarium, after which the number of surviving abalones in each aquarium was recorded. These results represented the survivorship at 24, 48, 72, and 96 h. Abalone hemolymph were collected randomly by 1 mL 25 gauge microsyring at 0, 6, 12, 24, 48, and 96 hours. The hemolymph samples were stored in -20°C until analyzed. Increase in temperature stress triggers more mucus production and hardness of the muscle and abnormal foot muscle structure. Furthermore, antioxidant enzyme activity increased in abalone exposed to relatively lower and higher temperatures (28, 32 and 32°C). Super oxide dismutase, catalase and phenol oxidase activity were determined in a time-dependent manner after high temperature pressure. Generally, production of heat shock 70 and 90 proteins also increased significantly in *H. squamata* which were treated in temperatures gradient (more than 30°C) for 6-12 hours. These results provide valuable information about the stress response to increasing temperature in *H. squamata*.
Key Words: *Haliotis squamata*, heat shock protein 70 and 90, superoxide dismutase, phenoloxidase, catalase, mucus production, muscle hardness, histology.

Introduction. Abalone, *Haliotis squamata*, is an indigenous species in Indonesia caught at the western coast of Bali with high commercial value and excellent meat taste. This species is one of the important marine gastropod species in Indonesian aquaculture (Susanto et al 2012; Permana et al 2017; Khotimah et al 2018). In recent years, this species also stocked in waters to improve stock enhancement. However, the market for *H. squamata* has suffered some economic losses because of its sensitivity to stress because of the increasing water temperatures during the grow out and excessive temperature during transportation process and adaptation to the stock enhancement site. Because of its sensitivity to environmental change, especially on adaptation to thermal variation, the molecular mechanisms of cellular stress response were urgently studied. Even though *H. squamata* has been successfully breed in Indonesia, but it still encountered various obstacles in the grow out phase (Permana et al 2017; Susanto et al 2012). The slow growth rate and high susceptible and high mortality to stress due to deteriorating environmental quality of seed are the main constraint in the commonly abalone culture (Cheng et al 2004a; Vosloo et al 2013; Hooper et al 2014).

The heat shock proteins (HSPs), also known as chaperones or stress proteins and extrinsic companions, are ubiquitous protein and widely preserved in prokaryotic and eukaryotic organisms (Kregel 2002; Roberts et al 2010). They play a crucial role in maintenance of structural integrity and regulation of a subset of cytosolic proteins such as degradation, folding, aggregation, stabilization, assembling, and transporting newborn proteins (Morimoto 1998; Sharma et al 2009), and also function as cellular defenses, prevent protein denaturation, and assist in the reintroduction and removal of proteins denatured by biotic and abiotic pressures (Feder & Hofmann 1999; Wang et al 2004). In aquatic organisms, HSP gene expression has been known to increase in response to stresses, such as heat (Cheng et al 2004a; Park et al 2015; Akbarzadeh et al 2018), organic pollutants (Sanders et al 1991), heavy metals (Qian et al 2012), and *Vibrio* infections (Rungrassamee et al 2010), osmolarity (Kultz 1996), low oxygen level (Cheng et al 2004b; Vosloo et al 2013). HSP in eukaryotes is generally categorized into six large families: small HSP, HSP60, HSP70, HSP90, HSP100 and HSP110 according to their molecular weight (Parsell & Lindquist 1993; Feder & Hofmann 1999). In eukaryotes, the HSP70 family consists of an inducible form of stress (HSP) and is stated constitutively (Hsc) differently in the structure and level of their expression under changing environmental conditions (Boutet et al 2003; Piano et al 2005; Cellura et al 2006).

In abalone a large number of antioxidant enzymes have been identified over the last decade and their response against environmental stress have been studied. Excessive production of ROS may, however, lead to oxidative stress and loss of cell function (Wu et al 2011; Wu et al 2014; Park et al 2015). Some antioxidants enzyme activity such as superoxide dismutase (SOD), phenoloxidase (PO) and catalase (CAT) in response to stress have been conducted in various aquaculture species. Study on the effect of SOD on severe heat stress have been conducted on hybrid abalone *H. laevigata* x *H. rubra* (Hooper et al 2014), on *H. discus discus* (Kim et al 2007), SOD and CAT on continuous thermal stress of *H. discus hannai* (Park et al 2015). The phenoloxidase system has been recognized as an efficient defense mechanism against the bacteria and parasites. This system is stored and produced by semi-granular and granular hemocytes, and it can be activated by a minimum presence of microbes (Sritunyalucksana & Söderhäll 2000). A study on PO activity as the key component of immune system in response to low salinity has been conducted on Sydney rock oysters, *Saccostrea glomerata* (Butt et al 2006).

Studies of HSP gene expression profiles in response to various environmental stresses have been conducted on a variety of aquaculture species such as tilapia, *Oreochromis mossambicus* (Molina et al 2000), bay shells, *Chamelea gallina* (Monari et al 2011), *Mytilus galloprovincialis* (Cellura et al 2006), European oyster, *Ostrea edulis* (Piano et al 2005), abalone, *Haliotis discus hannai* (Park et al 2015), white shrimp, *Litopenaeus vannamei* (Qian et al 2012), *Macrobrachium rosenbergii* (Liu et al 2004), tiger shrimp, *Penaeus monodon* (Lo et al 2004) etc. However, most of these studies focus only on one or two HSP genes on temperature, salinity, oxygen, overproduction, and heavy metals. Comparative investigations between biological responses such as mucus production, muscle hardness, biochemical activities, and HSP70 and 90 gene expression profiles are rarely performed in response to these environmental stresses.

Because of its importance in aquaculture, and its sensitivity to environmental variations, this tropical abalone is an ideal mollusk model for studying physiological responses, biochemical activities and molecular mechanisms of cellular stress response. The expression responses of HSP genes (HSP70 and HSP90) under different conditions of heat shock and on different tissues are described in previous studies (Wu et al 2008; Zhou et al 2010; Huang et al 2011). However, there is no comprehensive information on the physiological stress responses, biochemical activities and changes in the expression profile of HSP70 and 90 gene mRNA in response to the environmental stresses caused by thermal shock in this species. To better understand the biological processes in which abalone cope with various environmental pressures, a comparative study of relative expression patterns of mRNA HSP70, and HSP90 in response to thermal stress were done here. Meanwhile, candidate biomarkers of a specific environmental stress in *H. squamata* were assessed on the basis of the results obtained.

Material and Method

Description of the study sites. The study was conducted from July 2017 to August 2018. Abalone juveniles were obtained from the abalone hatchery unit at Sukadana Village, Kubu Sub-District, Karangasem Regency in Bali Province. It took 6 months for abalone seeds production starting from the newly hatched larvae. Larval rearing up to juvenile size of 1 cm was carried out on the rearing plate which was hung on the rearing tank with a total water volume of 1 m³. At this stage abalones were fed with bentic diatoms (*Nitzschia* sp.) attached to the rearing plate. The abalone was graded and transferred into floating baskets and fed with *Gracilaria edulis* in ad libitum when the abalone reached 1 cm. Maintenance with this basket was carried out for 4 months until the abalone seed reaches of 3-4 cm in size.

Animal maintenance. *H. squamata* with total length and weight of 32.97±1.83 mm and 5.13±0.83 g, respectively were collected from the floating baskets and distributed in 20 cm PVC pipe and lied in 1 m³ fiberglass tank with flow through system for 1 week (temperature, 29-30°C; salinity, 34-35 ppt) in the laboratory. The abalones were fed every day with fresh seaweed, *G. edulis* before doing the research. Three pipes were placed in a 100 L glass aquarium as triplicate.

Shock treatment experiment. Three hundred and sixty abalons were distribute into 4 aquaria with the total volume of 100 L for the experiment. Abalones were inserted into 30 cm, 3 inch PVC pipe which both ends cover by PE nets #0.5 cm and tied by rubber bands. Each PVC pipe consisted of 30 abalone juveniles. After acclimation of seawater, six abalones were randomly selected as 0 h samples prior to thermal shock for hemolymph collection. For the experiment abalones were divided into four groups for temperature (28, 30, 32 and 34°C), each with three replicates. During the experiment, salinity was maintained at 34 ppt and pH 7.8 to 8.3.

Evaluation of mucus production. To determine the mucus production we measured the absorbed floating mucus release on the upper layer of water surface by trapping it using the tissue paper. Briefly, 1 pcs of dry tissue paper were weighed and then put on the water surface to absorb the floating mucus. The absorbed mucus was then weighted by analytical measuring unit (Kern ABJ Type 320-4), finally the difference of wet and dry tissue was weighted as the value of mucus production per aquarium. The control group was only absorbing seawater which was free of mucus.

Survival rate of abalones. To determine the survival rate of abalone, 30 juveniles were transferred into each of the triplicate temperature treatment from the beginning of the experiment. The temperatures were adjusted to experimental group (28, 30, 32 and 34°C). Following 4 days of exposure, the dead abalones were collected from each aquarium, after which the number of surviving abalones in each aquarium was recorded. This result represented the survivorship at 24, 48, 72 and 96 h.

Muscle hardness. To observe the effect of temperature gradients on the muscle hardness of abalone, we tested foot muscle of abalone by using a digital shore A hardness Durometer 100HA (Zhengzhou Nanbei Instrument Equipment Co., Ltd. Henan, China) be equipped by type A indenter with pointer journey was 0-2.5 mm. Stress at the end of pointer was 0.55N-8.06N. Type A durometer was used to test hardness of low and medium level of materials like rubber bands and plastics. We used rubber ring as a calibrator.

The tests were done in 0, 12, 24, 48, 72, and 96 hour post treatment. Foot muscle was sliced off 0.7 mm before hardness test. The sample was put on a solid plate before durometer was applied. Durometer was hold to make the pointer at least 12 mm away from the sample. Thus made the pointer straightly goes into the sample till the legs completely get to the sample. The readings were checked in 1 s after test. Such test was repeated 5 times at different place at least 6mm away from test point and find the average readings.

SOD, CAT and PO enzyme activities. Abalone hemolymph was collected randomly by 25 gauge microsyring at 0, 12, 24, 48, and 96 hours. The hemolymph of six abalones at each sampling time was separated and immediately used for antioxidant enzyme assay.

SOD activity was determined by measuring the ability to inhibit the reduction of photochemical nitroblue tetrazolium chloride (NBT), as described previously (Datkhile et al 2009). SOD activity in this experiment was measured with SOD Kit-WST (water soluble tetrazolium salt - www.dojindo.com). The rate of the reduction of WST-1 with O₂ is linearly related to the xanthine oxidase (XO) activity, and this reduction is inhibited by SOD. A unit of SOD activity is defined as the amount of enzyme needed to induce 50% inhibition at the rate of NBT reduction under certain conditions. The result is expressed as unit activity (U) U mg⁻¹ protein extract. The measurement was conducted spectrophotometrically by an ELISA Reader (Heales® MB-580, Shenzhen Huisong Technology Development Co., Ltd. China) at 450 nm.

Catalase activity was measured colorimetrically by CAT activity Assay Kit (GeneWay, Biotech, Inc) according to the manufacturer instruction. The level of H₂O₂ loss (measured at 492 nm) was used to measure CAT activity of the hemolymph sample. The assay was measured by an ELISA reader (Heales® MB-580, Shenzhen Huisong Technology Development Co., Ltd. China).

Phenoloxidase activity was measured spectrophotometrically according to Hooper et al (2014) by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA). Hemolymph plasma (100 µL) was transferred in duplicate to 96-well micro-plates and 100 µL of L-DOPA (30 mM L-3,4-dihydroxyphenylalanine, Sigma D9628, in HCl 0.2 M, pH 8) was added to each well. The microplate was rapidly mixed for 10 s before each plate reading. The absorbance at 492 nm was recorded every 5 min at 20°C over 30 min, using an ELISA reader (Heales® MB-580, Shenzhen Huisong Technology Development Co., Ltd. China). Negative controls, without hemolymph, but containing L-DOPA and Tris HCl buffer, were run in parallel and the values subtracted from test values to correct for possible auto-oxidation of the L-DOPA. Enzyme activity was measured as the change in absorbance min⁻¹ mg⁻¹ of protein.

RNA extraction. Total RNA was extracted from abalone hemolymph from all treatments using Quick-RNA™ MiniPrepPlus Kit (R1058) (Zymo Research, USA) following the manufacturer protocol. The integrity of RNA was assessed by electrophoresis on 1.2% agarose gel. RNA purity was verified by measuring absorbance at 260 and 280 nm with NDD 2000 (Nano Drop Technologies, USA).

Quantification of mRNA expression of HSP genes by real-time PCR. HSP gene expression in hemolymph after heat shock challenge was measured by reverse transcription real-time PCR in Applied Biosystem (ABI, USA). Generally, total RNA was treated with DNaseI (Toyobo, Japan) to remove genomic DNA before reverse transcription. cDNA for each sample was synthesized from the same amount of RNA (50 ng) by ReverTra Ace® qPCR RT Master Mix with gDNA removal (FSQ-301) (Toyobo, Japan) in accordance with the manufacturer protocol with 3 pair primers (Table 1). The cDNA obtained as described above was stored at -20°C until used as a template for PCR reactions.

Table 1

Primer used in the present study

Primer	Sequence (5'-3')	Gene bank accession number	Reference
HSP90 F	CCAGGAAGAATATGCCGAGT	AM283515	Farcy et al (2007)
HSP90 R	CACGGAAGTCCAACTGACC		
HSP70 F	CCGCTCTAGAACTAGTGGAT	AM283516	Farcy et al (2007)
HSP70 R	CCGCCAAGTGGGTGTCT		
β-actin F	GGGTGTGATGGTCGGTAT	AM236595	Farcy et al (2007)
β-actin R	AGCGAGGGCAGTGATTTC		

The stress genes HSP70 and HSP90 were adopted based on Farcy et al (2007). The reference genes β -actin (access number GenBank AM236595) were used as internal controls to calibrate the cDNA template, and the primary pair (β -actin F and β -actin R) for 142 bp product (Farcy et al 2007). The real-time PCR reaction was performed in a 20 μ L reaction system with a mixture of 2 μ L Thunderbird SYBR® qPCR, 2 μ L forward primer (10 μ M), 2 μ L reverse primer (10 μ M), 2 μ L cDNA template equivalent to total RNA (total 50 ng), and 4 μ L free water nuclease. Each reaction was executed in triplicate. The thermal cycle condition was 95°C for 30 seconds, followed by 40 cycles 95°C for 5 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. Melt curve analysis was added (65 to 95°C, with 0.5°C s⁻¹ addition) to show PCR product specimens, as indicated by a single peak. The nuclease free water (Promega, USA) was used instead of the cDNA template as the PCR negative control.

Histological analysis. To observe the effects of temperature gradients on the foot muscle, 30 abalone were transferred into each temperature treatment aquarium at the beginning of the experiment. After 96 h of treatment, the shells were removed, the tissues were immersed and fixed in Bouin's solution, and samples were prepared via paraffinisation. The embedded tissues were then sectioned at a thickness of 5 μ m. The sections were treated with H&E double stain. The stained *H. squamata* tissues were then analyzed for organ morphology by light microscopy (ZEISS Primovert P35-C 2/3" 0,65x). The level of histological alteration in the foot determined from the individual number were divided into 4 quantitative scoring systems according to Park et al (2015).

Statistical analysis. All of the collected data were analyzed statistically by one-way ANOVA analysis using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA) followed by Tukey's test was conducted to compare the means between temperature treatments. P-value < 0.05 was considered statistically significant.

Results

Mucus production of *H. squamata*. The mucus production in 12 h was not significantly different between the 32 and 30°C (control) ($p > 0.05$) but the control was significantly different with two treatment groups (28 and 32°C) in 24 h ($p < 0.05$). However, all of abalones exposed to 34°C died in 12 h and release mucus around 1.8 ± 0.1 g to the water. Although there was no mortality in the control groups (30°C) but they keep continuously release the mucus 2.5 ± 0.173 g/aquarium every day. The mucus production of abalone exposed to 32°C was increased by 50% after 48 h and the production remained stable until 96 h. At the time of 96 h, abalones in the 32°C treatment had an 40% survival rate and abalones in the 28 and 30°C treatments had a > 80% survival rate. Finally, in 96 h, abalones at the 28°C treatment had a 2.2 ± 0.1 g mucus production and those in the 30°C treatment had a 3.3 ± 0.15 g mucus release to the water. The increasing of the mucus production was temperature dependent at higher temperatures (Figure 1).

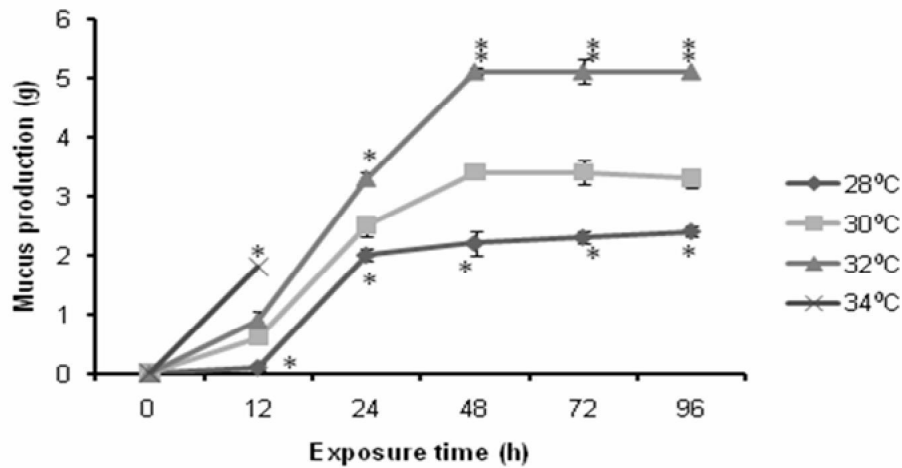


Figure 1. Mucus production of abalone *H. squamata* exposed to temperature gradients for 0 to 96 h. The temperature at 30°C is indicated as the control. An asterisk indicates statistically significant difference, * $p < 0.05$ and ** $p < 0.01$ compared with the control group.

Muscle hardness of *H. squamata*. The foot muscle of abalone was investigated to identify its hardness caused by different temperature shock based on durometer reading for 0 to 96 h (Figure 2). The results showed that muscle hardness was significantly higher in abalones exposed to temperatures over 32°C than in 30°C controls in 12 h ($p < 0.05$). On the other hand, for the abalones which were transferred from temperature 30 to 28°C the muscle hardness was lower than the control ($p < 0.05$). The most significant increase in muscle hardness was observed in abalones at the 34°C in 12 h ($p < 0.05$). The durometer scale showed that the value of muscle hardness was 27.18 ± 1.85 before all of abalone in this group died. After 48 h, muscle hardness was significantly decreased in the 32°C treatment ($p < 0.05$). The activity pattern of muscle hardness was affected in a time-dependent manner at relatively high temperatures.

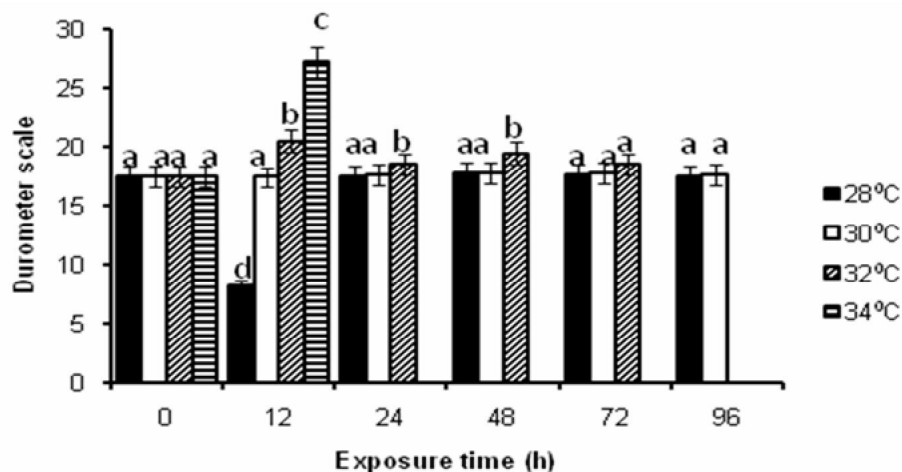


Figure 2. Muscle hardness of abalone *H. squamata* exposed to temperature gradients for 0 to 96 h. The temperature at 30°C is indicated as the control. Different letter indicates a statistically significant difference, $p < 0.05$ compared with the control group.

Survival rate of *H. squamata*. The survival rate after 12 h was similar in between the 28 and 30°C (control) but significantly different with 32°C treatment groups until 96 h ($p < 0.05$). However, abalones exposed to 32 and 34°C, relatively high temperatures, started to die earlier, before 12 h, and all abalones in the 34°C treatment died in 12 h exposure. Otherwise there were only a little mortality in the 28 and 30°C treatments. The survival rate for 72 h was reduced by 50% in the 32°C treatment. At 96 h, abalones in

the 32°C treatment had an 40% survival rate and abalones in the 28 and 30°C treatments had a > 90% survival rate. Finally, at the time of 96 h, abalones in the 28°C treatment had a 96% survival rate and those in the 30°C treatment had a 100% survival rate. The reduction of the survival rate was time-dependent at higher temperatures (Figure 3).

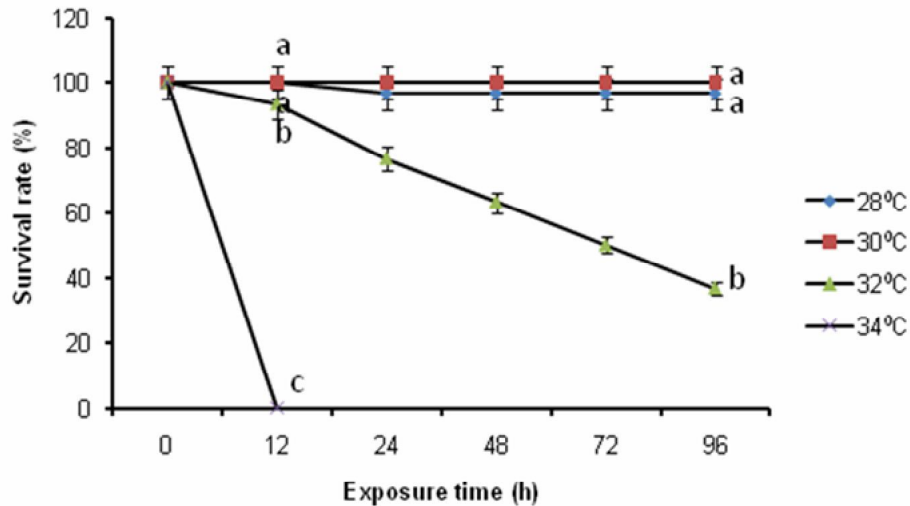


Figure 3. Survival rate (%) of abalone *H. squamata* exposed to temperature gradients for 0 to 96 h. The survival rate at 30°C is indicated as the control. Different letter indicates a statistically significant difference, $p < 0.05$ compared with the control group.

SOD, PO and CAT enzyme activities. The activities of the antioxidant enzymes SOD, PO and CAT were investigated to identify the changes in biochemical activity occurring in response to temperature gradients between 0 and 96 h (Figures 4, 5, 6). The results showed that SOD activity was higher in abalones exposed to temperatures over 30 and 32°C than that in 30°C (control) after 12 h (Figure 4). The most significant increase in SOD activity was observed in abalones at the 32°C treatment in 12 h. After 24 h, SOD activity was significantly decreased in the 32°C treatment ($p < 0.05$) and remained stable until 96h.

Overall PO activity for all of the treatments on (Figure 5) showed that there was no significant difference between control and treatment groups ($p > 0.05$) during 0 to 96 h. The PO activity of all the treatments remained stable until 96 h. PO activity during respected heat shock treatment didn't have significantly effect to the abalone.

Changes in the *H. squamata* CAT enzyme were observed after exposure to temperature gradients for short or long periods (Figure 6). Activities of the CAT enzyme were significantly reduced in the 32 and 34°C treatments in 12 h ($p < 0.05$). Activities of the CAT enzyme were significantly induced at the 32°C treatment in 24 h ($p < 0.05$) and 28°C treatments in 48 h ($p < 0.05$). At the time of 72 h, there was a trend for CAT activities to decrease with temperature 28 and 32°C, although the pattern of CAT activity of 28°C significantly increase in 96 h, but the 32°C treatment remained stable at 0.1413 ± 0.036 nmol mg^{-1} protein/sec. Measurement of CAT enzyme activity at 34°C finished in 12 h after all of abalones died after this treatment. CAT activities were significantly decreased in abalones exposed to high temperatures (32 and 34°C) in 12 h. Afterward CAT activity for 28 and 32°C were significantly higher than control ($p < 0.05$) in 24 h and 48 h respectively.

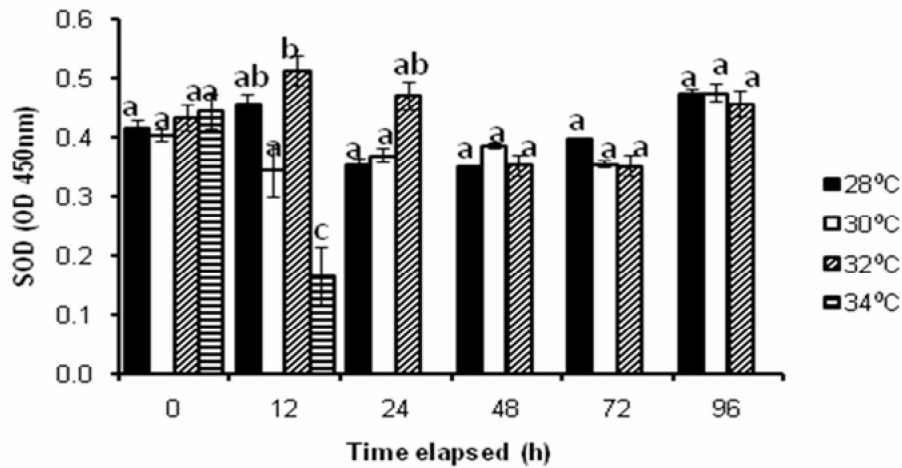


Figure 4. SOD activity in the abalone *H. squamata* exposed to temperature gradients for 0 and 96 h. The experiments were conducted in triplicate, and data represent the mean±SD. Differences between treatments and the 30°C control were considered significant at $p < 0.05$.

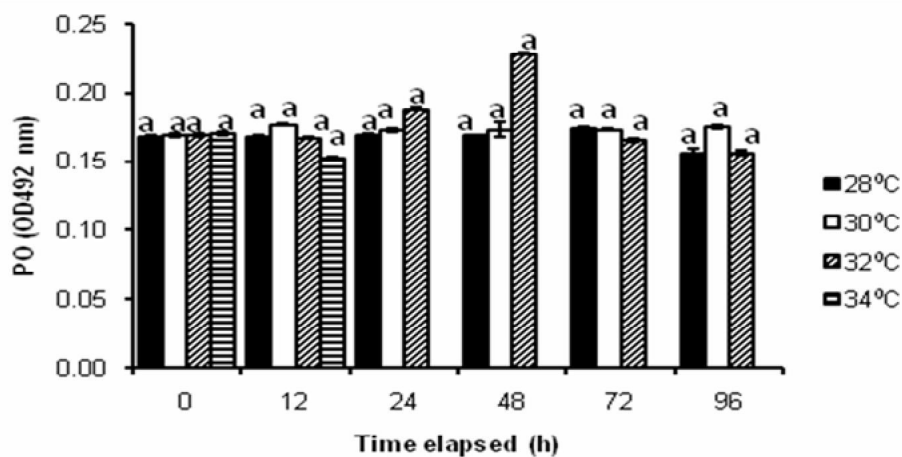


Figure 5. PO activity in the abalone *H. squamata* exposed to temperature gradients for 0 and 96 h. The experiments were conducted in triplicates, and data represent the mean±SD. Differences between treatments and the 30°C control were considered significant at $p < 0.05$.

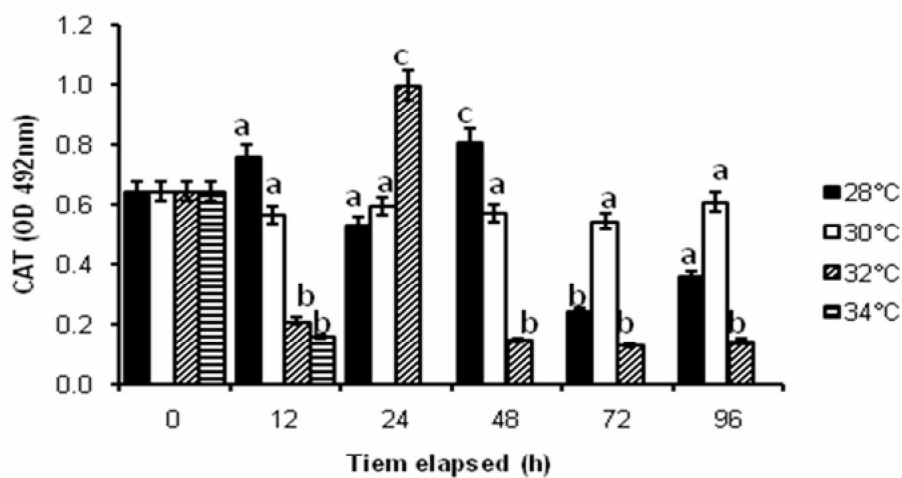
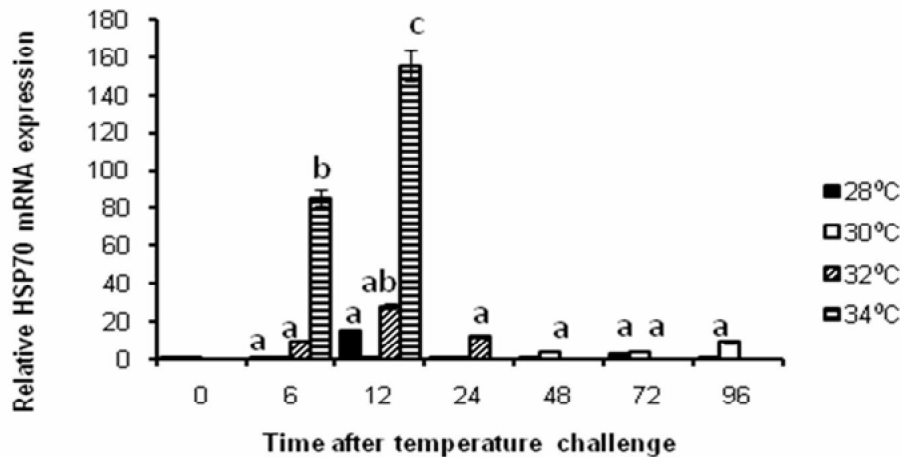
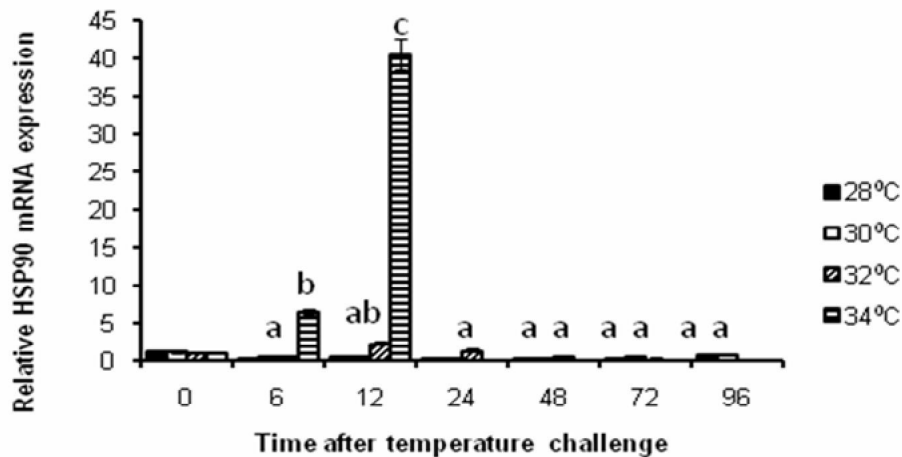


Figure 6. CAT activity in the abalone *H. squamata* exposed to temperature gradients for 0 to 96 h. The experiments were conducted in triplicates, and data represent the mean±SD. Differences between treatments and the 30°C control were considered significant at $p < 0.05$.

Responses of HSP70 and HSP90 genes to continuous thermal stress. Both genes were induced under similar temperature, and similar expression patterns were observed. The relative expression of HSP70 increased rapidly in the first 6-12 hours, peaking (85-100 fold control) for 34°C treatment after thermal exposure, and then gradually decreasing to pretreatment levels after 12 h (Figure 7A). On the other hand we found that HSP90 peaking (6-40 fold control) at 6-12 h. However, the transcription rate of HSP90 is reduced by thermal exposure in 24 hours after treatment and the expression level decreases gradually to the pretreatment level until 96h (Figure 7B).



(A)



(B)

Figure 7. HSP70 (A) and HSP90 (B) relative expression levels after thermal shock to the abalone. The 0, 12, 24, and 48 h denote 0 (untreated). The untreated group was used as the control group, and the ratio of which was considered as 1. Data are presented as means±SD (standard deviation) from independent samples with three technical replicate each (n = 30). Different lowercase letters indicate statistically significant differences (p < 0.05) in relative expression.

Histological analysis of foot structure of *H. squamata*. The histological foot structure of *H. squamata* consisted of muscle layer and hemolymph sinus (Figure 8). The muscle layer was broad and consisted of muscle fiber bundles and hemolymph sinus. Based on the Figure 8 below with the similar magnification, the diameter of hemolymph sinus of the foot structure was increased along with the increasing water temperature. The histological foot structure for 28°C and control relatively only shown a mild alteration on hemolymph sinus diameter, otherwise for 32 and 34°C treatments showed moderate and severe histological alteration on muscle layer respectively. Thus, high water temperatures clearly induced degeneration of mucous cells, swelling of hemolymph sinus

and thus histological changes to the foot structure of abalones. The elevated temperature induced degeneration of muscle fiber, hemocyte infiltration and expansion in the hemolymph sinus (Hs) at the muscle layer (ML) of the foot. The size of hemolymph sinus (Hs) in the muscle layer (ML) was obtained by recording a score of 0-3 for each temperature shock treatment:

- score 0 = 0-10% expansion of hemolymph sinus;
- score 1 = 10-30% expansion of hemolymph sinus;
- score 2 = 30-50% expansion of hemolymph sinus;
- score 3 = 50-70% expansion of hemolymph sinus.

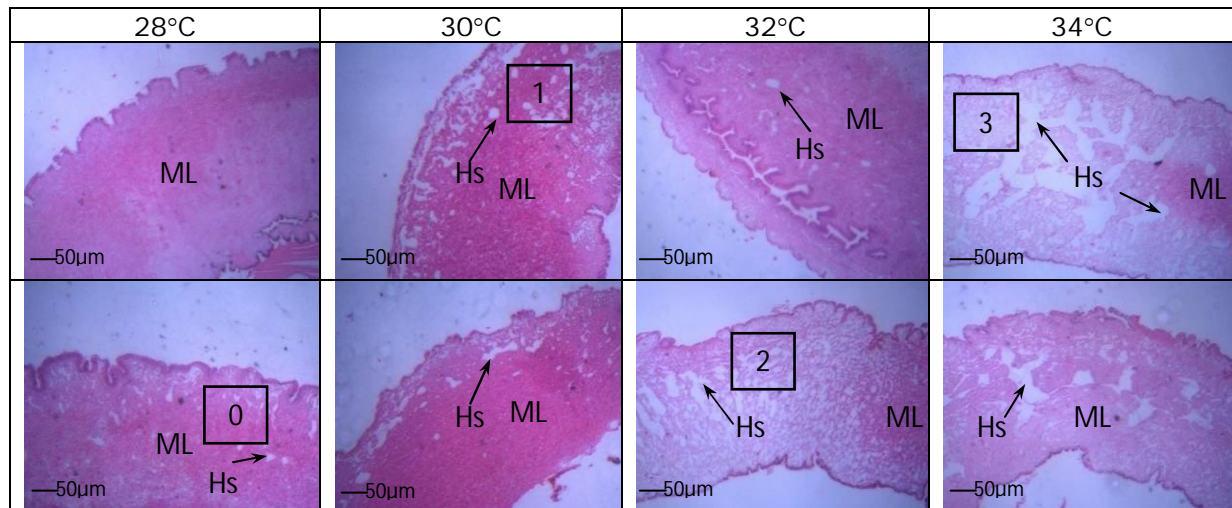


Figure 8. Histological foot structure of abalone *H. squamata* after thermal shock at 28, 30, 32 and 34°C. The 30°C was used as the control group. ML, muscle layer; Hs, hemolymph sinus.

Discussion. In the aquatic environment, almost all animals are vulnerable, being constantly exposed to various environmental stresses that can cause physiological, biochemical, gene expression, and histological changes. One of the protection mechanisms known to be developed by animals in response to environmental pressure are mucus secretion (Peck et al 1993; Connor 1986), increased activity of antioxidant enzymes (Butt et al 2006; Tanner et al 2006) and HSP induction (Zhang et al 2011; Park et al 2015). Eventhough under normal conditions abalone will continuously release the mucus, and its production increases as long as the environmental temperature increases. The mucus production of abalone exposed to 32°C was increased by 50% after 48 h and the production remained stable until 96 h (Figure 1).

Mucus is essential for gastropod protection, locomotion, adhesion and lubrication, as well as the importance of mucus to the gastropod energy budget is well known (Davies et al 1990; Peck et al 1993; Di et al 2011; Smith 2002). Horn (1986) found that mucus production accounted for 70% of consumed energy in *Chiton pelliserpentis*. Most values are not that high. For example, Calow (1974) estimated mucus production to represent 13-32% of absorbed energy in *Planorbis contortus*. Davies & Hawkins (1998) showed that the limpet *Patella vulgata* produced varying amounts of mucus depending on season and habitat, perhaps due to differences in activity level. Peck et al (1993) found that 80% of the daily mucus secretion of the limpet *Nacella concinna* could be attributed to adhesion, with the rest presumably due to locomotory activity. Likewise, Davies et al (1990) found increased mucus secretion in motile compared with stationary limpets. Not only does the amount of mucus secreted change during locomotion, but there is evidence that the nature of the mucus is different. Connor (1986) found that mucus secreted by stationary limpets in the field persisted significantly longer on the substratum than trail mucus.

Abalone muscle which was given excessive heat treatment will experience rheological changes such as elasticity, texture, viscosity so that the hardness of the meat increased (Gao et al 2001). Furthermore Gao et al (2002) reported that in cooked abalone meat heated for 3 hour, they found that changes in texture were attributed

mainly to heat denaturation of the protein. In this experiment heat treatment for more than 30°C for 12 hour had caused increased in muscle hardness and mass mortality for 34°C treatments.

Hemolymph is a major circulating fluid in mollusc that plays an important role in the immune defense. The haemocyte is the main defense cell of mollusk, they are capable of recognition, chemotaxis, attachment followed by agglutination and phagocytosis and exocytosis of antimicrobial factors, scavenging reactive oxygen species (ROS), xenobiotic detoxification (Soderhall & Cerenius 1998; Bachere 2000; Sahaphong et al 2001). Due to its non self recognition capabilities, hemolymph may be sensitive to environmental changes. Post heat shock exposure, hemolymph was taken for the analysis of SOD, CAT, PO and Hsp70 and Hsp90 gene expressions. ROS will be formed when abalones were experienced cellular stress (Farcy et al 2007; Park et al 2015).

At elevated temperature activities of cytosolic and microsomal enzyme that generate ROS in extra mitochondrial compartments also increase and contribute to both elevated rates of metabolism and increase ROS production. The rise in SOD activity suggests that this enzyme is produced in response to ROS production during 12 h heat shock phase can be due to the increased production of superoxide radical as a consequence of pO₂ reestablishment. On the other hand, the low and stable SOD activity during heat shock can be explained by the reduced production of this enzyme's substrate (O₂⁻) as a consequence of limited oxygen availability, similar to what was observed in *Littorina littorea* (Pannunzio & Storey 1998) and *Chasmagnathus granulata* (Oliveira et al 2005). In fact, several studies revealed an increase of SOD activity as a first line of protection against oxidative stress in different marine organisms such as mollusks and corals (Teixeira et al 2013; Park et al 2015).

In this study the provision of heat shock did not result in a change activity of the phenoloxidase enzyme. Butt et al (2006) reported that stress effect by low salinity causes PO activity to decline, resulting in opportunistic infection. In other studies, decreases in salinity have also been shown to negatively affect to PO expression or activity in the yellow leg shrimp, *Farfantepenaeus californiensis* (Vargas-Albores et al 1998). Tanner et al (2006) investigated the effect of hypoxia hypercapnia and pH on PO activity in the Atlantic blue crab, *Callinectes sapidus*. Phenoloxidase activity declined significantly with decreases in pH and oxygen (Tanner et al 2006). Our results suggest that antioxidant SOD and CAT enzymes are potential stress response markers of *H. squamata* following temperature change. Moreover, the activity of SOD and CAT were more sensitive to the temperature change than PO in *H. squamata*.

HSP gene involvement in the heat shock response has been well documented in many aquatic animals. It has been shown that the expression levels of HSP70 and HSP90 on *H. discus* hemolymph were significantly induced within the first 24 hours after exposure to heat (Park et al 2015). Another case was observed in Chinese shrimp *Fenneropenaeus chinensis*, where HSP70 and HSP90 were dramatically induced under heat stings, while hsc70 cognate was slightly affected (Li et al 2009; Luan et al 2010). Similar results were obtained in *M. rosenbergii* (Liu et al 2004). The HSP gene exhibits a very strong response to acute thermal stress at *Crassostrea gigas* with mRNA levels changing up to 40-fold (Farcy et al 2009).

In other aquatic animals such as abalone *H. tuberculata* (Farcy et al 2007), Zhikong *Chlamys farreri* (Gao et al 2007), sea crabs *Portunus trituberculatus* (Zhang et al 2009b) and Chinook salmon *Oncorhynchus tshawytscha* (Palmisano et al 2000), HSP90 transcription can also be caused by heat stings. The above results indicate the important role of HSP genes in overcoming heat stress in some organisms. In this study, we compared expression patterns of HSP70 and HSP90 in response to different heat shock using the similar stocking density conditions in abalone experiment. Our results show that the two hsp genes indicate almost similar expression pattern.

HSP70 and HSP90 are multigene protein families whose expression were caused by various stress factors (Farcy et al 2007). It has been shown that HSP70 expression can be regulated by the thermal exposure which is dependent on temperature and time exposure (Figure 7A). HSP70 induction may modulate cytosolic redox status in cells for protection against oxidative stress (Kalmar & Greensmith 2009). Up-regulation of HSP70

is caused by many denaturation proteins caused by the accumulation of toxic ROS, and is then used to refold and reassemble this protein (Zhang et al 2009a). In temperature treatment, the HSP70 and HSP90 abalone genes were up-regulated from 6 to 12 hours of exposure compared to control (Figure 7A, B).

The abalone defence mechanism can be weakly activated caused by oxidative stress (Huang et al 2011; Vosloo et al 2013). Thus, up-regulation of HSP70 and HSP90 may be attributed to long-term protection mechanisms (Zhang et al 2011). HSP90 influences the folding of newly synthesized polypeptides by providing an environmental companion coupled with translation (Frydman 2001). However, in thermal shock treatment, the mRNA level of the HSP70 transcript was lower than control after 48 h. This could be due to negative regulatory mechanisms (Morimoto et al 1993).

The maximum expressions of HSP70 and HSP90 were observed at 12 hours after exposure to the temperature of 34°C, and it was 155 times and 40 times to the control respectively. After the abalones were exposed to 24 hours, the HSP70 and HSP90 expression decreases very rapidly and becomes the same level as the control groups. The above results show that HSP70 transcription rate was higher than HSP90 against thermal exposure. When HSPs reach the highest point of abundance, the heat shock transcription factor will bind to HSPs and loss of DNA binding activity to heat shock element and decreasing the expression levels of HSPs (Morimoto et al 1993; Ali et al 1998).

The elevation of water temperature dramatically increases expression of both HSP70 and HSP90 gene. Based on current data, we suspect that, among the two known HSP genes in *H. squamata* HSP70 plays a more dominant role in protecting cells from damage caused by acute thermal pressure than HSP90, and this may be the best candidate gene for use as a biomarker to assess temperature increasing in the abalone, *H. squamata* culture.

During elevated temperature exposure, oxidative stress was induced in *H. squamata* and caused histological alterations in foot. Thus, high water temperatures clearly induced production of mucous cells and swelling of hemolymph sinus and histological changes to the foot structure of abalones. The histological alterations of the foot muscle in abalones increased with increasing water temperature. The abnormal foot may be harmful to abalone locomotion, community behavior, protection from external threats, and overall health activity. The elevated temperatures induced expansion in the hemolymph sinus (Hs) at the muscle layer (ML) of the foot of abalone, *H. squamata*.

Conclusions. The expression profiles of HSP70 and HSP90 in hemolymph abalone under thermal shock which are induced under the treatment of temperature in this study, both of them show a similar pattern of expression after different thermal shock. The profile of antioxidant enzyme activity such as SOD and CAT also distinguished from different HSP genes and the presence of different time exposure of temperature. Our results provide useful insights for investigating cellular stress related responses and for identifying potential stress-causing environmental biomarkers in abalone, *H. squamata*. Besides, there is also a need to know how abalone response when exposed simultaneously to several stress factors in the environmental context, where many stress factors are regulatory rather than exception. Our study is the first to report on the physiological stress response by using mucus production and muscle hardness parameter, differential expression of the mRNA gene of HSP70 and HSP90 genes in response to different thermal pressures on hemolymph of abalone, *H. squamata*. And also the first to evaluate the potential of the SOD and CAT as biomarker molecules to overcome the environment pressure on this abalone.

Acknowledgements. This study was supported by a grant from the National Agency of Management Education and Fund (LPDP) of the Republic of Indonesia based on Kep. No. KEP-14 / LPDP / 2016. We appreciated Mr. B. R. Basuki and Ni Putu Sumaryati for their assistance in this experiment.

References

- Akbarzadeh A., Günther O. P., Houde A. L., Li S., Ming T. J., Jeffries K. M., Hinch S. G., Miller K. M., 2018 Developing specific molecular biomarkers for thermal stress in salmonids. *BMC Genomics* 19:749.
- Ali A., Bharadwaj S., O'Carroll R., Ovsenek N., 1998 HSP90 interacts with and regulates the activity of heat shock factor 1 in *Xenopus* oocytes. *Molecular and Cellular Biology* 18(9):4949-4960.
- Bachère E., 2000 Shrimp immunity and disease control. *Aquaculture* 191:3-11.
- Boutet I., Tanguy A., Moraga D., 2003 Organization and nucleotide sequence of the European flat oyster *Ostrea edulis* heat shock cognate 70 (hsc70) and heat shock protein 70 (hsp70) genes. *Aquatic Toxicology* 65(2):221-225.
- Butt D., Shaddick K., Raftos D., 2006 The effect of low salinity on phenoloxidase activity in the Sydney rock oyster, *Saccostrea glomerata*. *Aquaculture* 251(2):159-166.
- Calow P., 1974 Some observations on locomotory strategies and their metabolic effects in two species of freshwater gastropods, *Ancylus fluviatilis* Mull, and *Planorbis contortus* Linn. *Oecologia* 16(2):149-161.
- Cellura C., Toubiana M., Parrinello N., Roch P., 2006 HSP70 gene expression in *Mytilus galloprovincialis* hemocytes is triggered by moderate heat shock and *Vibrio anguillarum*, but not by *V. splendidus* or *Micrococcus lysodeikticus*. *Developmental and Comparative Immunology* 30(11):984-997.
- Cheng W., Hsiao I. S., Hsu C. H., Chen J. C., 2004a Change in water temperature on the immune response of Taiwan abalone (*Haliotis diversicolor supertexta*) and its susceptibility to *Vibrio parahaemolyticus*. *Fish and Shellfish Immunology* 17(3):235-243.
- Cheng W., Li C. H., Chen J. C., 2004b Effect of dissolved oxygen on the immune response of *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus*. *Aquaculture* 232:103-115.
- Connor V. M., 1986 The use of mucous trails by intertidal limpets to enhance food resources. *Biological Bulletin* 171(3):548-564.
- Datkhile K. D., Mukhopadhyaya R., Dongre T. K., Nath B. B., 2009 Increased level of superoxide dismutase (SOD) activity in larvae of *Chironomus ramosus* (Diptera: Chironomidae) subjected to ionizing radiation. *Comparative Biochemistry and Physiology C. Toxicology and Pharmacology* 149(4):500-506.
- Davies M. S., Hawkins S. J., 1998 Mucus from marine mollusk. *Advances in Marine Biology* 34:1-71.
- Davies M. S., Jones H. D., Hawkins S. J., 1990 Seasonal variation in the composition of pedal mucus from *Patella vulgata* L. *Journal of Experimental Marine Biology and Ecology* 144(2-3):101-112.
- Di G., Ni J., Zhang Z., You W., Wang B., Ke C., 2012 Types and distribution of mucous cells of the abalone *Haliotis diversicolor*. *African Journal of Biotechnology* 11(37):9127-9140.
- Farcy E., Serpentine A., Fiévet B., Lebel J. M., 2007 Identification of cDNAs encoding HSP70 and HSP90 in the abalone *Haliotis tuberculata*: transcriptional induction in response to thermal stress in hemocyte primary culture. *Comparative Biochemistry and Physiology B. Biochemistry and Molecular Biology* 146(4):540-550.
- Farcy E., Voiseux C., Lebel J. M., Fievet B., 2009 Transcriptional expression levels of cell stress marker genes in the Pacific oyster *Crassostrea gigas* exposed to acute thermal stress. *Cell Stress and Chaperones* 14:371-380.
- Feder M. E., Hofmann G. E., 1999 Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology* 61:243-282
- Frydman J., 2001 Folding of newly translated protein *in vivo*: the role of molecular chaperones. *Annual Review of Biochemistry* 70:603-647.
- Gao Q., Song L., Ni D., Wu L., Zhang H., Chang Y., 2007 cDNA cloning and mRNA expression of heat shock protein 90 gene in the hemocytes of Zhikong scallop *Chlamys farreri*. *Comparative Biochemistry and Physiology B. Biochemistry and Molecular Biology* 147(4):704-715.

- Gao X., Ogawa H., Tashiro Y., Iso N., 2001 Rheological properties and structural changes in raw and cooked abalone meat. *Fisheries Science* 67(2):314-320.
- Gao X., Tashiro Y., Ogawa H., 2002 Rheological properties and structural changes in steamed and boiled abalone meat. *Fisheries Science* 68(3):499-508.
- Hooper C., Day R., Slocombe R., Benkendorff K., Handlinger J., Goulias J., 2014 Effects of severe heat stress on immune function, biochemistry and histopathology in farmed Australian abalone (hybrid *Haliotis laevis* × *Haliotis rubra*). *Aquaculture* 432:26-37.
- Horn P. L., 1986 Energetics of *Chiron pelliserpenris* (Quor & Gaimard, 1835) (Mollusca: Polyplacophora) and the importance of mucus in its energy budget. *Journal of Experimental Marine Biology and Ecology* 101:119-141.
- Huang W. J., Leu J. H., Tsau M. T., Chen J. C., Chen L. L., 2011 Differential expression of LvHSP60 in shrimp in response to environmental stress. *Fish and Shellfish Immunology* 30(2):576-582.
- Kalmar B., Greensmith L., 2009 Induction of heat shock proteins for protection against oxidative stress. *Advanced Drug Delivery Reviews* 61(4):310-318.
- Khotimah F. H., Permana G. N., Rusdi I., Susanto B., 2018 Pemeliharaan larva abalon *Haliotis squamata* dengan pemberian jenis pakan berbeda dalam bentuk tepung. *Jurnal Riset Akuakultur* 13(1):39-46. [in Indonesian]
- Kim K. Y., Lee S. Y., Cho Y. S., Bang I. C., Kim K. H., Kim D. S., Nam Y. K., 2007 Molecular characterization and mRNA expression during metal exposure and thermal stress of copper/zinc- and manganese-superoxide dismutases in disk abalone, *Haliotis discus discus*. *Fish and Shellfish Immunology* 23(5):1043-1059.
- Kregel K. C., 2002 Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *Journal of Applied Physiology* 92(5):2177-2186.
- Kultz D., 1996 Plasticity and stressor specificity of osmotic and heat shock responses of *Gillichthys mirabilis* gill cells. *American Journal of Physiology* 271(4):1181-1193.
- Li Z. W., Li X., Yu Q. Y., Xiang Z. H., Kishino H., Zhang Z., 2009 The small heat shock protein (sHSP) genes in the silkworm, *Bombyx mori*, and comparative analysis with other insect sHSP genes. *BMC Evolutionary Biology* 9:215.
- Liu J., Yang W. J., Zhu X. J., Karouna-Renier N. K., Rao R. K., 2004 Molecular cloning and expression of two HSP70 genes in the prawn, *Macrobrachium rosenbergii*. *Cell Stress and Chaperones* 9(3):313-323.
- Lo W. Y., Liu K. F., Liao I. C., Song Y. L., 2004 Cloning and molecular characterization of heat shock cognate 70 from tiger shrimp (*Penaeus monodon*). *Cell Stress and Chaperones* 9(4):332-343.
- Luan W., Li F., Zhang J., Wen R., Li Y., Xiang J., 2010 Identification of a novel inducible cytosolic Hsp70 gene in Chinese shrimp *Fenneropenaeus chinensis* and comparison of its expression with the cognate Hsc70 under different stresses. *Cell Stress and Chaperones* 15(1):83-93.
- Molina A., Biemar F., Müller F., Iyengar A., Prunet P., Maclean N., Martial J. A., Muller M., 2000 Cloning and expression analysis of an inducible HSP70 gene from tilapia fish. *FEBS Letters* 474(1):5-10.
- Monari M., Foschi J., Rosmini R., Marin M. G., Serrazanetti G. P., 2011 Heat shock protein 70 response to physical and chemical stress in *Chamelea gallina*. *Journal of Experimental Marine Biology and Ecology* 397(2):71-78.
- Morimoto R. I., 1998 Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes and Development* 12(24):3788-3796.
- Morimoto R. I., Tissieres A., Cesrgopoulos C., 1993 Cell in stress: transcriptional activation of heat shock genes. *Science* 259(5100):1409-1410.
- Oliveira U. O., Araújo A. S. R., Belló-Klein A., da Silva R. S. M., Kucharski L. C., 2005 Effects of environmental anoxia and different periods of reoxygenation on oxidative balance in gills of the estuarine crab *Chasmagnathus granulata*. *Comparative Biochemistry and Physiology B. Biochemistry and Molecular Biology* 140(1):51-57.
- Palmisano A. N., Winton J. R., Dickhoff W. W., 2000 Tissue-specific induction of Hsp90 mRNA and plasma cortisol response in Chinook salmon following heat shock, seawater challenge, and handling challenge. *Marine Biotechnology* 2(4):329-338.

- Pannunzio T. M., Storey K. B., 1998 Antioxidant defenses and lipid peroxidation during anoxia stress and aerobic recovery in the marine gastropod *Littorina littorea*. *Journal of Experimental Marine Biology and Ecology* 221(2):277-292.
- Park K., Lee J. S., Kang J. C., Kim J. W., Kwak I. S., 2015 Cascading effects from survival to physiological activities, and gene expression of heat shock protein 90 on the abalone *Haliotis discus hannai* responding to continuous thermal stress. *Fish and Shellfish Immunology* 42(2):233-240.
- Parsell D. A., Lindquist S., 1993 The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annual Review of Genetics* 27:437-496.
- Peck L. S., Prothero-Thomas E., Hough N., 1993 Pedal mucus production by the Antarctic limpet *Nacella concinna* (Strebel, 1908). *Journal of Experimental Marine Biology and Ecology* 174(2):177-192.
- Permana G. N., Khotimah F. H., Susanto B., Rusdi I., Haryanti, 2017 Keragaan pertumbuhan dan reproduksi abalon *Haliotis Squamata* Reeve (1846) turunan ketiga. *Jurnal Riset Akuakultur* 12(3):197-202.
- Piano A., Franzellitti S., Tinti F., Fabbri E., 2005 Sequencing and expression pattern of inducible heat shock gene products in the European flat oyster, *Ostrea edulis*. *Gene* 361:119-126.
- Qian Z., Liu X., Wang L., Wang X., Li Y., Xiang J., Wang P., 2012 Gene expression profiles of four heat shock proteins in response to different acute stresses in shrimp, *Litopenaeus vannamei*. *Comparative Biochemistry and Physiology, Part C* 156(3-4):211-220.
- Roberts R. J., Agius C., Saliba C., Bossier P., Sung Y. Y., 2010 Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: a review. *Journal of Fish Diseases* 33(10):789-801.
- Rungrassamee W., Leelatanawit R., Jiravanichpaisal P., Klinbunga S., Karoonuthaisiri N., 2010 Expression and distribution of three heat shock protein genes under heat shock stress and under exposure to *Vibrio harveyi* in *Penaeus monodon*. *Developmental and Comparative Immunology* 34(10):1082-1089.
- Sahaphong S., Linthong V., Wanichanon C., Riengrojpitak S., Kangwanrangsan N., Viyanant V., Upatham E. S., Pumthong T., Chansue N., Sobhon P., 2001 Morphofunctional study of the hemocytes of *Haliotis asinina*. *Journal of Shellfish Research* 20(2):711-716.
- Sanders B. M., Martin L. S., Nelson W. G., Phelps D. K., Welch W., 1991 Relationship between accumulation of a 60 kDa stress protein and scope-for-growth in *Mytilus edulis* exposed to a range of copper concentrations. *Marine Environmental Research* 31(2):81-97.
- Sharma S. K., Christen P., Goloubinoff P., 2009 Disaggregating chaperones: an unfolding story. *Current Protein and Peptide Science* 10(5):432-446.
- Smith A. M., 2002 The structure and function of adhesive gels from invertebrates. *Integrative and Comparative Biology* 42(6):1164-1171.
- Soderhall K., Cerenius L., 1998 Role of prophenoloxidase-activating system in invertebrate immunity. *Current Opinion in Immunology* 10(1):23-28.
- Sritunyalucksana K., Söderhäll K., 2000 The proPo and clotting system in crustaceans. *Aquaculture* 191:53-69.
- Susanto B., Rusdi I., Giri I. N. A., 2012 Optimalisasi pemeliharaan yuwana abalon (*H. squamata*) dengan kepadatan dan jenis pakan yang berbeda. *Jurnal Riset Akuakultur* 7(1):21-31.
- Tanner C. A., Burnett L. E., Burnett K. G., 2006 The effects of hypoxia and pH on phenoloxidase activity in the Atlantic blue crab, *Callinectes sapidus*. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 144(2):218-223.
- Teixeira T., Diniz M., Calado R., Rosa R., 2013 Coral physiological adaptations to air exposure: heat shock and oxidative stress responses in *Veretillum cynomorium*. *Journal of Experimental Marine Biology and Ecology* 439:35-41.

- Vargas-Albores F., Hinojosa-Baltazar P., Portillo-Clark G., Magallon-Barajas F., 1998 Influence of temperature and salinity on the yellow leg shrimp, *Penaeus californiensis* Holmes, prophenoloxidase system. *Aquaculture Research* 29(8):549-553.
- Vosloo D., van Rensburg L., Vosloo A., 2013 Oxidative stress in abalone: the role of temperature, oxygen and L-proline supplementation. *Aquaculture* 416-417:265-271.
- Wang W., Vinocur B., Shoseyov O., Altman A., 2004 Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in Plant Science* 9(5):244-252.
- Wu C., Zhang W., Mai K., Xu W., Zhong X., 2011 Effects of dietary zinc on gene expression of antioxidant enzymes and heat shock proteins in hepatopancreas of abalone *Haliotis discus hannai*. *Comparative Biochemistry and Physiology, Part C* 154(1):1-6.
- Wu C., Wang J., Xu W., Zhang W., Mai K., 2014 Dietary ascorbic acid modulates the expression profile of stress protein genes in hepatopancreas of adult Pacific abalone *Haliotis discus hannai* Ino. *Fish and Shellfish Immunology* 41(2):120-125.
- Wu R., Sun Y., Lei L. M., Xie S. T., 2008 Molecular identification and expression of heat shock cognate 70 (HSC70) in the Pacific white shrimp *Litopenaeus vannamei*. *Molecular Biology* 42(2):234-242.
- Zhang H. Y., Hormi-Carver K., Zhang X., Spechler S. J., Souza R. F., 2009a In benign Barrett's epithelial cells, acid exposure generates reactive oxygen species that cause DNA double-strand breaks. *Cancer Research* 69(23):9083-9089.
- Zhang X. Y., Zhang M. Z., Zheng C. J., Liu J., Hu H. J., 2009b Identification of two hsp90 genes from the marine crab, *Portunus trituberculatus* and their specific expression profiles under different environmental conditions. *Comparative Biochemistry and Physiology, Part C* 150(4):465-473.
- Zhang W., Wu C., Mai K., Chen Q., Xu W., 2011 Molecular cloning, characterization and expression analysis of heat shock protein 90 from Pacific abalone, *Haliotis discus hannai* Ino in response to dietary selenium. *Fish and Shellfish Immunology* 30(1):280-286.
- Zhou J., Wang L., Xin Y., Wang W. N., He W. Y., Wang A. L., Liu Y., 2010 Effect of temperature on antioxidant enzyme gene expression and stress protein response in white shrimp, *Litopenaeus vannamei*. *Journal of Thermal Biology* 35:284-289.

Received: 09 May 2019. Accepted: 23 August 2019. Published online: 12 October 2019.

Authors:

Ngurah Sedana Yasa, School Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Jl. Flora, Bulaksumur, Yogyakarta, 55281, Indonesia; National Broodstock Centre for Shrimp and Mollusk, Karangasem, Bali, 80811, Indonesia, e-mail: ngurahsyasa@gmail.com

Murwantoko, School Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Jl. Flora, Bulaksumur, Yogyakarta, 55281, Indonesia, e-mail: murwantoko@yahoo.com

Alim Isnansetyo, School Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Jl. Flora, Bulaksumur, Yogyakarta, 55281, Indonesia, e-mail: isnansetyo@yahoo.com

Niken Satuti Nur Handayani, Department of Genetics, Faculty of Biology, Gadjah Mada University, Jl. Flora, Bulaksumur, Yogyakarta, 55281, Indonesia, e-mail: niken_satuti@ugm.ac.id

Gemi Triastutik, National Broodstock Centre for Shrimp and Mollusk, Karangasem, Bali, 80811, Indonesia, e-mail: gtriastutik@yahoo.com

Lutfi Anshory, National Broodstock Centre for Shrimp and Mollusk, Karangasem, Bali, 80811, Indonesia, e-mail: lutfi.anshory@gmail.com

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

How to cite this article:

Yasa N. S., Murwantoko, Isnansetyo A., Handayani N. S. N., Triastutik G., Anshory L., 2019 Physiological stress response and gene expression of the Hsp70 and Hsp90 in abalone *Haliotis squamata* under thermal shock. *AAFL Bioflux* 12(5): 1672-1687.