

RESEARCH ARTICLE

Effect of water temperature on diel feeding, locomotion behaviour and digestive physiology in the sea cucumber *Apostichopus japonicus*

Jiamin Sun¹, Libin Zhang^{1,2,*}, Yang Pan^{1,3}, Chenggang Lin¹, Fang Wang⁴ and Hongsheng Yang^{1,2}

ABSTRACT

This study used controlled laboratory conditions to directly assess the role of water temperature in controlling diel feeding and locomotion behaviours, and digestive physiology, in the sea cucumber *Apostichopus japonicus*. The results revealed that both the proportion of feeding individuals and ingestion rate were highest at 16°C. Regardless of water temperature, sea cucumbers appeared to be nocturnal and their peak feeding activity occurred at 00:00 h to 04:00 h. Tentacle insertion rate was not significantly correlated with water temperature (<24°C). In all temperature treatments except 24°C, the proportion of moving sea cucumbers was also observed to be higher at night than during the day. The water temperature above thermal threshold (24°C) for aestivation may alter the diel locomotion rhythm. The highest lipase and amylase activities were both observed at 20°C. The highest activities of lipase and amylase at all temperature treatments were observed at 22:00 h to 02:00 h, which was slightly earlier than the feeding peak. In conclusion, even in total darkness, *A. japonicus* showed more active feeding and moving activities, and higher digestive enzyme activities, at night than during the day. These results demonstrated that diel feeding and locomotion behaviours, at least in the short term, were not controlled by light or low water temperature (<24°C) but by an endogenous rhythm, and *A. japonicus* had the ability to optimize the digestive function for the coming feeding peak. These findings should provide valuable information for the development of the aquaculture of this species.

KEY WORDS: *Apostichopus japonicus*, Water temperature, Feeding, Locomotion, Digestive enzyme

INTRODUCTION

Trade in sea cucumbers dates back several centuries owing to their health benefits and medicinal properties. In response to a dwindling supply from wild stocks and increasing demands causing a surge in market prices, commercial aquaculture of sea cucumbers has begun recently in many countries (Purcell et al., 2014; Toral-Granda et al., 2008). *Apostichopus japonicus* (Selenka 1867) is one of the most

important commercial aquaculture species. The *A. japonicus* industry has now overtaken the traditional shrimp and fish aquaculture industries, and *A. japonicus* generates the highest single-species output value and profit in China (Yang et al., 2015). It is distributed mainly along the coasts of Northeastern Asia, including eastern Russia, northern China, Japan, North Korea and South Korea (Hamel and Mercier, 2008; Okorie et al., 2008). Usually, *A. japonicus* is cultured in shallow seas (sea ranching) or ponds (cofferdam). In recent years, intensive indoor culture has expanded rapidly, becoming an important rearing method in China (Yang et al., 2015). Understanding the behavioural and physiological features of *A. japonicus* under different environmental conditions is beneficial not just to understand the natural behaviour of the species, but also to improve the rearing techniques for sea cucumbers.

Water temperature represents the most pervasive aspect of the environment affecting marine ectotherms, varies markedly on a variety of spatial and temporal scales and is one of the most important environmental factors affecting growth and physiological performance in aquatic animals (Buentello et al., 2000). Previous studies have demonstrated the effects of water temperature on growth (An et al., 2007; Dong and Dong, 2006; Dong et al., 2006; Yang et al., 2005), immune response (Wang et al., 2008) and body composition (Dong et al., 2006) of *A. japonicus*. However, our understanding of the behavioural responses of *A. japonicus* to temperature variation lags well behind our understanding of physiological responses.

The diel cycles of sea cucumbers vary among different species, with maximal activity being diurnal, nocturnal or crepuscular (Wheeling et al., 2007). However, the activity may be scheduled to coincide with regular changes in many environmental factors. The effect of light on rhythms and diel cycles in sea cucumbers has been well described (Chen et al., 2007; Dong et al., 2011; Dong et al., 2010; Mercier et al., 1999), but the effect of water temperature on their behavioural rhythms has received less attention. Temperature-induced changes in diel activity have been observed mainly in fish (Brown and Mackay, 1995; Fraser et al., 1993; Hurst and Duffy, 2005; Reeb, 2002). To our knowledge, relevant studies on sea cucumbers have been conducted mainly in the tropical species *Holothuria scabra* (Mercier et al., 2000; Purcell, 2010; Wolkenhauer, 2008).

Another potential technique to study diurnal variations in feeding is based on the measurement of digestive enzyme activities (Mata-Sotres et al., 2016). Previous studies have indicated that the diel digestive physiology of fish, such as sea bass (Tillner et al., 2014; del Pozo et al., 2012), sea bream (Zeytin et al., 2016; Montoya et al., 2010) and goldfish (Vera et al., 2007), was mainly entrained by the light–dark cycle, food types and feeding times. Water temperature was one of the important factors affecting the digestive physiology of sea cucumbers (Gao et al., 2009). The diel digestive rhythms of *A.*

¹Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, Shandong, China. ²Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, 1 Wenhai Road, Qingdao 266237, Shandong, China. ³Chinese Academy of Fishery Sciences, 150 Qingtao Road, Beijing 100039, China. ⁴Ocean University of China, Fisheries College, 5 Yushan Road, Qingdao 266003, Shandong, China.

*Author for correspondence (zhanglibin@qdio.ac.cn)

© L.Z., 0000-0002-4611-1852

japonicus at different temperatures remain unclear, and it is crucial to determine the optimum feeding time and frequency in aquaculture practice.

This study was carried out under controlled laboratory conditions to ensure that the effects of temperature were separate from that of other environmental factors. In this study, we describe the behavioural patterns and determine the digestive physiology of the sea cucumber *A. japonicus* under different water temperatures based on laboratory experiments. In particular, we examined diel feeding activity patterns and tentacle insertion rate, as well as the change of digestive enzyme activities, across the range of temperatures likely encountered throughout the year by *A. japonicus*. In addition, diurnal and nocturnal ingestion rate (IR) and locomotion behaviour of *A. japonicus* were compared.

MATERIALS AND METHODS

Collection and maintenance

Sea cucumbers were collected from Tianheng Sea Cucumber Farm, Shandong Province, China, and transported to the laboratory (Qingdao National Ocean Science Research Center). All individuals were acclimated in six large tanks (1000 l⁻¹) at 6–14°C, 30–32 ppt salinity, a pH of 7.8–8.2, >5.5 mg l⁻¹ dissolved oxygen and at a 14 h–10 h light–dark photoperiod. During the acclimation period, the sea cucumbers were fed once at 08:00 h using a homemade diet containing 40% *Sargassum* powder and 60% sea mud processed into a cylindrical form. Healthy undamaged sea cucumbers (body length: 5.2±0.6 cm, wet body weight: 22.7±1.2 g; means±s.d.) were selected and used in the series of experiments.

Experimental design

Experimental conditions

Five temperatures (8, 12, 16, 20 and 24°C) were selected to approximately represent the overall temperature range (0–30°C) found in the natural habitat of *A. japonicus* (Dong and Dong, 2006). The experiments at different water temperatures were conducted consecutively. When the ambient water temperature reached the lower experimental temperatures (8 and 12°C), the experiments were initiated. Aerated water in a 4000 l tank was heated to the corresponding higher experimental temperatures (16, 20 and 24°C) using one or two 2000 W electric heaters controlled by a thermostat, then pumped into the experimental tanks. The air temperature of the experimental room was maintained at the corresponding higher experimental water temperature (16, 20 and 24°C) by air conditioning. The sea cucumbers were fed the same food as during the acclimation period for all studies except the tentacle observation trials. The test individuals were moved into the experimental tank, where they were raised from holding temperature to the experimental temperature at a rate of 0.2°C h⁻¹. Each experiment began with a 24 h (8, 12 and 16°C) or 48 h (20 and 24°C) period of temperature acclimation. All experiments were conducted in a dark environment except the tentacle observation. In this study, 08:00 h to 20:00 h was defined as ‘day’ and 20:00 h to 08:00 h was defined as ‘night’.

Diel feeding activity pattern and locomotion behaviour

The experiments were conducted in four glass tanks (60×60×50 cm height×width×depth). To decrease possible interference from external factors, the sides and bottom of the tanks were covered with opaque paper. Ten sea cucumbers were placed in each of the four experimental tanks for the 24 or 48 h acclimation period. Each trial was run for 4 days, after which the animals were removed and the tanks were drained, cleaned and refilled. The animals were fed

once per day, and half of the water was exchanged at 08:00 h. The water depth was maintained at 30 cm during the experiment. Charge-coupled device (CCD) cameras (Hikvision, DS-2CC11A2P-IR3, China) with the infrared systems were mounted 1.5 m above the experimental tank and connected to a video recorder to record the feeding and locomotion behaviours of sea cucumbers.

Feeding behaviour was determined by observing the individuals’ position in the aquarium and the decrease of food on the aquarium bottom. The proportion of feeding sea cucumbers was determined every 30 min based on the videos. Four consecutive feeding values from 30 min intervals were used to generate a single mean value every 2 h. Locomotion behaviour observations were based on analyses of the final 30 min of the video recordings made during each 2 h segment of the experiment. A grid dividing the tank into four quadrants sections was overlaid on the monitor during playback, and the number of line crossings was used as an index of horizontal distance travelled along the bottom of the tank. A sea cucumber was considered actively moving if it made a horizontal movement of more than three body lengths during the 30 min. The mean proportion of moving sea cucumbers from 08:00 h to 20:00 h was used as the day value, and the mean from 20:00 h to 08:00 h was used as the night value. Four consecutive days’ values of feeding and locomotion behaviours were used to generate a single mean value.

Tentacle locomotion observation

Tentacle locomotion during feeding was recorded using an upward-looking digital camera (Canon IXUS 125 HS) located below the glass aquarium (60×60×50 cm) according to the method of Hudson et al. (2005). A lamp with dim light beneath the tank allowed observations to be made in darkness. Four individuals of each temperature treatment were observed and the rates of tentacle insertion were determined from 100 consecutive tentacle movements.

Ingestion rate

The experiment was designed to examine the differences of diurnal and nocturnal feeding activities indicated by food intake at different water temperatures. A group of six sea cucumbers were introduced into each of the four experimental tanks (45×35×30 cm). Sea cucumbers were acclimated to the experimental tank for 1–2 days, and the experiment was conducted on four consecutive days. During the period of acclimation and experimentation, the sea cucumbers were fed twice per day at 08:00 h and 20:00 h. The remaining uneaten feed was siphoned before exchanging water twice per day, followed by rinsing with freshwater to remove salt. The dry weight of the uneaten feed was determined by drying the glass beaker (50 ml) with feed at 60°C to constant mass and weighing. The ingestion rates (IR; mg g⁻¹ h⁻¹) of day and night were calculated as described by Maxwell et al. (2009):

$$IR = [(W_o - W_u)/W_{sc}]/t, \quad (1)$$

where W_o is the dried mass of the offered food (mg); W_u is the dried mass of the uneaten feed (mg); W_{sc} is the wet mass of the sea cucumber in the tank (g); and t is time (h).

Digestive physiology

For each temperature treatment, 72 sea cucumbers were introduced into 12 tanks (45×35×30 cm), acclimated for 1–2 days and maintained for 10 days. The sea cucumbers were fed excessively

at 08:00 h every day. At the end of the experiment, six individuals were chosen randomly every 2 h during a 24 h cycle (12 sampling points) from the tanks and dissected to obtain intestines. The intestines were then cut longitudinally and washed thoroughly in ice-cold 0.84% normal saline. After rinsing, the gut was blotted dry with filter paper, frozen quickly in liquid nitrogen and then stored at -80°C until analyzed. Two replicate samples were mixed into one sample to make sure each one was enough to determine the activities of lipase and amylase enzymes.

Activities of lipase and amylase were measured using commercial assay kits (Nanjing Jiancheng, Bioengineering Institute, Nanjing, China). Lipase activity was assayed by the simplified turbidimetric assay. Amylase activity was assayed via iodine spectrophotometry. Activities of lipase and amylase were expressed as U g^{-1} protein and U mg^{-1} protein, respectively.

Statistical analysis

All statistical analysis was performed with the SPSS 19.0 for Windows statistical package. A two-way repeated measures (RM) analysis of variance (ANOVA) followed by Bonferroni test for *post hoc* multiple comparisons was used to test the effect of the interaction between time and temperature on the proportion of feeding sea cucumbers. The time of tentacle insertion, IR, proportion of moving sea cucumbers and mean digestive enzyme activities at different temperatures and digestive enzyme activities at different times were subjected to one-way ANOVA followed by *post hoc* multiple comparisons with Tukey's test. The differences between day and night of IR and the proportion of moving sea cucumbers were compared using an independent sample *t*-test. The probability level of 0.05 was used for rejection of the null hypothesis, and all data were presented as means \pm s.d.

RESULTS

Feeding behaviour

Diel feeding rhythm

A two-way repeated measures ANOVA showed that there was no significant interaction between temperature and time (interaction, $F_{44,132}=0.66$, $P=0.944$) on the proportion of feeding sea cucumbers.

However, both temperature and time had an independent significant influence on the proportion of feeding sea cucumbers (temperature, $F_{4,12}=122.08$, $P<0.001$; time, $F_{11,33}=8.30$, $P<0.001$). Concerning the effect of temperature alone, the maximum proportion of feeding sea cucumbers was observed at 16°C ($23\pm 4\%$), with no significant difference when compared with 12 and 20°C groups (*post hoc* analysis with a Bonferroni adjustment test, $P>0.05$). However, the proportions of feeding sea cucumbers in these three groups were significantly higher than those of the 8 and 24°C groups ($P<0.05$). The proportion of feeding sea cucumbers at 24°C was the lowest, significantly lower than that observed at 8°C (Fig. 1, $P>0.05$).

All temperature treatments displayed a similar feeding pattern, with the proportion of feeding sea cucumbers increasing gradually from 14:00 h to 20:00 h, then reaching feeding peak before decreasing (Fig. 1). The 8 and 24°C treatments showed a feeding peak at 02:00 h to 04:00 h, whereas the feeding peak of other treatments occurred during 00:00 h to 02:00 h (Fig. 1).

Tentacle locomotion

Frame-by-frame analysis of the video footage showed that the feeding process of *A. japonicus* involved several coordinated movements. Usually, 50% of all tentacles (10) extended from the oral crown and moved towards the sediment surface. Each tentacle contained several tree-like branches, and each branch comprised several sub-branches. As the tentacle approached the sediment, it slowly opened outwards, presumably to maximize the available surface area for subsequent particle adhesion. The tentacle was then pressed onto the sediment surface and spread out. Once the tentacle contacted enough food particles, it retracted and closed the distal branched apparatus inwards and upwards towards the mouth. The tentacle released and the food was dropped into the oral mouth (Fig. 2). The sea cucumber would repeat the action continuously during its feeding.

No significant difference in the tentacle insertion rate was observed between the treatments of 8, 12, 16 and 20°C (one-way ANOVA, $F_{3,12}=0.45$, $P=0.722$). However, no tentacle locomotion was observed at 24°C . The total time for a tentacle to be placed onto the sediment, collect food, pass it to the mouth and feed again was

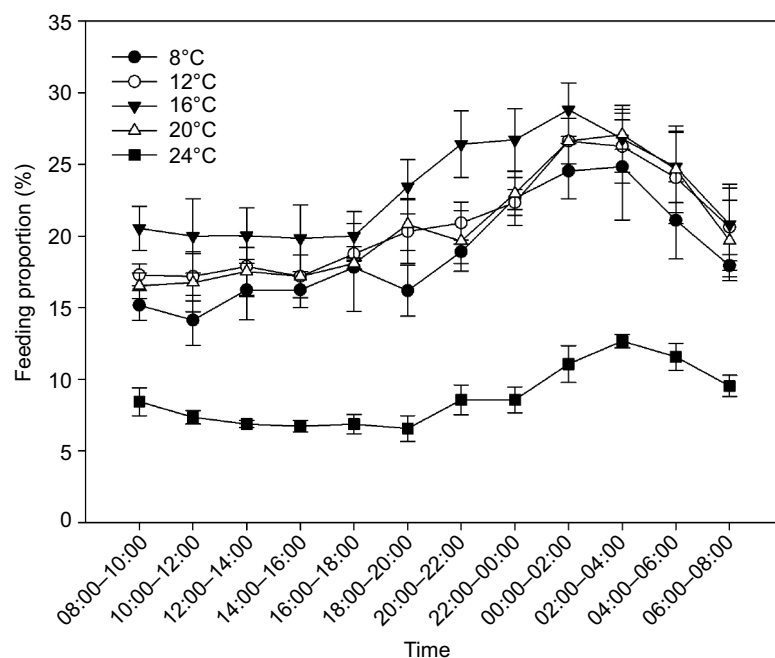


Fig. 1. Diurnal changes in the proportion of feeding *Apostichopus japonicus* at 8, 12, 16, 20 and 24°C . Data are shown as means \pm s.d. ($n=4$).

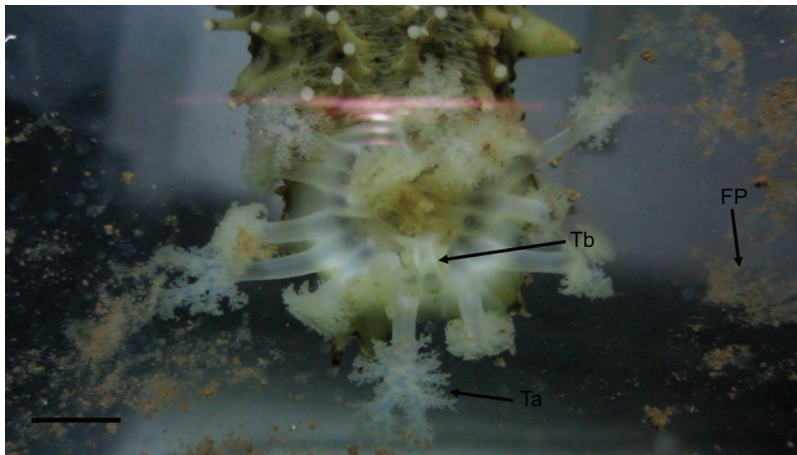


Fig. 2. Example of image obtained from videos of feeding tentacles of *A. japonicus* at 16°C. The tentacle marked 'Ta' is outstretched; a tentacle that has collected food particles (FP) and is transferring these to the mouth is labelled 'Tb'. Scale bar: 5 mm.

46.21±2.79, 44.80±2.22, 45.74±3.16 and 47.02±2.80 s at 8, 12, 16 and 20°C, respectively (Table 1).

Ingestion rate

The IR of sea cucumbers was significantly affected by water temperature (one-way ANOVA, $F_{4,15}=31.85$, $P<0.001$, Fig. 3A). Specifically, the IR of sea cucumbers at 16°C (4.9 ± 0.4 mg g⁻¹ h⁻¹) was significantly higher than at other temperatures (*post hoc* Tukey's test, $P<0.05$) and IR at 24°C was significantly lower than those observed at other temperatures ($P<0.05$). No significant difference in IR was observed between the 8, 12 and 20°C treatments ($P>0.05$). Significant differences in IR were observed between day and night within all temperature treatments (*t*-test, $P<0.05$, Fig. 3B).

Locomotion behaviour

The proportion of moving sea cucumbers was also significantly affected by water temperature (one-way ANOVA, $F_{4,15}=30.87$, $P<0.001$, Fig. 4A). The proportion of moving sea cucumbers increased as the temperature increased from 8 to 16°C, then decreased above 16°C. The proportion of moving sea cucumbers at 16°C ($42.9\pm4.4\%$) was significantly higher than that observed at 8, 20 and 24°C (*post hoc* Tukey's test, $P<0.05$). No significant differences were observed between 12 and 16°C ($P>0.05$). At 24°C, the proportion of moving sea cucumbers was significantly lower than that at 8, 12 and 16°C ($P<0.05$). No significant difference was observed between 20 and 24°C ($P>0.05$). Significant differences in the proportion of moving sea cucumbers were also observed between day and night within all temperature treatments except 24°C (Fig. 4B).

Digestive physiology

The lipase activity of sea cucumbers at all temperatures showed significant differences over time during the 24 h cycle (one-way

ANOVA, $P<0.05$, Fig. 5A). Peak lipase activity of the sea cucumbers at 8, 12 and 24°C occurred at 24:00 h, whereas the highest activity for individuals at 16 and 20°C occurred at 22:00 h. Amylase activity showed significant differences over time at 8 and 12°C (one-way ANOVA, $P<0.05$), but no significant differences over time were found for the 16, 20 and 24°C treatments ($P>0.05$, Fig. 5B). The highest amylase activity at 8°C appeared at 22:00 h, whereas peak amylase activity for individuals at 12°C occurred at 02:00 h.

Both the mean lipase and amylase activities were significantly affected by water temperature (lipase: $F_{4,10}=83.93$, $P<0.001$; amylase: $F_{4,10}=9.14$, $P=0.002$, Fig. 5). Specifically, mean lipase activities at 12, 16 and 20°C were significantly higher than at 8 and 24°C (*post hoc* Tukey's test, $P<0.05$). The highest lipase activity was 12.88 ± 0.20 U g⁻¹ protein at 20°C, and the lowest lipase activity was 6.92 ± 0.34 U g⁻¹ protein at 24°C (Fig. 5A). No significant differences in mean amylase activity were found between the 8, 12, 16 and 20°C ($P>0.05$), but they were all significantly higher than at 24°C ($P<0.05$, Fig. 5B).

DISCUSSION

The responses of feeding activity to different water temperature vary among species. As a tropical species, *H. scabra* decreased its feeding activity from 9.8 to 0.8 h day⁻¹ as the temperature changed from 24 to 17°C (Wolkenhauer, 2008). The sea cucumber species in the present study, *A. japonicus*, is a temperate species that survives in a temperature range of 0–30°C (Dong and Dong, 2006). However, growth occurs only between 12 and 21°C, and the optimal temperature for food consumption and growth is 15–18°C (An et al., 2007; Dong et al., 2006; Yang et al., 2005). The present study showed that feeding proportion peaked at 16°C, and the proportion of feeding sea cucumbers at the highest temperature treatment (24°C) was significantly lower than in the other groups.

Table 1. Tentacle locomotion of *Apostichopus japonicus* under different temperatures as tested by video observations in an aquarium

Specimen no.	Tentacle insertion (s)				
	8°C	12°C	16°C	20°C	24°C
1	42.95±4.09	47.45±3.82	45.89±3.69	48.69±4.59	–
2	47.48±4.25	43.68±3.85	47.23±4.07	49.58±5.01	–
3	45.07±3.94	42.39±4.52	48.56±3.95	46.52±3.21	–
4	49.35±5.02	45.67±3.52	41.29±2.93	43.28±3.58	–
Mean	46.21±2.79 ^a	44.80±2.22 ^a	45.74±3.16 ^a	47.02±2.80 ^a	–

Data are means±s.d. ($n=100$). Means followed by the same letter in the same row are not significantly different ($P>0.05$). Tentacle insertion time is the total time (in seconds) for a tentacle to be placed onto the sediment, collect food, pass it to the mouth and feed again.

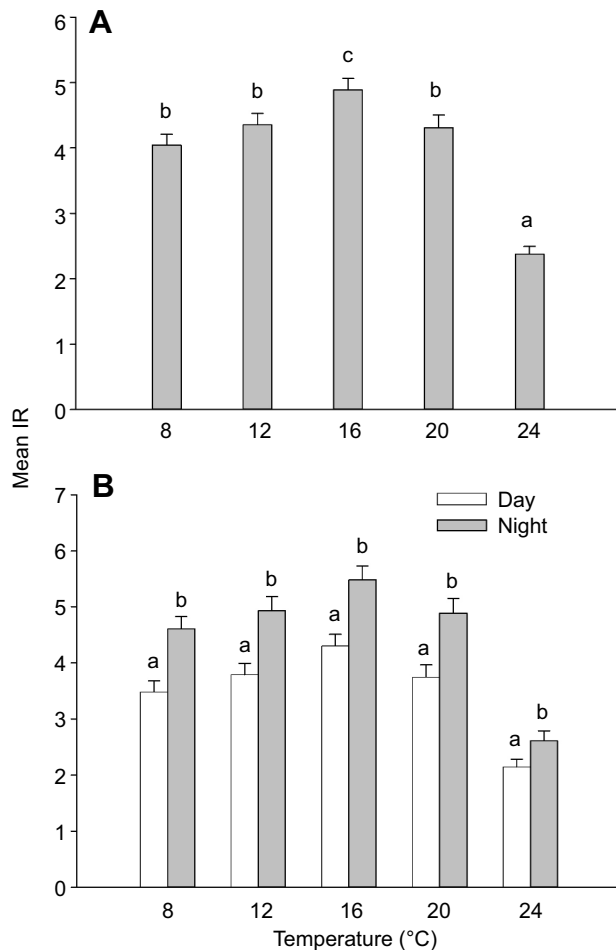


Fig. 3. Mean ingestion rates (IRs) at different temperatures. (A) Mean IR of *A. japonicus* at different temperatures (8, 12, 16, 20 and 24°C). (B) Mean day and night IR of *A. japonicus* at different temperatures. Different letters within each treatment represent significant differences (A: one-way ANOVA; B: t-test, $n=4$, $P<0.05$).

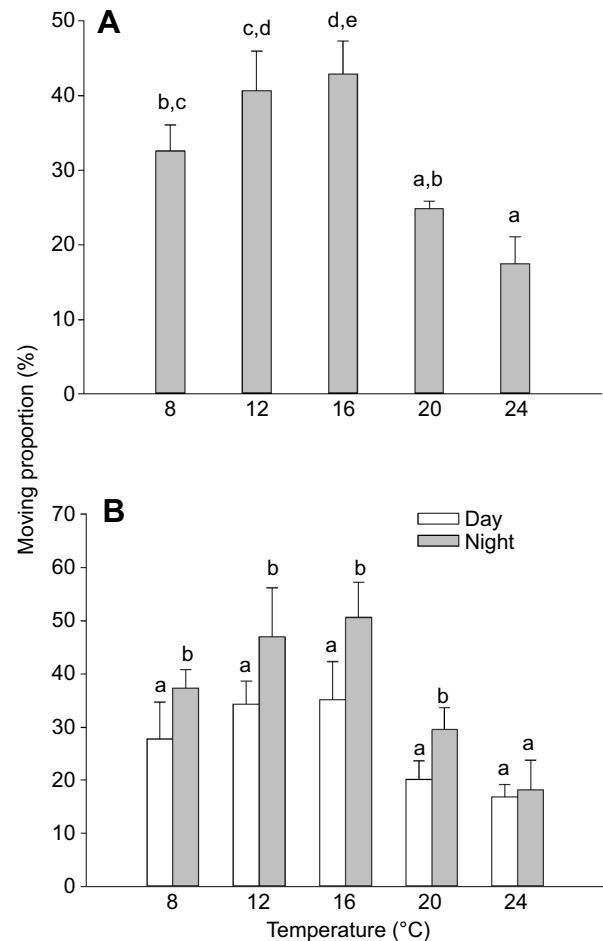


Fig. 4. The proportion of moving *A. japonicas* at different temperatures. (A) The proportion of moving *A. japonicus* at different temperatures (8, 12, 16, 20 and 24°C). (B) Day and night proportion of moving *A. japonicus* at different temperatures. Different letters within each treatment represent significant differences (A: one-way ANOVA; B: t-test, $n=4$, $P<0.05$).

This finding was consistent with the results obtained for IR, which demonstrated that the highest and the lowest IRs were found at 16 and 24°C, respectively. These results were supported by previous studies by Dong et al. (2006), which showed that juvenile *A. japonicus* displayed the highest specific growth rate (SGR) at 16–18°C and the lowest SGR at 24°C. It can thus be concluded from the present study that 16°C is the optimal water temperature for the feeding activity of *A. japonicus*, and high temperature ($\geq 24^\circ\text{C}$) had negative effects on feeding activity of this species.

Sea cucumbers have previously been shown to exhibit physiological adaptations that parallel the behavioural switches that occur with seasonal changes in temperature (Hamel and Mercier, 1996, 1998; Singh et al., 1999; Swan, 1961). The present study examined the direct effects of water temperature on the diurnal variation of feeding activity of sea cucumbers using infrared-light-assisted laboratory observations. *Apostichopus japonicus* exhibited a higher feeding proportion from 22:00 h to 06:00 h, and the feeding peak occurred at 00:00 h to 04:00 h. Also, they had higher IRs at night than during the day. Our study showed that *A. japonicus* demonstrated a distinct nocturnal feeding activity pattern at all temperature treatments. While water temperature significantly affected the activity levels of *A. japonicus*, it did not alter the overall feeding rhythm or timing of feeding peaks.

Sea cucumbers feed by extending their buccal tentacles either into or over the sediment surface (deposit feeders) or into the water column (suspension feeders) using a variety of tentacle forms (Roberts and Moore, 1997). Factors that are known to cause variation in the tentacle insertion rate of sea cucumbers include body size (Sun et al., 2015), current speed (Holtz and MacDonald, 2009; Singh et al., 1999), and food quality or concentration (Singh et al., 1998, 1999). These factors might counteract or exacerbate the effect of temperature on tentacle locomotion in the wild. This study aimed specifically to exclude those variable factors to find a possible underlying pattern in response to water temperature alone. Our results indicated that water temperature had no influence on the tentacle insertion rate, and this corresponded well with a previous report, which showed that tentacle insertion rates of *Cucumaria frondosa* were not significantly related to temperature (Singh et al., 1999). Combined with the results that water temperature affected the IR, we may assume that the influence of water temperature on feeding behaviour was mainly reflected in the duration of feeding. There are two possible reasons for the lack of tentacle locomotion at 24°C. First, high temperature stopped the feeding behaviour of *A. japonicus*. Second, the light or the presence of humans for observation exacerbated the cessation of feeding, given that *A. japonicus* prefers to feed in a dark environment.

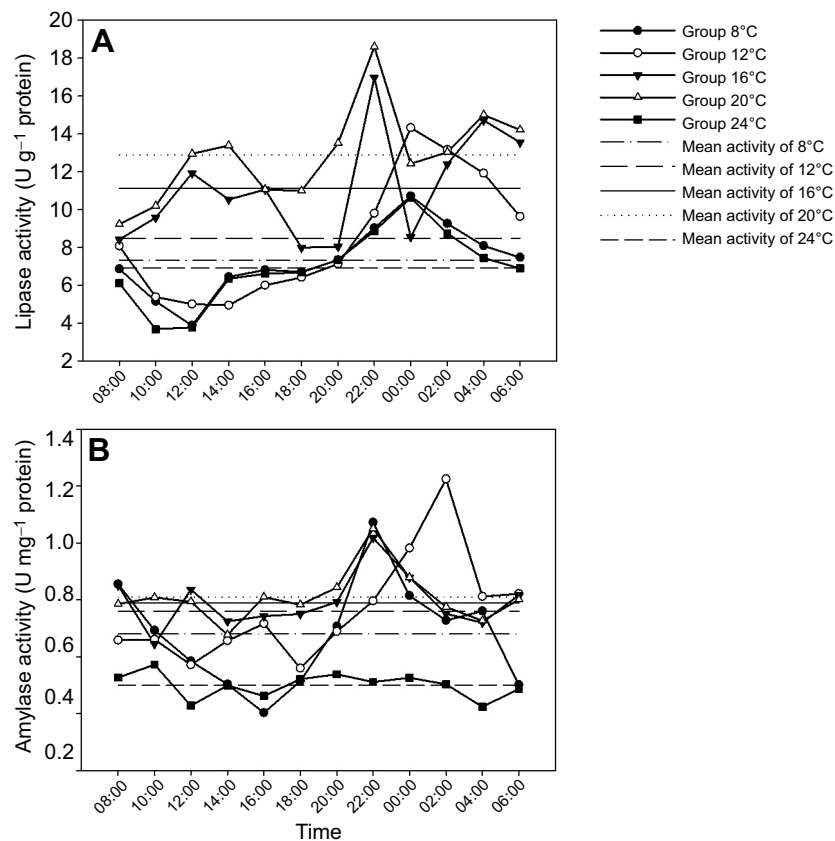


Fig. 5. Digestive enzyme activity levels over time. (A) Diurnal pattern of lipase activities and mean activities at different temperatures. (B) Diurnal pattern of amylase activities and mean activities at different temperatures. Data are presented as means ($n=3$).

The locomotion of many aquatic animals is significantly related to water temperature (Reynolds, 1977). For example, the sea urchin *Abatus ingens* increased their displacement activity with only a 1°C increase in temperature (Thompson and Riddle, 2005). The amount of time during the day that individuals of the sea cucumber species *H. scabra* spend buried (no movement) increased with decreasing temperature from 6.7 h per day at 24°C to 14.5 h per day at 17°C (Wolkenhauer, 2008). A long period of high water temperature would induce *A. japonicus* to enter aestivation, during which they exhibited little movement and depressed metabolic activity (Yang et al., 2015). In the present study, the proportion of moving sea cucumbers increased and then decreased with increasing temperature. *Apostichopus japonicus* became inactive in the present study at 24°C, which is consistent with the reported thermal threshold (~24°C) for aestivation in the field (Yang et al., 2005). A laboratory investigation conducted by Kato and Hirata (1990) also found that *A. japonicus* moved more (49.6 m day⁻¹ on average) at temperatures <17°C and moved less (21.6 m day⁻¹ on average) at temperatures >18°C (Kato and Hirata, 1990). This may be because the high temperature resulted in an increased energetic cost and oxygen consumption (Dong et al., 2006, 2008), which made the sea cucumber reduce movement to save energy for other metabolic activities. In the wild, one of the primary reasons for movement by sea cucumbers is to search for food (Uthicke, 1999; Mercier et al., 1999). Therefore, the decrease in movement may in part have reduced the feeding activity.

A study conducted on one Indo-Pacific species showed that *Holothuria edulis* had a higher movement rate at night than during the day (Wheeling et al., 2007). In our study, a nocturnal locomotion behaviour was also evident at all temperatures but 24°C. At 24°C, *A. japonicus* was relatively inactive and displayed a nearly equal

moving proportion during the day and night. This suggests that the high temperature (>24°C) may alter diel locomotion rhythm. Similar results have also been reported in some other species of sea cucumbers. Most adult *H. scabra* on the surface did not follow their usual burying cycle when the water temperature was increased to more than 30°C (Mercier et al., 2000).

Even in complete darkness, *A. japonicus* still showed more active feeding and moving at night than during the day in the present study. Dong et al. (2011) reported that the nocturnal activity patterns (i.e. emergence and feeding at night, sheltering during the day) of *A. japonicus* were observed under continuous darkness as well as continuous light. Sea cucumbers have evolved multiple behavioural strategies to increase their chances of survival in nature. It is believed that nocturnal activity results in the avoidance of diurnal predation (Ebling et al., 1966; Lawrence and Hughes-Games, 1972; Nelson and Vance, 1979). Many predators have been reported to capture *A. japonicus* at different developmental stages, such as copepods for larval *A. japonicus*, and carnivorous fish, sea stars, sea urchins and crabs for juveniles and adults less than 10 cm (Yang et al., 2015). In the present experiment, sea cucumbers were kept with a natural photoperiod before the experiment began. Perhaps the length of the experiment was too short or the attempt to change their behaviour was too rapid in relation to their internal clock. Additional studies should, therefore, be conducted to determine whether longer exposure to the different external environment can change the activity patterns of *A. japonicus*. Thus, it can be concluded from our results and previous studies on this species that diel feeding and locomotion within a short period were not controlled by light but by an endogenous rhythm.

The effects of water temperature on the activities of digestive enzymes of the poikilotherm *A. japonicus* were mainly reflected in

two aspects. First, changes in water temperature caused body temperature changes, which then directly altered the activities of enzymes within the digestive tract. Second, the water temperature affected the feeding behaviour, which then had an indirect impact on digestive activity (Gao et al., 2009). In this study, both lipase and amylase activities increased with the water temperature and reached peaks at 20°C, which is in line with the previous study (Gao et al., 2009). The lowest activities of both digestive enzymes were found at 24°C. The decrease in digestive enzyme activities at 24°C may therefore be related to a reduction in food intake. Maintaining a high level of digestive enzyme activity within the digestive tract would consume energy and, thus, a reduction in digestive enzyme activity during aestivation is conducive to energy conservation in sea cucumber (Gao et al., 2009). The declining trends in digestive enzyme activity observed in *A. japonicus* during the aestivation phase are consistent with data from lobster and shrimp, either in aestivation or under food deprivation (Comoglio et al., 2004; Johnston et al., 2004).

Digestive activity was positively correlated with both ingestion and assimilation of food (Baars and Oosterhuis, 1984). In our study, the highest activities of lipase and amylase were observed between 22:00 h and 02:00 h (depending on temperature), which was slightly earlier than their corresponding feeding peak (00:00 h to 04:00 h). The results demonstrated that *A. japonicus* may have the ability to optimize digestive function by secreting digestive enzymes before the feeding peak. This mechanism from ancestral sea cucumbers may allow them to concentrate on feeding in a short time period and thus lower predation risks (Sánchez-Vázquez et al., 1997). In addition, the uptake of food as a physical and biochemical trigger will further increase digestive enzyme secretion into the gut (Zeytin et al., 2016).

In conclusion, the present study highlighted the shift in the diel activity of *A. japonicus* in response to different water temperatures, which will refine our ecological understanding of *A. japonicus*. The knowledge of the preferred conditions of water temperature in *A. japonicus* will assist in the design of suitable holding conditions in the context of captive breeding. In addition, understanding diel activity pattern will be of strategic value to the design of the optimal feeding schedule to maximize feeding efficiency and minimize food waste.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Methodology: J.S.; Formal analysis: J.S.; Investigation: J.S.; Data curation: J.S.; Writing - original draft: J.S.; Writing - review & editing: J.S., L.Z., Y.P., C.L., F.W.; Supervision: L.Z., F.W., H.Y.; Funding acquisition: L.Z., H.Y.

Funding

This work was supported by the National Natural Science Foundation of China (41676136), the Strategic Priority Research Program of Chinese Academy of Sciences (XDA11020703), the Funding of Youth Innovation Promotion Association, CAS, and the Nature Science Foundation of Shandong Province (ZR2016CQ04).

References

- An, Z., Dong, Y. and Dong, S. (2007). Temperature effects on growth-ratios of juvenile sea cucumber *Apostichopus japonicus* (Selenka). *Aquaculture* **272**, 644-648.
- Baars, M. and Oosterhuis, S. (1984). Diurnal feeding rhythms in North Sea copepods measured by gut fluorescence, digestive enzyme activity and grazing on labelled food. *Neth. J. Sea Res.* **18**, 97-119.
- Brown, R. S. and Mackay, W. C. (1995). Fall and winter movements of and habitat use by cutthroat trout in the Ram River, Alberta. *Trans. Am. Fish Soc.* **124**, 873-885.
- Buentello, J. A., Gatlin, D. M. and Neill, W. H. (2000). Effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization and growth of channel catfish (*Ictalurus punctatus*). *Aquaculture* **182**, 339-352.
- Chen, Y., Gao, F., Liu, G. S., Shao, L. P. and Shi, G. F. (2007). The effects of temperature, salinity and light cycle on the growth and behavior of *Apostichopus japonicus*. *J. Fish China* **5**, 687-691.
- Comoglio, L., Gaxiola, G., Roque, A., Cuzon, G. and Amin, O. (2004). The effect of starvation on refeeding, digestive enzyme activity, oxygen consumption, and ammonia excretion in juvenile white shrimp *Litopenaeus vannamei*. *J. Shellfish Res.* **23**, 243-249.
- del Pozo, A., Montoya, A., Vera, L. M. and Sánchez-Vázquez, F. J. (2012). Daily rhythms of clock gene expression, glycaemia and digestive physiology in diurnal/nocturnal European seabass. *Physiol. Behav.* **106**, 446-450.
- Dong, Y. and Dong, S. (2006). Growth and oxygen consumption of the juvenile sea cucumber *Apostichopus japonicus* (Selenka) at constant and fluctuating water temperatures. *Aquac. Res.* **37**, 1327-1333.
- Dong, Y., Dong, S., Tian, X., Wang, F. and Zhang, M. (2006). Effects of diel temperature fluctuations on growth, oxygen consumption and proximate body composition in the sea cucumber *Apostichopus japonicus* Selenka. *Aquaculture* **255**, 514-521.
- Dong, Y., Dong, S. and Ji, T. (2008). Effect of different thermal regimes on growth and physiological performance of the sea cucumber *Apostichopus japonicus* Selenka. *Aquaculture* **275**, 329-334.
- Dong, G., Dong, S., Wang, F. and Tian, X. (2010). Effects of light intensity on daily activity rhythm of juvenile sea cucumber, *Apostichopus japonicus* (Selenka). *Aquac. Res.* **41**, 1640-1647.
- Dong, G., Dong, S., Tian, X. and Wang, F. (2011). Effects of photoperiod on daily activity rhythm of juvenile sea cucumber, *Apostichopus japonicus* (Selenka). *Chin. J. Oceanol. Lim.* **29**, 1015-1022.
- Ebling, F., Hawkins, A., Kitching, J., Muntz, L. and Pratt, V. M. (1966). The ecology of Lough Ine XVI. Predation and diurnal migration in the Paracentrotus community. *J. Anim. Ecol.* **35**, 559-566.
- Fraser, N. H. C., Metcalfe, N. B. and Thorpe, J. E. (1993). Temperature-dependent switch between diurnal and nocturnal foraging in salmon. *Proc. R. Soc. Lond. Ser. B. Biol. Sci.* **252**, 135-139.
- Gao, F., Yang, H., Xu, Q., Wang, F. and Liu, G. (2009). Effect of water temperature on digestive enzyme activity and gut mass in sea cucumber *Apostichopus japonicus* (Selenka), with special reference to aestivation. *Chin. J. Oceanol. Lim.* **27**, 714-722.
- Hamel, J.-F. and Mercier, A. (1996). Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Can. J. Fish Aquat. Sci.* **53**, 253-271.
- Hamel, J.-F. and Mercier, A. (1998). Diet and feeding behaviour of the sea cucumber *Cucumaria frondosa* in the St. Lawrence estuary, eastern Canada. *Can. J. Zool.* **76**, 1194-1198.
- Hamel, J.-F. and Mercier, A. (2008). Population status, fisheries and trade of sea cucumbers in temperate areas of the Northern Hemisphere. *FAO Fish. Aquac. Tech. Paper* **516**, 257-291.
- Holtz, E. H. and MacDonald, B. A. (2009). Feeding behaviour of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) in the laboratory and the field: relationships between tentacle insertion rate, flow speed, and ingestion. *Mar. Biol.* **156**, 1389-1398.
- Hudson, I. R., Wigham, B. D., Solan, M. and Rosenberg, R. (2005). Feeding behaviour of deep-sea dwelling holothurians: Inferences from a laboratory investigation of shallow fjordic species. *J. Mar. Syst.* **57**, 201-218.
- Hurst, T. P. and Duffy, T. A. (2005). Activity patterns in northern rock sole are mediated by temperature and feeding history. *J. Exp. Mar. Biol. Ecol.* **325**, 201-213.
- Johnston, D. J., Ritar, A. J. and Thomas, C. W. (2004). Digestive enzyme profiles reveal digestive capacity and potential energy sources in fed and starved spiny lobster (*Jasus edwardsii*) phyllosoma larvae. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **138**, 137-144.
- Kato, A. and Hirata, H. (1990). Effects of water temperature on the circadian rhythm of the sea-cucumber, *Stichopus japonicus* in culture. *Aquac. Sci.* **38**, 75-80.
- Lawrence, J. and Hughes-Games, L. (1972). The diurnal rhythm of feeding and passage of food through the gut of *Diadema setosum* (Echinodermata: Echinoidea). *Isr. J. Zool.* **21**, 13-16.
- Mata-Sotres, J. A., Moyano, F. J., Martínez-Rodríguez, G. and Yúfera, M. (2016). Daily rhythms of digestive enzyme activity and gene expression in gilthead seabream (*Sparus aurata*) during ontogeny. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **197**, 43-51.
- Maxwell, K. H., Gardner, J. P. A. and Heath, P. L. (2009). The effect of diet on the energy budget of the brown sea cucumber, *Stichopus mollis* (Hutton). *J. World Aquacult. Soc.* **40**, 157-170.
- Mercier, A., Battaglene, S. C. and Hamel, J.-F. (1999). Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. *J. Exp. Mar. Biol. Ecol.* **239**, 125-156.
- Mercier, A., Battaglene, S. C. and Hamel, J.-F. (2000). Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. *Hydrobiologia* **440**, 81-100.
- Montoya, A., López-Olmeda, J., Yúfera, M., Sánchez-Muros, M. and Sánchez-Vázquez, F. (2010). Feeding time synchronises daily rhythms of behaviour and

- digestive physiology in gilthead seabream (*Sparus aurata*). *Aquaculture* **306**, 315–321.
- Nelson, B. V. and Vance, R. R.** (1979). Diel foraging patterns of the sea urchin *Centrostephanus coronatus* as a predator avoidance strategy. *Mar. Biol.* **51**, 251–258.
- Okorie, O. E., Ko, S. H., Go, S., Lee, S., Bae, J. Y., Han, K. and Bai, S. C.** (2008). Preliminary study of the optimum dietary ascorbic acid level in sea cucumber, *Apostichopus japonicus* (Selenka). *J. World Aquacult. Soc.* **39**, 758–765.
- Purcell, S. W.** (2010). Diel burying by the tropical sea cucumber *Holothuria scabra*: effects of environmental stimuli, handling and ontogeny. *Mar. Biol.* **157**, 663–671.
- Purcell, S. W., Polidoro, B. A., Hamel, J.-F., Gamboa, R. U. and Mercier, A.** (2014). The cost of being valuable: predictors of extinction risk in marine invertebrates exploited as luxury seafood. *Proc. R. Soc. B* **281**, 20133296.
- Reebs, S. G.** (2002). Plasticity of diel and circadian activity rhythms in fishes. *Rev. Fish Biol. Fish.* **12**, 349–371.
- Reynolds, W. W.** (1977). Temperature as a proximate factor in orientation behavior. *J. Fish. Board Can.* **34**, 734–739.
- Roberts, D. and Moore, H.** (1997). Tentacular diversity in deep-sea deposit-feeding holothurians: implications for biodiversity in the deep sea. *Biodivers. Conserv.* **6**, 1487–1505.
- Sánchez-Vázquez, F., Madrid, J., Zamora, S. and Tabata, M.** (1997). Feeding entrainment of locomotor activity rhythms in the goldfish is mediated by a feeding-entrainable circadian oscillator. *J. Comp. Physiol. A* **181**, 121–132.
- Singh, R., MacDonald, B. A., Lawton, P. and Thomas, M. L. H.** (1998). Feeding response of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) to changing food concentrations in the laboratory. *Can. J. Zool.* **76**, 1842–1849.
- Singh, R., MacDonald, B. A., Thomas, M. L. H. and Lawton, P.** (1999). Patterns of seasonal and tidal feeding activity in the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) in the Bay of Fundy, Canada. *Mar. Ecol. Prog. Ser.* **187**, 133–145.
- Sun, J., Zhang, L., Pan, Y., Lin, C., Wang, F., Kan, R. and Yang, H.** (2015). Feeding behavior and digestive physiology in sea cucumber *Apostichopus japonicus*. *Physiol. Behav.* **139**, 336–343.
- Swan, E. F.** (1961). Seasonal evisceration in the sea cucumber, *Parastichopus californicus* (Stimpson). *Science (New York, NY)* **133**, 1078.
- Thompson, B. A. W. and Riddle, M. J.** (2005). Bioturbation behaviour of the spatangoid urchin *Abatus ingens* in Antarctic marine sediments. *Mar. Ecol. Prog. Ser.* **290**, 135–143.
- Tillner, R., Rønnestad, I., Dhert, P. and Ueberschär, B.** (2014). The regulatory loop between gut cholecystokinin and tryptic enzyme activity in sea bass (*Dicentrarchus labrax*) larvae is influenced by different feeding regimes and trigger substances. *Aquaculture* **420**, 139–146.
- Toral-Granda, V., Lovatelli, A. and Vasconcellos, M.** (2008). *Sea Cucumbers: a Global Review of Fisheries and Trade*. FAO Fish. Aquac. Tech. Paper. Rome: FAO.
- Uthicke, S.** (1999). Sediment bioturbation and impact of feeding activity of *Holothuria (Halodeima) atra* and *Stichopus chloronotus*, two sediment feeding holothurians, at Lizard Island, Great Barrier Reef. *Bull. Mar. Sci.* **64**, 129–141.
- Vera, L., De Pedro, N., Gomez-Milan, E., Delgado, M. J., Sanchez-Muros, M. J., Madrid, J. A. and Sanchez-Vazquez, F. J.** (2007). Feeding entrainment of locomotor activity rhythms, digestive enzymes and neuroendocrine factors in goldfish. *Physiol. Behav.* **90**, 518–524.
- Wang, F., Yang, H., Gao, F. and Liu, G.** (2008). Effects of acute temperature or salinity stress on the immune response in sea cucumber, *Apostichopus japonicus*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **151**, 491–498.
- Wheeling, R. J., Verde, E. A. and Nestler, J. R.** (2007). Diel cycles of activity, metabolism, and ammonium concentration in tropical holothurians. *Mar. Biol.* **152**, 297–305.
- Wolkenhauer, S. M.** (2008). Burying and feeding activity of adult *Holothuria scabra* (Echinodermata: Holothuroidea) in a controlled environment. *SPC Béche-de-mer Information Bulletin* **27**, 25–28.
- Yang, H., Yuan, X., Zhou, Y., Mao, Y., Zhang, T. and Liu, Y.** (2005). Effects of body size and water temperature on food consumption and growth in the sea cucumber *Apostichopus japonicus* (Selenka) with special reference to aestivation. *Aquac. Res.* **36**, 1085–1092.
- Yang, H., Hamel, J.-F. and Mercier, A.** (2015). *The Sea Cucumber Apostichopus japonicus: History, Biology and Aquaculture*. Amsterdam: Elsevier.
- Zeytin, S., Schulz, C. and Ueberschär, B.** (2016). Diurnal patterns of tryptic enzyme activity under different feeding regimes in gilthead sea bream (*Sparus aurata*) larvae. *Aquaculture* **457**, 85–90.