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Effect of temperature and thermal stress on the hemodynamics of the scallop *Chlamys farreri*, as indicated by Doppler ultrasonography

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A B S T R A C T

The effect of temperature and thermal stress on cardiac activity and hemodynamics of a temperate scallop species *Chlamys farreri* was revealed using Doppler echocardiography. The variation scope of eight hemodynamic parameters within the optimal temperature range (8–26 °C) was determined, including heart beat rate (HR), acceleration of blood flow (ABF), peak systolic velocity (PS), end diastolic velocity (ED), maximum & minimum instantaneous blood flow (Max & Min-IBF), resistive index (RI) and PS/ED.The effects of two thermal stress factors were examined, prolonging high water temperature (28–30 °C) and fast heating (2 °C h^{-1}), on hemodynamics of gill blood vessels. The HR-based Arrhenius break temperatures (ABTs) of the scallop under two stress factors were 29.32 °C and 32.37 °C respectively. Results showed that the scallop has a short-term tolerance to acute thermal stress compared with prolonging high temperature. When temperature rose above 28 °C, the PS/ED ratio and RI exceeded the thresholds of 3.0 and 0.63, respectively, indicating potential damage and malfunction of the blood vessels. Low water temperature (6 °C) may also have negative impacts on blood circulation. When faced with a rapidly rising temperature, the scallop cannot adjust its heartbeat in time, but in compensation, the scallop still supplied sufficient hemolymphs to the gill by expanding the diameter of the arteries. The cause of the scallop's death may be different under two thermal stresses: prolonging high temperature caused cardiac failure and physical damage of the artery, whereas rapid heating caused serious tachycardia accompanied by cardiac failure.

1. Introduction

Weather extremes associated with global climate change have occurred more frequently in the last decade, such as heat waves in the summer and sudden cold waves in the winter (Van Aalst 2006). These have been threatening the survival of marine organisms around the world, especially in coastal areas where many species are thought to be living at or near their upper lethal limits of stress tolerance (Somero 2002). The immediate modulation of physiological conditions is particularly important for coastal animals when faced with different kinds of abiotic stresses. Revealing the physiological modulation process is essential in order to understand the response and adaptation mechanisms of the animals to abiotic stresses.

Circulatory physiology activities, such as the cardiac rhythm or hemodynamics, have been shown to serve as an effective index to reveal the entire physiological condition of the organism. Quantifying this parameter under the variation of natural or experimental conditions can help us to understand the general physiological responses of the organisms to abiotic stresses in the environment (Williams et al. 2005; Ungherese et al. 2008; Logan et al. 2012). In particular, these data can also improve our ability to predict the responses of species to weather extremes, such as accurate changes in water temperature.

Bivalves are a large community that contribute major diversity to coastal benthic biota. They're also effective indicators of environmental variations. Under adverse conditions, bivalves maintain homeostasis through stress response. Proper modulation of circulatory physiological activities plays a key role in accelerating the recovery of the body. Taking cardiac activity as an example, reduced oxygen tensions elicited significant bradycardia and positive heart inotropism (indicative of greater heart output) of the mussel *Perna viridis*, showing an energetically advantageous mechanism of reducing the heart rate while increasing stroke volume to maintain hemolymph circulation to maintain digestion and basal metabolism (Nicholson 2002). Faced with a sudden drop of salinity, the heart rate of the mussel *Mytilus edulis*

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slowed down remarkably to decrease the oxygen consmption when its valves were completely closed (Kholodkevich et al. 2009). The sudden transfer of the scallop *Pecten maximus* to deoxygenated water for 3 h resulted in very rapid bradycardia; when returned to well-oxygenated seawater, there was a rapid recovery and initial overshoot of the normal heart rate (Brand and Roberts 1973).

The response of cardiac activity to environmental stresses have been studied in many littoral invertebrate species including mollusks and crabs. Non-invasive measurements of cardiac activity of limpets from four sites with different pollution level showed between site differences in baseline heart rates under standard seawater; when acutely exposed to water borne copper, limpets from clean sites showed a significantly higher increase in inter-beating time (bradycardia) than those from polluted sites (Chelazzi et al. 2004). When exposed to moderate hypo- and hypersalinity (23 and 43) for 24 min, the limpet *P. caerulea* inhabiting the lower midlittoral zone showed no significant variation in heart rate with respect to the control salinity, while *P. aspera* inhabiting infralittoral fringe exhibited a significant increase in heart rate in both conditions, suggesting a rise in metabolic rate due to activation of behavioral responses or physiological regulation (De Pirro et al. 1999). There have been several studies concerning on the heartbeat of mussels and its response to environmental variations such as temperature and pollutant (Burnett et al. 2013; Bakhmet and Khalaman 2006). For example, sudden salinity drop or hydrochinon pollution can both result in peculiar dynamic change of heart rate together with valve movement (Kholodkevich et al. 2009). More studies also confirmed the sensibility of cardial activity as an indicator for the physiological response of mollusks inhabiting variable marine environment (Chelazzi et al. 1999; Calosi et al. 2005; Bini and Chelazzi 2006; Ungherese et al. 2008; Marshall et al. 2011).

Hemodynamics is also an effective way to indicate the circulatory physiological condition of animals, yet for lack of an appropriate method, it is seldom studied in mollusks. Noninvasive echocardiography uses high-frequency sound waves to produce detailed two-dimensional images of the heart and large blood vessels. It permits a noninvasive hemodynamic assessment of the circulatory system by measuring flow velocities, pressure gradients, valve areas, and intracardiac pressure in various blood vessels (Coucelo et al. 1996; Gowda et al. 2004). Echocardiography has been used successfully to study the cardiac function and structure in nonmammalian vertebrates (Franklin and Davie 1992; Lai et al. 2004; Martirosyan 2011). With all the inherent potential of this technique, its application is supposedly well explored in the study of physiology of marine organisms. Airriess & Mcmahon (1996) found that the hemolymph flow of the crab *Cancer magister* through all the arteries originating at the heart declined markedly together with sharp decline of the heart beats during experimental emersion period, owing to the adaptation response to conserve energy and protect the supply of hemolymph to the central nervous system. Ultrasonography also found that blood pulses in the anterior vena cava of the cephalopod *Sepia officinalis* are obligatorily coupled to ventilatory pressure pulses, both in frequency and phase; when it is at rest, the mantle does not compress any of the large veins including the anterior vena cava, and that peristaltic contractions of the large veins might be important in returning cephalopod blood to the hearts (King et al. 2005; Melzner et al. 2007). As for studies in bivalves, it is found that the acceleration of the cardiac rate of the mussel *M. edulis* relative to changes in temperature varied: the acceleration was slower over the temperature range from 10 to 14 °C than from 15 to 20 °C (Haefner et al. 1996), but unfortunately, no hemodynamic profile was described in the study.

Compared with mussels inhabiting intertidal zone where it is exposed to tidal variations in temperature, scallops living in sublittoral zone experiences more stable water temperature conditions, hence has poor tolerance to temperature fluctuation. It is reported that debility from temperature fluctuations might be expected to interfere with escape reactions of the scallop when attacked by predators (Dickie and Medcof 1963). *Chlamys farreri* (Zhikong scallop) is a temperate species distributed in North China, Korea and Japan coast (latitude from 40°N to 25°N) at depths of 5–60 m. It is also one of the most important commercial mollusk species cultured in Liaoning and Shandong province in China. The probability of heat-related mortality event is gradually increasing due to heat waves in the summer in recent years. At high temperature, sufficient hemolymph circulation besides cardiac activity is essential for mollusks to acquire enough oxygen supply and excrete wastes to avoid metabolite poisoning. The HR-based Arrhenius break temperatures (ABTs, the temperature at which heart rate decreases dramatically) have been defined successfully as a thermal tolerance indicator of four scallop species *Patinopecten yessoensis, C. farreri, Argopecten irradians* and *Argopecten ventricosus*, which were 22.0, 29.1, 32.2, and 34.1 °C, respectively (Xing et al. 2016). Previously, we compared the acclimation mechanism of circulatory physiology between the scallops *C. farreri* and *P. yessoensis* when exposed to cold water, based on hemodynamic measurement using Doppler ultrasonography (Hao et al. 2016). The aims of the present work are 1) to identify and characterize detailed cardiac activities and hemolymph flow in the scallop in response to temperature changes; and 2) to determine how the hemodynamics profile would change under prolonged high temperatures and rapidly rising temperature stresses. Results are expected to be used in the diagnosis and prediction of scallop's stress response condition in a timely way.

2. Material and methods

Two-year-old edible scallops, *C. farreri*, were obtained from a raft-culture farm in Jiaozhou Bay (Qingdao, Shandong Province) in early March 2014. After removing fouling organisms on the shell, they were maintained in 1000 l tanks with aerated grit-filtered seawater (salinity 31.3) and acclimated at 6 °C for 2 weeks before experimental handling. During this period, scallops were daily fed with the microalgae *Phaeodactylum tricornutum*, and the water was renewed every day. After acclimation, 30 healthy scallops (shell hight 55–63 mm; wet weight 21–29 g) with powerful and active heartbeats were seleted for the following experiments by preliminary ultrasonic scanning. Then they were devided into two heating speed experiment groups (15 ind. each) and put into six 100 l plastic bucket (5 ind. each) with aerated grit-filtered seawater. The scallops were reared on a punched polyethylene plate in the middle layer of the water; a temperature-controlled heater (power 1000 W, precision ± 0.2 °C) was set on the bottom to keep it away from the scallops.

2.1. Monitoring system

The ultrasonic hemodynamic measure system includes two parts: a recycling aquarium (50 × 40 × 40 cm) and a Doppler ultrasound imaging system (Fig. 1). The outer tank of the aquarium (80 l) was fulfilled with fresh water, which was recycled by a precise temperature-controlled water bath (Scientz® SDC-6; Ningbo Scientz biotechnology Co., Ltd.; Ningbo, China; temperature fluctuation range ± 0.1 °C). The stainless steel inner tank (36 l), which has a good thermal conductivity, was filled with sand-filtered seawater (salinity 31.3) for scallop rearing.

The Doppler ultrasonography system (Mindray® Z6 vet, Shenzhen Mindray Bio-Medical Electronics Co., Ltd.; Shenzhen, China) was equipped with a 14-MHz high frequency waterproof linear emission probe. The black & white mode (B mode) and color mode were mostly used. The B mode indicates the anatomical image of the tissues and organs, while color plus Doppler pulse mode shows the hemodynamics, such as the real-time velocity and direction of the hemolymph.

2.2. Parameter introduction

The Doppler ultrasonography system can measure a series of hemodynamic parameters (listed in Table 1). These parameters can