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Light cycle effects on *Haliotis discus hannai* Ino growth, energy budget, and related gene expression

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ABSTRACT

This study investigated the effects of different light cycles on growth, food intake, energy budget, and related gene expression of the abalone *Haliotis discus hannai* Ino. Abalones (mean ± standard error shell length: 30.28 ± 1.64 mm, body weight: 4.51 ± 0.49 g) were exposed to the following light cycles: 0L:24D, 4L:20D, 8L:16D, 12L:12D, and 16L:8D for 60 days, four repetitions were provided for each light cycle. At the end of the experiment, there was no significant difference in shell length or body weight growth rate between 0L:24D and 4L:20D ($P > 0.05$), the values of which were significantly higher than in 16L:8D ($P < 0.05$). In 0L:24D, abalone food intake was significantly higher compared with in the other groups ($P < 0.05$), but the food conversion efficiency was significantly lower than in 4L:20D ($P < 0.05$). At the end of the experiment, in 16L:8D, the activity of hexokinase was significantly higher than in any other group ($P < 0.05$), but in 12L:12D and 16L:8D, it significantly increased compared with Day 30 ($P < 0.05$). In 0L:24D, 4L:20D, and 8L:16D, cellulase and pyruvate kinase activity was significantly higher than in 12L:12D and 16L:8D ($P < 0.05$). There was no significant difference in the energy acquired from food in 0L:24D and 4L:20D ($P > 0.05$). However, the energy loss via feces and respiratory metabolism in 0L:24D was significantly higher than in 4L:20D ($P < 0.05$); therefore, the energy accumulated for individual growth was significantly lower than in 4L:20D ($P < 0.05$). Although the energy loss via feces, excretion, and respiratory metabolism was significantly lower in 16L:8D compared with 0L:24D, 4L:20D, and 8L:16D, the ratio of the energy loss via feces and respiratory metabolism to the energy acquired from food intake was significantly higher than in any other group ($P < 0.05$); therefore, the net growth efficiency K_2 was significantly lower ($P < 0.05$). In 16L:8D, gene expression levels of manganese superoxide dismutase (Mn-SOD), catalase (CAT), TP_x, heat shock protein (HSP) 26, HSP70, and HSP90 were significantly higher than in any other group ($P < 0.05$). At the end of the experiment, *Mn-SOD*, *CAT*, and *HSP70* expression in 16L:8D significantly increased compared with Day 30 ($P < 0.05$). Therefore, considering aquaculture production and business costs, it would be appropriate to select the light cycle of 4L:20D for the culture of *H. d. hannai*.

1. Introduction

The light environment is a complex, volatile, and important ecological factor (Taylor et al., 2006; Wang et al., 2013), which mainly comprises light intensity, light cycle, and spectral composition. Its changes show strong stability and marked regularity, with multiple properties. Change in each light characteristic has a specific ecological role, which may directly or indirectly affect the physiological and ecological properties of cultured organisms (Begtashi et al., 2004; Felip et al., 2008). The light cycle is considered the primary zeitgeber that provides the biological rhythm for animal growth and development (Bromage et al., 2001). It is the only environmental factor that provides animals with a stable seasonal rhythm, which is essential for normal physiological development of aquatic organisms (Wood and Loudon, 2014).

Eyes are the critical photoreceptors of aquatic organisms. The retina is the physiological basis for visual formation in fish and its morphological characteristics are closely associated with fish ecotype. In this sense, the retinal structure is suitable for the study of fish ecological habits and environmental adaptation (Bailes et al., 2006; Gehring, 2004). *Haliotis discus hannai* Ino is a typically nocturnal abalone and its food intake activities show significant circadian rhythm; 2–3 h after

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sunset and 2–3 h before sunrise comprise the most intensive activity timeframes (Ahmed et al., 2013). Gao et al. (2016a, 2016b) found that abalone eyes have a complete retinal structure comprising the retinal pigment epithelium, outer nuclear layer, photoreceptor inner segment, inner nuclear layer, melanin granule deposit layer, and visual fiber layer from inside out. Accordingly, based on the structural features of abalone retina structure and food intake rhythm, it is possible to manually construct and optimize the appropriate light environment for individual growth.

Garcia-Esquivel et al. (2007) found that *Haliotis fulgens* had the highest growth rate in a 00:24 light (L):dark (D) cycle. In a dark setting, the survival rate and food intake rate of *Haliotis corrugata* larvae were significantly higher than those of the group under constant lighting (Gorrostieta-Hurtado et al., 2009). In light conditions, the oxygen consumption rate and ammonia excretion rate of *Haliotis discus* were significantly higher than those of a group in a dark setting (Ahmed et al., 2008). In a lightproof water tank, the growth rate of *Haliotis rubra* was 15.65% faster than that in the light (Day et al., 2004). Therefore, the light cycle has been shown to have a significant effect on abalone growth, food intake, and metabolism, but how does it affect the food intake of abalone that lead to the growth differences? Furthermore, how do abalone form physiological adaptation mechanisms? These questions remain to be clarified.

Therefore, this study aimed to examine the effects of different light cycles on the growth, food intake, and energy metabolism of *H. d. hannai* using bioenergy and molecular biology in order to elucidate the causes of abalone growth differences and physiological adaptation mechanisms in different light cycles. It also aimed to provide a reference for the optimization and regulation of light environment factors during the course of aquaculture production.

2. Materials and methods

2.1. Abalone source and acclimation

This experiment was performed at the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, Shandong, China from November 18, 2016 to January 18, 2017. Juvenile abalones were purchased from Changqing Ocean Science & Technology Co., Ltd. (Weihai, Shandong, China), and all experimental abalones were sourced from the same batch after artificial hatching. After purchasing, abalones were acclimated in four culture containers (1.2 m × 1 m × 1 m, water volume: 1200 L) for 15 days. The culture conditions were as follows: water temperature: 18 °C, salinity: 30 ± 1, pH: 8.2, concentration of dissolved oxygen > 6 mg/L, and natural light cycle. Aquaculture water comprised natural seawater that was supplied after sedimentation and sand filtration. Fresh seawater was exchanged daily at 9 am, equaling 2/3 of water volume to ensure good water quality. In the acclimation period, *Laminaria japonica* Aresch (crude protein: 13.11% ± 0.19%; fat: 1.45% ± 0.16%; ash: 16.02% ± 0.43%; humidity: 88.52% ± 0.24%) was added to the container to feed abalone daily at 17:00 pm, with the feeding quantity equivalent to 6% of wet body weight of abalone, in order to keep them fully fed.

2.2. Experimental design

Light-emitting diodes were provided by the Institute of Semiconductors, Chinese Academy of Sciences, the white-light lamp (main peak: 460 nm) was in full spectrum to simulate the natural light, which hung above the aquarium in a serial connection. The light intensity in different groups was kept the same as much as possible by adjusting the distance between each lamp and the water surface, which was measured at 6.28 ± 1.17 μmol/m^2/s with a spectroradiometer (JETI LiVal Spectroradiometer Model Specbos 1xx1, Jena, Germany). Five different light cycle groups were designed; 0L:24D, 4L:20D, 8L:16D, 12L:12D, and 16L:8D, each lamp was opened or closed by the clock controller. Twenty aquariums (50 cm × 40 cm × 35 cm, water volume: 70 L) were used throughout the experiment and four repetitions were provided for every treatment group.

A total of 24 abalones were placed into each aquarium (shell length: 30.28 ± 1.64 mm, body weight: 4.51 ± 0.49 g), different groups of light cycles were divided with a black-out cloth to avoid mutual interference. Each group was exposed to only a few light rays during the dark periods from the water exchange, feeding, and collecting residual food and feces. Throughout the experiment, the indoor temperature was maintained at 17 °C with an air conditioner, the salinity and pH were the same as in the acclimation period. Abalones were fed only once daily at 17:00 pm and the feeding amount was 6% of the wet weight. The next morning, residual food and feces were siphoned; after precipitation, they were dried in an oven at 70 °C until they reached constant weight, they were then stored in a refrigerator at − 20 °C for the subsequent determination of energy value and nitrogen content. At the same time, fresh kelp (*L. japonica*) as a feeding stuff was added to another three aquariums to measure the possible loss of food. The next morning, the kelps were collected, weighed, and dried, then the amount of residual food was calculated and used for the numerical correction of food intake. During the course of the experiment, 4/5 of water was exchanged daily, and each aquarium was washed once every 3 days. Each aquarium was provided with continuous aeration to maintain the concentration of dissolved oxygen above 6 mg/L, but the aeration was stopped 20 min before the collection of residual food. The ammonia nitrogen concentration throughout the experiment did not exceed 0.2 mg/L.

2.3. Sample collection

All abalones were starved for 24 h and were randomly placed in different light cycle groups before the start of the experiment. Prior to the experiment, 15 abalones were randomly selected and used as the initial samples for later analysis to determine the initial weight, energy, and crude protein content. After the start of the experiment, 40 abalones were randomly selected from each light cycle group every month for the measurement of shell length and weight, and the survival rate of abalones in each light cycle group was counted. Ten abalones were randomly collected from each light cycle group, and the activity of hexokinase (HK), pyruvate kinase (PK), pepsin (PES), cellulase (CL), and α-amylase (AMS) in the hepatopancreas tissues, and the gene expression levels of manganese superoxide dismutase (Mn-SOD), catalase (CAT), $TP_{x,}$ heat shock protein (HSP) 26, HSP70, and HSP90 in the samples were determined. The abalones were then dried in the oven at 75 °C until constant weight for the determination of energy values. Four replicates were set in the experiment, each replicate contained 10 individuals, the samples were measured three times, and the mean value was recorded.

2.4. Sample analysis

2.4.1. Enzyme activity

The abalone body surface water content was removed using absorbent paper, then the abalone was rapidly dissected on the ice tray. After being shelled, the hepatopancreas was removed and placed in a 1.5-mL centrifuge tube, the sample was evenly divided into two sections, quickly placed in liquid nitrogen, and later placed in a refrigerator at − 80 °C for subsequent testing. Following this, 0.2–0.4 g of hepatopancreas tissues were collected and fully ground with the addition of 1.8 mL 0.86% saline in an ice-water bath. The sample was then ground, centrifuged at 3500 × *g*/min for 10 min, until 10% tissue homogenates were obtained to determine the activity of HK and PK. For the determination of PES, CL, and AMS, 0.2–0.4 g of tissues was added to 1.8 mL 0.86% normal saline to be fully ground in an ice-water bath. After this, the tissues were centrifuged at 2500 × *g*/min, 8000 × *g*/min,

Table 1
Abalone antioxidant enzymes and heat shock proteins' genes analyzed in this study.

Gene	Basic function	Cellular localization	Reference
Mn-SOD	Detoxification of O_2^-	Mitochondria	Ekanayake et al. (2006)
CAT	Detoxification of H_2O_2	Cytoplasm, nucleus mitochondria	Ekanayake et al. (2008)
TPx	Detoxification of H_2O_2	Cytoplasm, nucleus mitochondria	Wickramaarachchilage et al. (2008)
HSP26	Chaperone, antioxidant biomarker of stress	Cytoplasm, nucleus	Brerro-Saby et al. (2010)
HSP70	Chaperone, antioxidant biomarker of stress	Cytoplasm, nucleus	Cheng et al. (2007)
HSP90	Chaperone, antioxidant biomarker of stress	Cytoplasm, nucleus	Zhang et al. (2011)

Mn-SOD: Mn-superoxide dismutase, CAT: catalase, TPx: thioredoxin peroxidase, HSP26: heat shock protein 26, HSP70: heat shock protein 70, HSP90: heat shock protein 90.

and 2500 × *g*/min at 4 °C for 15 min, until 10% tissue homogenate was produced. The activity of HK, PK, PES, CL, and AMS was determined using a kit supplied by Nanjing Jiancheng Bioengineering Institute (China). The protein content in homogenates was determined using Bradford's (1976) Coomassie Brilliant Blue, and bovine serum albumin was used as the standard protein.

The activity of HK, PK, PES, CL, and AMS was determined according to Gao et al. (2016a, 2016b). For the determination of HK (EC 2.7.1.1) activity: the coupling reaction of glucose-6-phosphate dehydrogenase was utilized at 37 °C and pH 7.6 based on the amount of issue protein per g generating 1 mmol nicotinamide adenine dinucleotide phosphate every 1 min (i.e., one enzyme activity unit). For the determination of PK (EC 2.7.1.40) activity: the ability of PK to catalyze the production of pyruvate by phosphoenolpyruvate (PEP) in the presence of adenine triphosphate, followed by lactate dehydrogenase converting pyruvate to lactic acid and nicotinamide adenine dinucleotide (NADH) to NAD was utilized. One unit of enzyme activity was defined as the conversion of 1 μmol PEP to pyruvate per min by 1 mg of tissue protein at 340 nm, 37 °C, and pH 7.6. PES activity was defined as: tissue protein per mg decomposed into 1 μg amino acid at 37 °C every min, namely one enzyme activity unit. CL activity was defined as: tissue per g catalyzed to 1 μg glucose in every min, namely one enzyme activity unit. AMS activity was defined as: protein per mg in the tissues that reacted with the substrate (3,5-dinitrosalicylic acid, 0.2 mol/L phosphate buffer, 6 mol/L NaOH, 0.3% NaCl) at 37 °C for 30 min to hydrolyze 10 mg of amylon, namely one AMS activity unit.

2.4.2. Energy determination and budget calculation

An oxygen bomb calorimeter (model 1341, Parr Instrument Company, Moline, IL, USA) was used to determine the energy value of food, feces, and abalone body. An elemental analyzer (Vario EL III, Hanau, Germany) were used to determine the content of N. Each sample was measured three times and the average was recorded.

Energy budgets was based on the model proposed by Carfoot (1987): C = F + U + R + G was changed to G = C − F − U − R. Where, C is the energy contained in food intake, G is the accumulated energy for growth, F is the energy loss via feces, U is the energy loss via excretion, and R is the energy loss via the process of respiration. Energy values of C, G, and F were determined via the oxygen bomb calorimeter (Parr Instrument Company) after samples were dried at 70 °C until constant weight.

The energy of excretion was calculated as: $U = U_N \times 24{,}830 = (C_N - G_N - F_N) \times 24{,}830$ (Lemos and Phan, 2001; Levine and Sulkin, 1979), where, U_N is the excreted N, C_N is the N contained in food intake, G_N is the N stored in abalone, F_N is the nitrogen loss in feces, and the factor 24,830 means the energy value contained in NH_3-N per g (J g^{-1}). Since most shellfish are ammoniotelic, NH_4^+-N is the major form of N excretion (Wang and Wang, 2008), with only a low quantity of carbamide; therefore, the latter is negligible in this study. An elemental analyzer (Vario EL III) was used to determine the N content, and the energy of respiration was expressed as follows: R = C − G − F − U. The assimilation efficiency (K_1) and net growth efficiency (K_2) were calculated in the form of energy, which can be expressed as: $K_1(\%) = 100 \times (G + R) / (G + R + U)$, $K_2(\%) = 100 \times G / (G + R)$ (Ye et al., 2009).

2.4.3. Gene expression

Another sample preserved in liquid nitrogen was removed from storage and added to liquid nitrogen in a mortar for tissue grinding; 0.05 mg of the obtained sample powders was rapidly mixed with the 1 mL TRIzol (Invitrogen, Carlsbad, CA, USA) to extract total RNA from the hepatopancreas. Total RNA was extracted by removing the residual DNA from the sample using RQI RNase-Free DNase (TaKaRa, Otsu Shiga, Japan), and then RNA was reversely transcribed to cDNA using the moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA). Real-time quantitative polymerase chain reaction (PCR) test was performed using the SYBR® Premix Ex Taq™ II kit (Tli RNaseH Plus) and the TaKaRa Thermal Cycler Dice™ Real Time System TP800 instrument (TaKaRa). The specific primer was designed as per cDNA complete sequence submitted in GenBank, and *Mn-SOD*, *CAT*, *TP_x*, *HSP26*, *HSP70*, *HSP90* expression, and the reference gene β-actin, genetic information, and primer sequence are shown in Tables 1 and 2. The sample was then evenly mixed in a PCR tube and placed into a PCR plate (Roche, Indianapolis, IN, USA). PCR amplification was performed after transient centrifugation, and the reaction conditions were: initial denaturation at 94 °C for 30 s; the cycling conditions were 94 °C for 5 s and 60 °C for 30 s, 40 cycles in total. The analysis of the solubility curve was performed the end of the experiment. For each RNA sample and gene, three repeats were set for all PCR analyses. The mRNA level of the target gene was calibrated using the real-time PCR Ct ($2^{-\Delta\Delta Ct}$) relative quantitative method with the reference gene β-actin as the quantitative standard.

2.5. Data calculation

In the experimental process, the survival rate (S, %), shell length/body weight specific growth rate (SGR, % day^{-1}), food intake (FI, % body weight day^{-1}), and food conversion efficiency (FCE, %) of

Table 2
Real-time quantitative PCR primers for antioxidant enzymes and heat shock proteins' genes of *Haliotis discus hannai*.

Gene	Sequence (5′–3′)	Reference
Mn-SOD	F: ACCTAGCTTTACCGAGTTA R: GCCACCTTACGGTAAATGGACC	Designed by author
CAT	F: ACTACCTGCAACTTCCCGTCAACT R: AGGTTGTTGATCAGATGGTCCCGT	Ekanayake et al. (2008)
TPx	F: TCAGACTACAGAGGGAAATA R: CATCCAAGGACCTCACAG	Wickramaarachchilage et al. (2008)
HSP26	F: ACCGCCTAAAGGTTTGCACC R: CGTTACCGAAACTTCGAAT	Designed by author
HSP70	F: ATGCCAATGGTATCCTC R: GTAATTCTCAGCCTCGTT	Cheng et al. (2007)
HSP90	F: CACTGTGGACCAGAAATGC R: ACAGCAAAGCACGGAACT	Designed by author
β-Actin	F: GCTGCGTTGGTTATCG R: GGTACTTGAGGGTGAGGA	Designed by author

F: forward primer; R: reverse primer.

abalone were calculated every other month as follows:

$$S\,(\%) = [(N_1 - N_2)/N_1] \times 100$$

$$SGR\,(\%day^{-1}) = (\ln W_2 - \ln W_1)/T \times 100 \text{ and } SGR\,(\%day^{-1}) = (\ln L_2 - \ln L_1)/T \times 100$$

$$FI\,(\%body\ weight\ day^{-1}) = F/[T \times (W_2 + W_1)/2] \times 100$$

$$FCE\,(\%) = (W_2 - W_1)/F \times 100$$

wherein, N_1 and N_2 are the numbers of abalone at the beginning and end stages, respectively, W_1 and W_2 are the wet weights (g) of abalone at the beginning and end stages, respectively, L_1 and L_2 are the shell length at the beginning and ending stages, respectively, T is the number of experimental days, and F is the weight of food intake during the experimental process (dry weight, g).

2.6. Statistical analysis

SPSS v. 18.0 (Armonk, NY, USA) was used for statistical analysis. Logarithmic transformation was needed to satisfy the homogeneity test of variances and standard normal distribution. One-way analysis of variance and Tukey's test were performed to examine the differences in growth, food intake, enzymes activity, energy value, and related gene expression of *H. discus hannai* among different light cycle groups. The differences in growth and food intake at different sampling times in the same light cycle group were also analyzed using a *t*-test, in which $P < 0.05$ was accepted as statistically significant. All experimental data were expressed by mean ± standard error and the data obtained through analysis were drawn using Sigmaplot (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Survival rate

At Day 30, the survival rate of abalones in 16L:8D was significantly lower than in 0L:24D, 4L:20D, and 8L:16D, but no significant difference was identified compared with 12L:12D ($P = 0.142$, Fig. 1). At the end of this experiment, the survival rate of abalones in 8L:16D was significantly reduced compared with Day 30 ($P = 0.002$), but was still significantly higher than in 16 L:8D ($P = 0.001$).

3.2. Growth and food intake

At Day 30, the specific growth rate of abalone shell length and body weight in 12L:12D and 16L:8D was significantly lower than in 0L:24D, 4L:20D, and 8L:16D (Fig. 2). At the end of this experiment, the specific growth rate of abalone shell length in 0L:24D was significantly higher than in 8L:16D, 12L:12D, and 16L:8D, but no significant difference was identified compared with 4L:20D ($P = 0.171$). In contrast, the specific growth rate of abalone shell length in 8L:16D and 16L:8D was significantly reduced at Day 30. At Day 60, no significant difference with respect to the specific growth rate of abalone body weight was identified between 0L:24D, 4L:20D, and 8L:16D groups, and in 8L:16D it was significantly reduced compared with Day 30 ($P = 0.003$).

At Day 30, the food intake of abalones in 0L:24D was significantly higher than in 12L:12D and 16L:8D, but no significant difference was

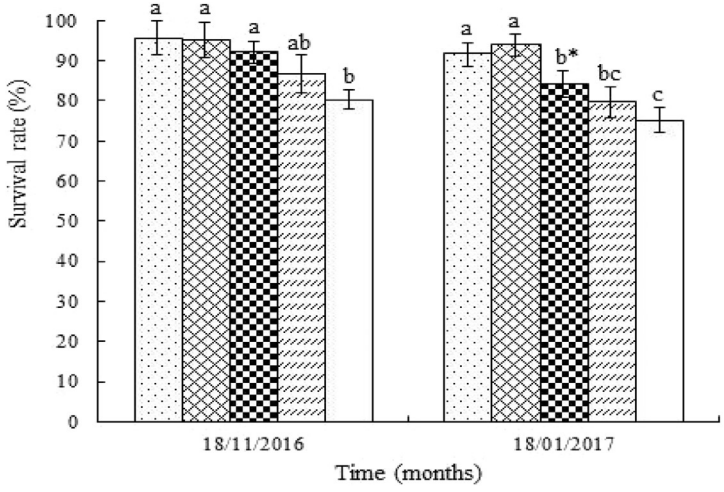
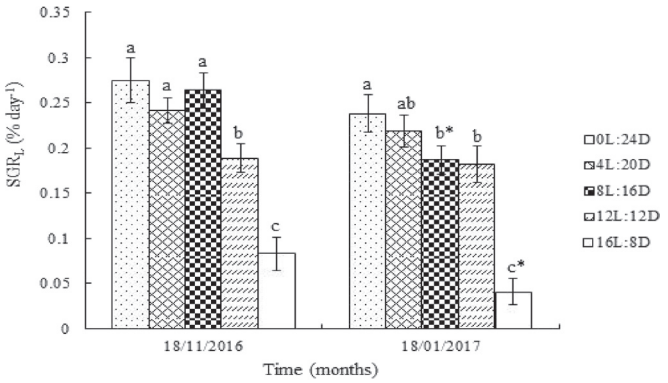
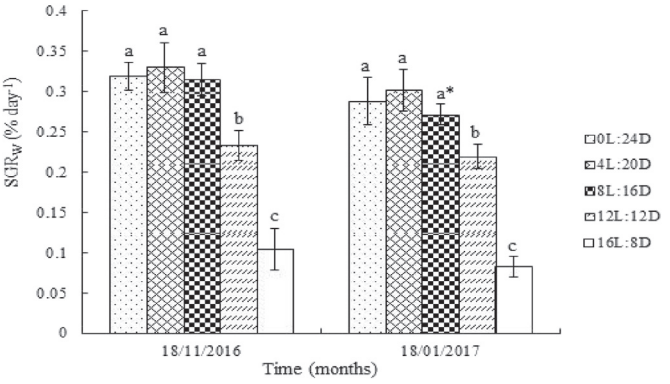


Fig. 1. Effects of light cycle on the survival rate of juvenile abalone (*H. discus hannai*). Values are expressed as mean ± SE ($n = 4$). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test, using SPSS version 18.0. Means with the different lower case letters are significantly different at $P < 0.05$ level. Asterisks indicates the significant differences in the same light cycle treatment at different time points, $P < 0.05$.



a. Shell-length specific growth rate of juvenile abalone (*H. discus hannai*)



b. Body-weight specific growth rate of juvenile abalone (*H. discus hannai*)

Fig. 2. Effects of light cycle on the shell-length (a) and body-weight (b) specific growth rate of juvenile abalone (*H. discus hannai*). Values are expressed as mean ± SE ($n = 4$). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test, using SPSS version 18.0. Means with the different lower case letters are significantly different at $P < 0.05$ level. Asterisks indicates the significant differences in the same light cycle treatment at different time points, $P < 0.05$.

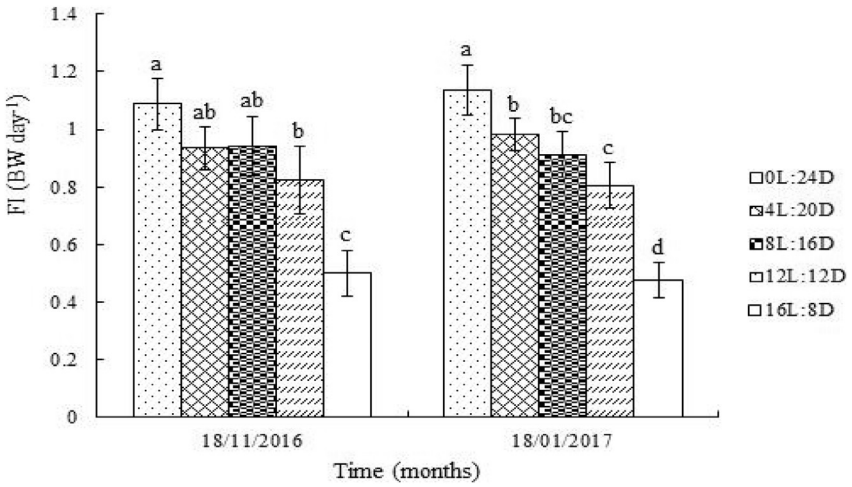


Fig. 3. Effects of light cycle on the food intake of juvenile abalone (*H. discus hannai*). Values are expressed as mean ± SE (n = 4). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test, using SPSS version 18.0. Means with the different lower case letters are significantly different at $P < 0.05$ level.

identified compared with 4L:20D and 8L:16D (Fig. 3). At Day 60, the food intake of abalones in 0L:24D was significantly higher than in any other group ($F = 43.72$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$). Abalone food intake in 4L:20D showed no significant difference compared with 8L:16D ($P = 0.183$), but it was significantly higher than in 12L:12D and 16L:8D.

At Day 30, the food conversion efficiency of abalones in 4L:20D was significantly higher than in 8L:16D, 12L:12D, and 16L:8D, but no significant difference was identified compared with 0L:24D ($P = 0.119$, Fig. 4). At Day 60, the food conversion efficiency of abalones in 4L:20D was significantly higher than in any other group ($F = 58.95$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$), but it showed no significant difference between 0L:24D and 8L:16D ($P = 0.191$). In contrast with Day 30, the food conversion efficiency of abalones in 16L:8D significantly decreased at Day 60 ($P = 0.001$).

3.3. Enzyme activity

The activity of HK in 16L:8D was significantly higher than in any other group ($F = 12.38$, $df_1 = 4$, $df_2 = 10$, $P = 0.006$, Table 3). At the end of the experiment, the activity of HK in 16L:8D and 12L:12D significantly increased compared with Day 30. At Day 30, the activity of PK in 16L:8D showed no significant difference compared with 12L:12D ($P = 0.147$), but was significantly higher than in 0L:24D, 4L:20D, and 8L:16D. At the end of the experiment, the activity of PK in 16L:8D and 12L:12D significantly increased compared with Day 30. At Day 30, the activity of PES in 4L:20D showed no significant difference compared with 0L:24D ($P = 0.135$), but it was significantly higher than in 8L:16D, 12L:12D, and 16L:8D. At Day 60, the activity of PES in all groups was ranked as: 0L:24D, 4L:20D and 8L:16D > 12L:12D and 16L:8D, and no significant difference was identified between 12L:12D and 16L:8D ($P = 0.327$). No significant difference with respect to the activity of CL was identified between 0L:24D, 4L:20D, and 8L:16D, and the value recorded in 16L:8D was significantly lower than in any other group ($F = 16.72$, $df_1 = 4$, $df_2 = 10$, $P = 0.004$). At Day 30, the activity of AMS in 0L:24D, 4L:20D, and 8L:16D was significantly higher than in 12L:12D or 16L:8D, and the activity of AMS in 12L:12D was significantly higher than in 16L:8D ($P = 0.001$). At the end of the experiment, the activity of AMS in all groups was described as: 4L:20D > 0L:24D and 8L:16D > 12L:12D > 16L:8D.

3.4. Energy parameters

In 0L:24D, 4L:20D, and 8L:16D, the energy acquired from food was significantly higher than in any other group and no significant difference was identified between the three groups (Table 4). At the end of the experiment, in 8 L:16D and 16L:8D, the energy acquired from food significantly decreased compared with Day 30. At Day 30, the energy loss via feces in 16L:8D was significantly lower than in any other group ($F = 78.02$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$). At the end of the experiment, the energy loss via feces in all groups was described as: 0L:24D > 4L:20D and 8L:16D > 12L:12D > 16L:8D. At Day 30, no significant difference with respect to energy loss via excretion was identified between 8L:16D and 12L:12D ($P = 0.221$), but values in both

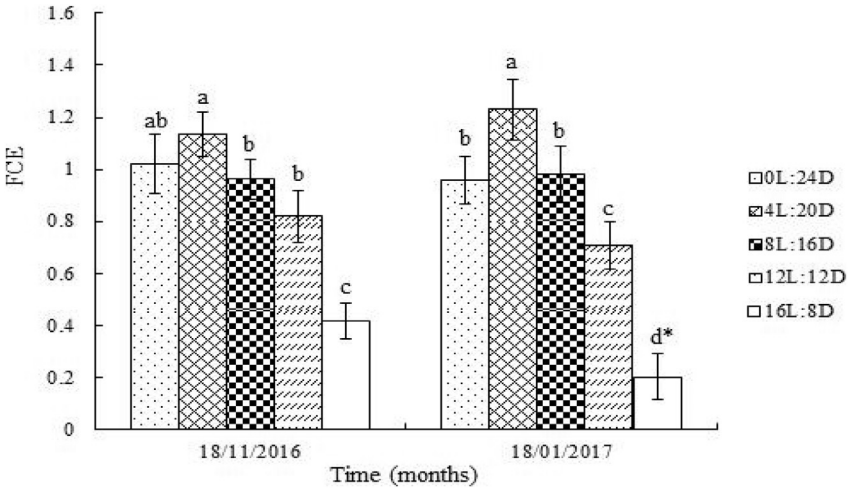


Fig. 4. Effects of light cycle on the food conversion efficiency of juvenile abalone (*H. discus hannai*). Values are expressed as mean ± SE (n = 4). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test, using SPSS version 18.0. Means with the different lower case letters are significantly different at $P < 0.05$ level. Asterisks indicates the significant differences in the same light cycle treatment at different time points, $P < 0.05$.

Table 3
Effects of light cycle on the activity of metabolic and digestive enzyme of juvenile abalone, *H. discus hannai*.

Light cycle	Time	Variable				
		Hexokinase (HK) (U/g prot)	Pyruvate kinase (PK) (U/g prot)	Pepsase (PES) (U/mg prot)	Cellulase (CL) (U/g prot)	Amylase (AMS) (U/mg prot)
0L:24D	18/11/2016	0.68 ± 0.13^{b}	5.92 ± 1.31^{b}	5.19 ± 0.82^{ab}	8.57 ± 0.71^{a}	10.15 ± 1.32^{a}
	18/1/2017	0.81 ± 0.16^{b}	5.33 ± 0.98^{b}	5.68 ± 0.53^{a}	9.09 ± 1.04^{a}	9.24 ± 0.87^{b}
4L:20D	18/11/2016	0.73 ± 0.13^{b}	5.68 ± 1.09^{b}	7.25 ± 1.19^{a}	8.12 ± 0.75^{a}	10.83 ± 0.59^{a}
	18/1/2017	0.70 ± 0.18^{b}	6.31 ± 1.22^{b}	6.42 ± 1.06^{a}	7.58 ± 0.83^{a}	11.69 ± 1.25^{a}
8L:16D	18/11/2016	0.85 ± 0.12^{b}	6.59 ± 0.75^{b}	3.87 ± 0.89^{b}	7.79 ± 1.14^{a}	10.02 ± 1.30^{a}
	18/1/2017	0.93 ± 0.16^{b}	5.75 ± 0.68^{b}	4.19 ± 0.44^{a}	8.54 ± 1.10^{a}	9.36 ± 0.72^{b}
12L:12D	18/11/2016	0.79 ± 0.17^{b}	8.27 ± 1.41^{ab}	3.26 ± 0.21^{b}	6.31 ± 0.69^{a}	7.41 ± 0.28^{b}
	18/1/2017	1.26 ± 0.23^{b*}	10.15 ± 1.07^{a*}	2.57 ± 0.26^{b}	5.08 ± 0.46^{b}	5.99 ± 0.84^{c}
16L:8D	18/11/2016	1.86 ± 0.35^{a}	10.92 ± 1.25^{a}	1.41 ± 0.15^{c}	3.19 ± 0.17^{b}	2.38 ± 0.39^{c}
	18/1/2017	2.15 ± 0.21^{a*}	13.89 ± 1.39^{a*}	1.28 ± 0.19^{b}	2.56 ± 0.11^{c}	2.76 ± 0.27^{d}

Values are expressed as mean ± SE (n = 4). Different small letters indicate significant differences in activity of metabolic and digestive enzyme for the same time points in different light cycle treatments, $P < 0.05$. Asterisks indicates the significant differences in the same light cycle treatment at different time points, $P < 0.05$.

groups were significantly lower than in 0L:24D. At the end of the experiment, the energy loss via excretion in 12L:12D and 16L:8D was significantly lower than in 0L:24D, 4L:20D, and 8L:16D. Compared with Day 30, the energy loss via excretion in 0L:24D significantly decreased at the end of the experiment ($P = 0.012$).

At Day 30, no significant difference with respect to energy loss via respiration was identified between 12L:12D and 16L:8D ($P = 0.303$, Table 4), but values in both groups were significantly lower than that in 0L:24D, 4L:20D and 8L:16D groups. At the end of the experiment, the energy loss via respiration in 0L:24D was significantly higher than in any other group ($F = 14.18$, $df_1 = 4$, $df_2 = 10$, $P = 0.005$). No significant difference with respect to energy loss via respiration was identified between 4L:20D and 8L:16D ($P = 0.267$), but the values in both groups were significantly higher than in 16L:8D. At Day 30, the energy accumulated for growth in all groups was described as: 0L:24D and 4L:20D, 8L:16D > 12L:12D > 16L:8D. At the end of the experiment, the energy accumulated for growth in 4L:20D was significantly higher than in any other group ($F = 38.19$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$). In 0L:24D and 16L:8D, the energy accumulated for growth significantly decreased compared with Day 30.

3.5. Energy budget

At Day 30, in 12L:12D, the ratio of the energy loss via feces to the energy from food intake was significantly higher than in 4L:20D and 8L:16D, but no significant difference was identified compared with 0L:24D and 16L:8D (Table 5). At the end of the experiment, no significant difference in the ratio of the energy loss via feces to the energy from food intake was identified between 0L:24D and 12L:12D ($P = 0.292$), but each was significantly higher than in any other group. In 16L:8D, the ratio of energy loss via excretion to the energy from food intake was significantly higher than in any other group ($F = 30.35$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$), and in 16L:8D, the ratio of the energy loss via respiration to the energy from food intake was significantly higher than that in any other group ($F = 62.08$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$). At Day 30, the energy accumulated for growth in all groups was described as: 0L:24D and 4L:20D, 8L:16D > 12L:12D > 16L:8D. At the end of the experiment, no significant difference in the energy accumulated for growth was identified between 4L:20D and 8L:16D ($P = 0.197$), but values in both groups were significantly higher than in any other group.

At Day 30, the assimilation efficiency K_1 of from food in 8L:16D was significantly higher than in 16L:8D ($P = 0.002$, Table 5), but no significant difference was identified between 0L:24D, 4L:20D, and 12L:12D. At the end of the experiment, the assimilation efficiency K_1 of from food in 16L:8D was significantly lower than that in 0L:24D, 4L:20D, and 8L:16D, but no significant difference was identified compared with 12L:12D ($P = 0.180$). At Day 30, the net growth efficiency K_2 of abalones in all groups was ranked as: 0L:24D and 4L:20D, 8L:16D > 12L:12D > 16L:8D. At the end of the experiment, the net growth efficiency K_2 in 0L:24D decreased, which was significantly lower than in 4L:20D and 8L:16D.

Table 4
Energy parameters in juvenile abalone, *H. discus hannai*, under different light cycles.

Light cycle	Time	Energy parameters				
		C (J g^{-1} d^{-1})	F (J g^{-1} d^{-1})	G (J g^{-1} d^{-1})	U (J g^{-1} d^{-1})	R (J g^{-1} d^{-1})
0L:24D	18/11/2016	1389.15 ± 128.71^{a}	633.04 ± 59.28^{a}	472.21 ± 62.75^{a*}	114.38 ± 16.52^{a*}	169.52 ± 45.83^{a}
	18/1/2017	1317.23 ± 142.38^{a}	702.85 ± 83.64^{a}	336.93 ± 48.06^{b}	89.06 ± 10.06^{a}	188.39 ± 57.39^{a}
4L:20D	18/11/2016	1293.88 ± 109.95^{a}	503.29 ± 57.11^{a}	541.62 ± 77.92^{a}	95.25 ± 9.85^{ab}	153.72 ± 41.08^{a}
	18/1/2017	1364.91 ± 136.29^{a}	528.03 ± 66.79^{b}	615.01 ± 92.54^{a}	84.73 ± 6.49^{a}	137.14 ± 68.17^{b}
8L:16D	18/11/2016	1302.29 ± 156.87^{a*}	518.28 ± 73.63^{a}	542.76 ± 68.73^{a}	75.68 ± 7.92^{b}	165.57 ± 54.38^{a}
	18/1/2017	1156.15 ± 127.51^{a}	472.85 ± 50.28^{b}	457.44 ± 56.25^{b}	86.44 ± 8.72^{a}	139.42 ± 48.29^{b}
12L:12D	18/11/2016	803.81 ± 121.33^{b}	433.92 ± 81.51^{a}	184.75 ± 60.85^{b}	60.75 ± 4.38^{bc}	124.39 ± 29.05^{b}
	18/1/2017	675.79 ± 91.02^{b}	351.62 ± 49.87^{c}	155.44 ± 32.79^{c}	66.09 ± 5.47^{b}	102.64 ± 26.67^{bc}
16L:8D	18/11/2016	384.92 ± 72.17^{c*}	169.07 ± 22.64^{b}	44.43 ± 8.08^{c*}	52.71 ± 6.96^{c}	118.71 ± 34.19^{b*}
	18/1/2017	235.89 ± 61.88^{c}	103.29 ± 30.57^{d}	18.83 ± 3.34^{d}	40.25 ± 7.09^{c}	73.52 ± 9.95^{c}

Values were expressed as mean ± SE (n = 4); C: feeding energy; F: energy loss in feces discharge; G: energy deposit for growth; U: energy loss in excretion; R: energy loss in respiration. Different small letters indicate significant differences in energy parameters for the same time points in different light cycle treatments, $P < 0.05$. Asterisks indicates the significant differences in the same light cycle treatment at different time points, $P < 0.05$.

Table 5
Estimated energy allocation in juvenile abalone, *H. discus hannai*, under different light cycles.

Light cycle	Time	Energy allocation						
		C	F (% C^{-1})	G (% C^{-1})	U (% C^{-1})	R (% C^{-1})	K_1	K_2
0L:24D	18/11/2016	100	45.57 ± 5.39^{ab}	33.99 ± 3.92^{a}	8.23 ± 0.51^{b}	12.20 ± 2.18^{b}	84.87 ± 7.72^{ab}	73.58 ± 7.04^{a}
	18/1/2017	100	53.36 ± 5.82^{a}	25.58 ± 2.77^{b}	6.76 ± 0.69^{b}	14.30 ± 1.42^{b}	85.50 ± 5.91^{a}	64.14 ± 5.64^{b}
4L:20D	18/11/2016	100	38.90 ± 3.96^{b}	41.86 ± 3.28^{a}	7.36 ± 0.78^{b}	11.88 ± 2.66^{b}	87.95 ± 9.25^{ab}	77.89 ± 4.98^{a}
	18/1/2017	100	38.69 ± 3.24^{b}	45.06 ± 4.94^{a}	6.21 ± 0.64^{b}	10.05 ± 2.03^{b}	89.88 ± 7.88^{a}	81.77 ± 8.62^{a}
8L:16D	18/11/2016	100	39.80 ± 7.04^{b}	41.68 ± 2.76^{a}	5.81 ± 0.42^{b}	12.71 ± 3.25^{b}	90.35 ± 9.34^{a}	76.63 ± 5.95^{a}
	18/1/2017	100	40.90 ± 4.85^{b}	39.57 ± 4.11^{a}	7.48 ± 0.74^{b}	12.06 ± 2.60^{b}	87.35 ± 6.87^{a}	76.64 ± 4.33^{a}
12L:12D	18/11/2016	100	53.98 ± 5.18^{a}	22.98 ± 3.67^{b}	7.56 ± 0.88^{b}	15.48 ± 3.57^{b}	83.58 ± 9.24^{ab}	59.76 ± 8.60^{b}
	18/1/2017	100	52.03 ± 6.74^{a}	23.00 ± 5.27^{b}	9.78 ± 0.79^{b}	15.19 ± 2.73^{b}	79.61 ± 8.69^{ab}	60.23 ± 6.29^{b}
16L:8D	18/11/2016	100	43.92 ± 7.85^{ab}	11.54 ± 1.86^{c}	13.69 ± 1.28^{a}	30.84 ± 4.64^{a}	75.58 ± 7.53^{b}	27.23 ± 3.71^{c}
	18/1/2017	100	43.79 ± 5.10^{b}	7.89 ± 1.32^{c}	17.06 ± 1.69^{a}	31.17 ± 4.30^{a}	69.65 ± 8.21^{b}	20.39 ± 3.13^{c}

Values were expressed as mean ± SE (n = 4); C: feeding energy; F: energy loss in feces discharge; G: energy deposit for growth; U: energy lost via excretion; R: energy lost via respiration; K_1: assimilation efficiency; K_2: net growth efficiency. Different small letters indicate significant differences in energy allocation for the same time points in different light cycle treatments, $P < 0.05$. Asterisks indicates the significant differences in the same light cycle treatment at different time points, $P < 0.05$.

3.6. Gene expression

The expression levels of *Mn-SOD* in 16L:8D were significantly higher than in any other group ($F = 81.90$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$, Fig. 5A), and at the end of the experiment, they significantly increased compared with Day 30 ($P = 0.001$). At Day 60, the expression levels of *Mn-SOD* in 12L:12D were significantly higher than in 0L:24D, 4L:20D, and 8L:16D, but no significant differences were identified between the three groups. At Day 30, no significant difference with respect to the expression levels of *CAT* were identified among these groups ($F = 1.40$, $df_1 = 4$, $df_2 = 10$, $P = 0.175$, Fig. 5B), but at the end of the experiment, the expression levels of *CAT* in 16L:8D was significantly higher than in any other group ($F = 70.35$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$), and significantly increased compared with Day 30 ($P < 0.001$). During the course of the experiment, the expression levels of TP_x in 16L:8D were significantly higher than in any other group ($F = 52.06$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$, Fig. 5C), but no significant difference with respect to the expression levels of TP_x was identified among the other four groups.

At Day 30, the expression levels of *HSP26* in 16L:8D were significantly higher than in any other group ($F = 96.89$, $df_1 = 4$, $df_2 = 10$, $P < 0.001$, Fig. 5D). At the end of the experiment, the expression levels of *HSP26* in 12L:12D significantly increased compared with Day 30, but were still significantly lower than in 16L:8D ($P = 0.003$). At Day 30, no significant difference with respect to the expression levels of *HSP70* was identified among groups ($F = 1.28$, $df_1 = 4$, $df_2 = 10$, $P = 0.204$, Fig. 5E). At the end of the experiment, the expression levels of *HSP70* in 16L:8D were significantly higher than in any other group, and significantly increased compared with Day 30 ($P < 0.001$). At Day 30, the expression levels of *HSP90* in 12L:12D were significantly higher than in 0L:24D and 4L:20D, but no significant difference was identified compared with 8L:16D ($P = 0.116$, Fig. 5F). At the end of the experiment, the expression levels of *HSP90* in 12L:12D were significantly higher than in 8L:16D ($P = 0.002$). In contrast with Day 30, the expression levels of *HSP90* at the end of the experiment significantly decreased ($P = 0.001$).

4. Discussion

In nature, most biological rhythms (day, night, and season) are closely related to the cyclical changes of light; aquatic organisms will adjust their physiological activities with light-dark cyclic changes to result in an adaptation mechanism (Kusmic and Gualtieri, 2000; Villamizar et al., 2011). Therefore, light-dark conversion is likely to play an important role in animal growth and food intake rhythm. In this experiment, no significant difference with respect to the specific growth rate of *H. d. hannai* shell length and body weight was identified between 0L:24D and 4L:20D, but both parameters were significantly higher than in 12L:12D and 16L:8D, indicating an adverse effect of a light period exceeding 12 h. In 0L:24D and 4L:20D, at the end of the experiment, abalone food intake and food conversion efficiency were significantly higher than in 12L:12D and 16L:8D. The growth advantage of abalone in 0L:24D and 4L:20D might be primarily attributed to the high food intake and food conversion efficiency.

The food intake of abalone in 16L:8D was significantly lower than in any other group, and compared with Day 30, it significantly decreased. This indicates that the food intake was less when the light exposure reached 16 h because abalones are typically nocturnal and long-time light exposure may cause oxidative damage. Even in the presence of food intake, the food conversion efficiency of abalone was also very low. At Day 60, food intake in 0L:24D was significantly higher than in 4L:20D, but the food conversion efficiency in 4L:20D was significantly higher than in 0L:24D. This indicates that although there was a greater food intake in full darkness, it was not properly converted for growth; therefore, it was presumed that an appropriate light exposure can enhance food utilization rate. The growth and food conversion rate of *Oncorhynchus mykiss* has been found to be promoted by an increasing light cycle (Mäkinen and Ruohonen, 1992). The light cycle of 16L:8D further promoted the growth of *Sebastes diploproa*, and its basic metabolic rate was relatively low when exposed to long light cycle compared with a light cycle of 12L:12D (Boehlert, 1981). With continuous light exposure, although the growth rate of *Dicentrarchus labrax* was relatively fast, its normal development was affected (Cerqueira and Chatain, 1991). The precocious puberty of male *D. labrax* is inhibited during long-term light exposure prior to the formation of gametes (4 months) or during the formation of gametes (6 months), thereby facilitating growth (Begtashi et al., 2004; Felip et al., 2008). Therefore, the selection of an appropriate light cycle will be critical for abalone growth and food intake.

The energy metabolism of many gastropods is based on the utilization of carbohydrates. A number of findings have demonstrated that abalone also follow this trend because the fat content of natural food for abalones is usually lower, but that of carbohydrates is higher (Knauer et al., 1996). At the end of the present experiment, the activity of CL in 0L:24D, 4L:20D, and 8L:16D was significantly higher than in 12L:12D and 16L:8D, indicating that abalone digestion and absorption of food would be enhanced when light exposure is under 8 h. Garcia-Esquivel and Felbeck (2006) verified that abalones in essence belong to herbivores. Compared with the activity of LPS, chymotrypsin, and aminopeptidase, the activity of CL and AMS in abalones was obviously higher. In 4L:20D, the activity of AMS was significantly higher than in any other group, which might be the primary cause of the high food conversion efficiency and prominent growth advantage of abalones in this light cycle. In most cases, there is specific relationship between food

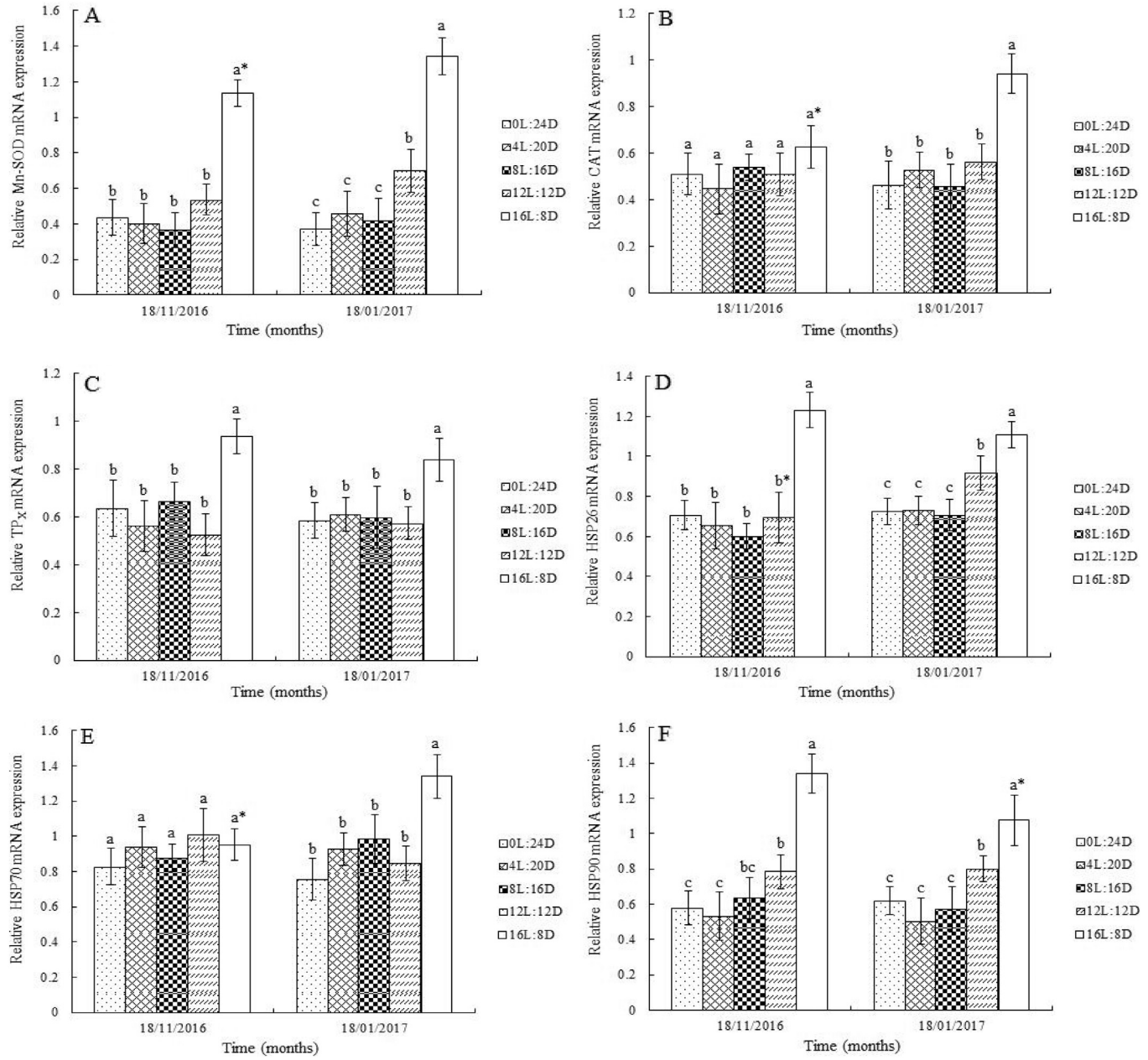


Fig. 5. Relative mRNA expression levels of antioxidant enzyme and heat shock proteins' genes in the hepatopancreas of abalone *H. discus hannai*. Values are expressed as mean ± SE (n = 3). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test, using SPSS version 18.0. Means with different lower case letters are significantly different at $P < 0.05$ level. Asterisks indicates the significant differences in the same light cycle treatment at different time points, $P < 0.05$.

intake and digestive enzyme activity, the indigested food stimulates the digestive system to secrete digestive enzymes, which in turn promotes the food intake behavior of organisms (Jones et al., 1997).

The activity of PES in the digestive tract is critical for the effective absorption and utilization of indigested proteins (Picos-García et al., 2000). At the end of the present experiment, the activity of PES in 0L:24D, 4L:20D, and 8L:16D was also significantly higher than in 12L:12D and 16L:8D, indicating that the secretion of the protease increased within 8 h light exposure, which further promoted the generation of abalone food intake behavior and accelerated individual growth. The activity levels of digestive enzymes directly reflect an organism's food digestion and nutrient absorption capability, as well as its immune state (Bolasina et al., 2006). In 16L:8D, the activity of CL and AMS was significantly lower than in any other group, indicating that a reduction in CL and AMS secretion might inhibit the generation of abalone food intake behavior when light exposure reaches 16 h. Such a light cycle may also cause an oxidative stress in the mechanism, leading to a reduction in food intake activities. When a limited availability of energy occurs over a long period of time, the oxidative damage on the mechanism needs to be dealt, resulting in increased mortality.

Carbohydrates are critical energy substances in an organism, and HK and PK are two key enzymes in the process of glycolysis; changes in their activity play an important role in maintaining blood glucose level and the rate of glycolysis (Allert et al., 1991; Sabourin and Stickle, 1981). HK is a key enzyme in the liver that regulates the concentration of blood glucose; it can be coupled with the function of glucose transporters on the cell membrane, and exerts a specific influence on the glucose flow and metabolism in cells (Sangiao-Alvarellos et al., 2006; Van Aardt and Wolmarans, 1987). During the course of the experiment, the activity of HK in 16L:8D was significantly higher than in any other group, and at the end of the experiment, it significantly increased compared with Day 30. This indicates that energy consumption,

glycolysis, and energy production increased in this light cycle, enhancing the environmental adaptation of organism (Metón et al., 2003). PK may play a critical role in controlling the rate of glycolysis by regulating adenosine triphosphate, adenosine diphosphate, and glycolysis intermediates. At the end of the experiment, no significant difference with respect to the activity of PK was identified between 12L:12D and 16L:8D, but values in both groups were significantly higher than in any other group. PK activity in these two groups significantly increased at the end of the experiment compared with Day 30, indicating that these two light cycles may cause an oxidative stress in abalones, resulting in a drastic increase in energy consumption. Abalones may have supplemented the required energy by enhancing the glycolysis. Therefore, glycolysis characteristics and physiological response mechanisms in different light cycles could be further understood through changes in HK and PK activity.

The abalone energy budget model showed different characteristics in different light cycles. In 0L:24D, 4L:20D, and 8L:16D, the energy acquired from food was significantly higher than in 12L:12D and 16L:8D, but no significant differences were identified between 0L:24D, 4L:20D, and 8L:16D. In 12L:12D and 16L:8D, although the energy loss via feces, excretion, and respiratory metabolism was significantly lower than in 0L:24D, 4L:20D, and 8L:16D, the ratio of the energy loss via feces, excretion, and respiratory metabolism to the energy from food intake was higher than in 0L:24D, 4L:20D and 8L:16D. Based on the energy budget equation, the energy accumulated for abalone growth in 12L:12D and 16L:8D significantly decreased. Differences in light cycles altered the energy budget model of abalone, in 12L:12D and 16L:8D, more energy accumulated was used for feces, excretion, and respiratory metabolism. This indicates that when light exposure is increased to 12 h and 16 h, the physiological function of the organism change and oxygen consumption increases as a response to the environmental stress. In addition to the reduction in energy acquired from external sources, more energy was needed to be allocated to respond to the environmental factor stress. Under this circumstance, the primary demand was to maintain homeostasis and ensure survival, so that no further energy would be allocated for individual growth. There was no significant difference in the energy acquired from food between 0L:24D and 4L:20D, but the energy loss via feces and respiratory metabolism in 0L:24D was significantly higher than in 4L:20D. The energy accumulation for growth and net growth efficiency K_2 were significantly lower in 0L:24D than in 4L:20D, which might be another primary cause of the significant increase in food conversion efficiency in 4L:20D at the end of this experiment. Paul and Fuji (1989) reported that changes in energy metabolism determine energy accumulation for individual growth. Therefore, based on bioenergetics, the present experiment initially elucidated the possible causes of abalone growth differences among different light cycles.

The dynamic equilibrium between the antioxidant system and reactive oxygen species is a critical factor in assuring the health status of animals. If such equilibrium tends to be oxidative, it would cause an oxidative stress to the organism and impose an oxidative damage. For aquatic animals, due to the complexity of the living environment, such internal balance is more likely to be disrupted by oxidative stress factors (Kim et al., 2007; Limón-Pacheco and Gonsebatt, 2009; Yu, 1994). In 16L:8D, the expression levels of *Mn-SOD*, *CAT*, and TP_x at the end of the experiment were significantly higher than in any other group, and compared with Day 30, the expression levels of *Mn-SOD* and *CAT* further significantly increased at the end of the experiment. This indicates that the enzyme system comprising SOD, CAT, and other types of antioxidant enzymes play an important regulatory role, enhancing the adaptability of organism to the external environment, and protecting it from oxidative damage. However, when ROS excessively accumulates in the organism and cannot be removed in time, it can cause oxidative damage in the tissues, leading to disease, growth reduction, and even mortality (Almroth et al., 2015; Li et al., 2016). This may be another primary cause of the low individual growth and high mortality in 16L:8D.

As one of the molecular chaperones, HSPs can contribute to the stress response of an organism, which plays an important role in protein folding, degradation, redox equilibrium, and innate immune response (Picard, 2002; Robert, 2003; Yang et al., 2007). In addition to responding to temperature change or hypoxia stress, HSPs also trigger a reaction to stress arising from heavy metals and free radicals, leading to expression and regulation at the gene and protein levels (Cara et al., 2005; Gao et al., 2007; Palmisano et al., 2000). At Day 30, the expression levels of *HSP90* or *HSP26* in 16L:8D were significantly higher than in any other group, the expression levels of *HSP90* in 12L:12D were also significantly different from in 0L:24D and 4L:20D. This indicates that a light exposure of 12 h and 16 h might induce HSP synthesis, thereby involving the repair process after the occurrence of abnormal protein. In contrast, no significant difference with respect to the expression level of *HSP70* was identified among these groups, while the expression level of *HSP90* significantly increased. However, at the end of the experiment, the expression level of *HSP70* significantly increased, while that of *HSP90* was significantly reduced. This might be attributed to the oxidation of cysteine residues and methionine residues in HSP90, resulting in the loss of biological activity of molecular chaperones to the long-term environmental stress. Wu et al. (2011) also found that *HSP90* mRNAs, which are stored in cells for the maintenance of normal physiological function, were highly expressed in the early stress induction period, which is similar to the present results. Therefore, the increase in the expression levels of *HSP26*, *HSP70*, and *HSP90* might be a tissue-specific mechanism and long-term cytoprotective mechanism, which will be of a great significance for improving the resistance of the organism to oxidative stress.

In summary, during a light cycle of 12L:12D and 16L:8D, the food intake activity of abalones was reduced. When the energy acquired from food is reduced and the ratio of the energy loss via feces, excretion, and respiratory metabolism to the energy from the food intake is relatively high, abalones can generate more energy to resist oxidative damage by accelerating the rate of glycolysis. In addition, the extra energy budget was also utilized to maintain the dynamic equilibrium between internal oxidation and antioxidation, so that no further energy accumulation was used for individual growth. In 0L:24D and 4L:20D, although there was no significant difference in the energy acquired from food, more energy was lost via feces and respiratory metabolism in 0L:24D, so that the energy accumulated for growth reduced. Furthermore, during the course of aquaculture production, full darkness did not facilitate routine water exchange, feeding, or other related operations. Therefore, considering aquaculture production and business costs, it would be more appropriate to select a light cycle of 4L:20D for *H. d. hannai* culture.

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