

## RESEARCH NOTE

### Illumination can change the periodic variation of the oxygen consumption rate of *Crassostrea gigas*

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Illumination is a complicated ecological factor for aquatic organisms (Zhou, Niu & Li, 2000). It has many ecological functions and is of considerable significance for biological evolution (Ruppert, Fox & Barnes, 2004). Molluscs—Gastropoda, Bivalvia, Polyplacophora and Cephalopoda—have been found to possess photosensitive organs (Serb & Earnisse, 2008), but the structure and function of these photosensitive organs vary among species (Kobak & Nowacki, 2007; Morton, 2008). In bivalves, species with ‘structured photosensitivity organs’ have strong environmental perception ability (Wu *et al.*, 2015); species with ‘unstructured photosensitivity organs’ can also use skin receptors to sense light (Ramirez *et al.*, 2011). Oysters are typical bivalves, but complex cephalic eyes have been reported only in oyster larvae (Morton, 2008), and these develop into a pair of light-sensitive eyespots that gradually degenerate soon after metamorphosis (Baker & Mann, 1994). Nevertheless, a gene that may be associated with photosensitivity in adult oysters has been reported (Wu *et al.*, 2018). To our knowledge, ‘structured photosensitivity organs’ in adult oysters have not been reported, and yet some researchers have explored the photosensitivity of adult oysters by observing the opening of their shells’ dark conditions, when light was provided (Wu *et al.*, 2015). These results indicate that adult oysters are sensitive to light.

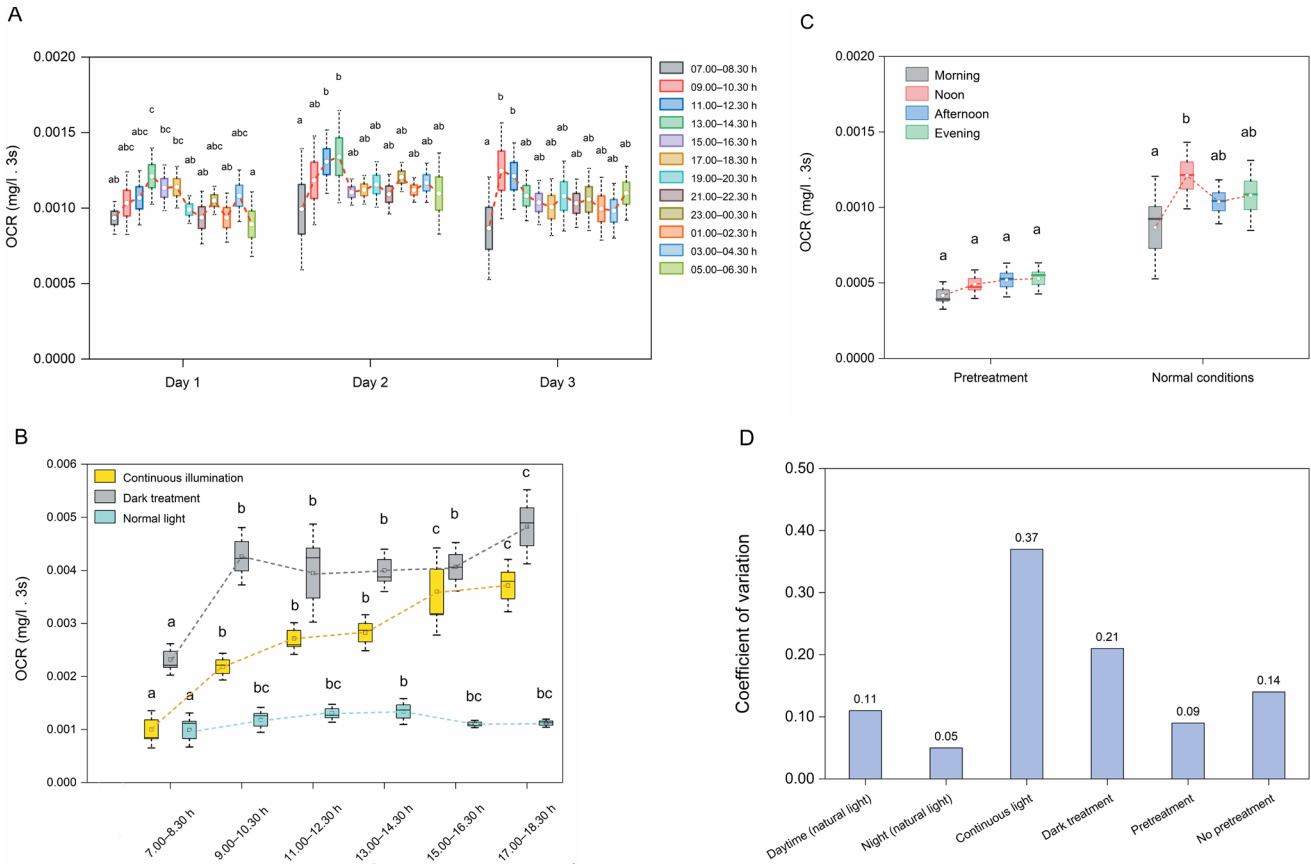
The behaviour, growth and development of molluscan species with photosensitive organs are dramatically affected by light. It has been elucidated that light could exert an influence on the sand-diving activities of clam larvae (Fonken & Nelson, 2014); under light conditions, the food intake of the benthic mud snail *Heleobia australis* was reduced (Magalhaes *et al.*, 2014). However, little is known about the effect of light on the physiology of bivalves, especially on sessile organisms such as adult oysters (Baker & Mann, 1994). Adult oysters are sensitive to various environmental factors owing to their high physiological plasticity (Zhang *et al.*, 2014, 2016).

Gaping behaviour provides a measurable response to light factors in shellfish (Andrade *et al.*, 2016). In *Mytilus galloprovincialis*,

the light/dark cycle may represent an environmental zeitgeber entraining the endogenous valve gaping rhythm (Comeau *et al.*, 2018). Similarly, *Pinna nobilis* shows cyclic gaping behaviour related only to light entrainment (Garcia-March *et al.*, 2016). While valve behaviour exhibited a strong tidal rhythm combined with a daily rhythm in *Crassostrea gigas* (Payton *et al.*, 2017), light is a significant driver of valve behaviour rhythm (Rodland *et al.*, 2009). Circadian clock genes have also been detected to be linked to the rhythmicity of oysters (Tran *et al.*, 2020). However, there are few reports on the detection of oyster rhythm at the physiological metabolism level. Oxygen consumption rate is a sensitive physiological and metabolic indicator frequently used to test the response of an organism to environmental changes (Prins, Smaal & Dame, 1998); as such, it is also an essential physiological ecological characteristic of marine bivalves (Widdows & Bayne, 1971). Oxygen consumption rate is highly detectable and reflects the physiological metabolism level of bivalves in real time compared with the valve gape. Hence, we hypothesize that oxygen consumption rate may be used to detect the physiological effects of light on molluscs.

In this study, oxygen consumption rate was used to assess the physiological effects of light on the dominant intertidal bivalve species, the oyster *C. gigas*. Two-year-old oysters of similar size (shell height:  $80.53 \pm 4.08$  mm; wet weight:  $75.79 \pm 3.66$  g) were collected from Aoshan Bay in Qingdao, China. To avoid the influence of periphyton, all individuals were cleaned and reared temporarily in a 500-l tank for 2 weeks before testing. The salinity (29‰) and temperature (20 °C) of the seawater were controlled to be consistent with the seawater of the sampled habitat. To ensure a sufficient supply of food in the tank, 4 g/m<sup>3</sup> of spirulina powder was added thrice a day. An air pump (Super Silent Power Air Pump, China, V-10) was used to ensure a sufficient and stable supply of oxygen, algae and the uniform distribution of spirulina powder.

During the experiment, the oysters were placed in sealed bottles (0.35 l), filled with air-saturated seawater from a 50-l bucket with 4 g/m<sup>3</sup> of spirulina powder. The temperature was regulated using a water bath at 20 °C and the salinity was maintained at 29‰.



**Figure 1.** **A.** Trend chart of oxygen consumption rate within 24 h. The x-axis shows the number of days for which the oxygen was monitored and the y-axis shows the oxygen consumption rate (OCR). There was no significant difference between groups marked with the same letter, and there were significant differences in components marked with different letters ( $P < 0.05$ ). **B.** The changing trend of the oxygen consumption rate of adult *Crassostrea gigas* in a 12-h period and under three treatments (natural, constant and shaded light). **C.** The oxygen consumption rate of adult *C. gigas* at different times and 2 d of constant light pretreatment and natural light treatment. **D.** The average variation coefficient of oxygen consumption rate at different periods under different conditions.

The seawater was refreshed every 2 h and mixed using a magnetic stirrer bar that was placed beneath the chamber to ensure adequate and uniform distribution of oxygen in the sealed bottle. The dissolved oxygen levels were measured using a ten-channel optical fibre oxygen meter (OXY-10 ST Prototype, PreSens Precision Sensing GmbH, Germany).

We tested the oxygen consumption rate of *C. gigas* in four different lighting conditions. (1) Natural light (duration of daytime: 07:00–19:00; night: 19:00–07:00); three replicates were conducted for 3 d, and the effects of other light sources were removed for the duration of the experiment. (2) Constant light: lamps with the same specifications (240 lx) were used for continuous lighting, and the oxygen consumption rate was measured for over 12 h. (3) Removal of light by placing oysters in dark conditions, with the oxygen consumption rate being measured for over 12 h. (4) Constant light after pretreatment: after 2 d of pretreatment with constant temperature and constant light source at a controlled temperature, the oxygen consumption rate was measured four times in 1 d.

The dissolved oxygen contents were measured every 3 s by the fibre optic probes. Due to the initial violent fluctuation of the dissolved oxygen, data points in the first 30 min were ignored and the subsequent 1,200 data points were adopted to obtain the fitting line and slope coefficient  $K$ . The oxygen consumption rate of the oysters was calculated as  $K \cdot V$ , where  $V$  is the volume of the sealed bottle (l) and  $K$  is the slope of the fitted line (Lihua et al., 2013). We plotted the trend with the average oxygen consumption rate and an ANOVA analysis was performed on the obtained data.

It was verified that under natural light conditions, the oxygen consumption rate of *C. gigas* varied regularly in the daily cycle: it gradually increased in the morning, reached a peak at noon and then decreased gradually, whereas at night it tended to fluctuate continuously over a small range. The variation in range of oxygen consumption rate during the daytime was more significant than that at night, and the trend over 24 h was approximately the same in the three replicate groups (Fig. 1A).

The regular variation of oxygen consumption rate in the daily cycle was disturbed by changing the light condition. Under continuous light, the oxygen consumption rate showed a gradual and constant increase during 12 h; under the shaded light treatment, it showed a more significant fluctuation compared to rates under the natural light conditions (Fig. 1B). After 2 d of constant pretreatment with light, the oxygen consumption rate under constant light was slightly lower than that under natural light conditions but became more stable over the day (Fig. 1C). Correspondingly, the variation coefficients at different periods under different conditions are shown in Fig. 1D. Under the 24-h natural light condition, there was a significant difference ( $P < 0.05$ ) in the coefficient of variation between daytime and nighttime. The variation coefficient was much higher than that under the normal light when the illumination conditions were controlled by shading or constant light.

Along with the ebb and flow of the tides, oysters in the intertidal zone are subjected to regular changes in salinity, temperature, food, oxygen content and light intensity, and the daily rhythm of

oysters may be affected in such a highly variable environment (Bulla *et al.*, 2017). In our indoor experiment, the oxygen consumption rate of *C. gigas* showed a daily rhythm, while it was not the case in another indoor experiment conducted at a temperature quite different from the original seawater (Lihua *et al.*, 2013). A similar oxygen consumption rate and its daily rhythm trend were also detected in a field experiment in a previously published study (Lihua *et al.*, 2013). Therefore, we deduce the temperature difference may explain the absence of a daily rhythm in an earlier indoor experiment (Lihua *et al.*, 2013). In our study, in order to reflect the oyster's natural state of the daily rhythm trend, we attempted to measure the illumination effect on oxygen consumption rate at ambient temperature and the indoor experiment temperature was selected to be consistent with the original seawater. In addition, studies on abalone wrinkling (Huang *et al.*, 2008) and *Hemifusus ternatanus* (Baojun, Wei & Xiong, 2015) have shown that the variability in the oxygen consumption rate was not significant when temperature and salinity conditions were stable; this might be due to the stable living environment of the two species. The dynamic pattern of the oxygen consumption rate under different light conditions suggests that the shaded and continuous light conditions significantly change the oxygen consumption rate, and that light has an effect on the physiology of the oyster.

Interestingly, the oysters pretreated with continuous light at a constant temperature exhibited more stable oxygen consumption rates when exposed to constant light compared to the oysters not subjected to a pretreatment of constant light. We think that the preconditioning of the oyster with constant light in the experiment was an acclimatization process (Bougrier *et al.*, 1995). Therefore, we suggest that an approach using a pretreatment and the measuring of oxygen consumption rate under constant light might yield more repeatable estimates of oxygen consumption rate. We also suggest that such an approach might be usable for oysters as well as other marine ectotherms.

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