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Thermal tolerance traits of the undulated surf clam *Paphia undulata* based on heart rate and physiological energetics

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ABSTRACT

Thermal tolerance traits are important factors that affect the ability of an aquaculture species to survive, grow, and reproduce in new habitats. *Paphia undulata*, a species of saltwater clam, has been farmed in China for over thirty years. Despite this, the ecophysiology of *P. undulata* and its effects on the aquaculture of this species are poorly understood. To investigate the thermal tolerance of *P. undulata*, the semi-lethal temperature, cardiac activity, and scope for growth (SFG) of the organisms were measured under different thermal stresses in a laboratory-simulated muddy habitat condition. Our results showed that the cardiac activity, feeding behaviors, and respiration rates of *P. undulata* were positively correlated with temperature changes from 10 °C to 32 °C. The upper incipient lethal temperature (192-h UILT_{50}) and Arrhenius break temperature (ABT) of this species were 33.49 ± 0.02 °C and 35.64 ± 0.77 °C, respectively. SFG trend had negative values at temperatures between 10 °C and 16 °C and positive values at temperatures between 18 °C to 32 °C. These data suggest that the suitable thermal window that provided the energy required for the efficient growth and reproduction of *P. undulata* in the Dongshan Bay was approximately nine months out of the year. Taken together, these results indicate that *P. undulata* is proficient at adapting to thermal stress in the subtidal zone. Moreover, our results may also provide information that assists in both the management of wild populations of *P. undulata* and the improvement of *P. undulata* aquaculture.

1. Introduction

Temperature is a critical abiotic factor that can influence chemical and biochemical reactions, thereby affecting the growth, reproduction, and survival of ectotherms (Sicard et al., 2006; Pörtner and Knust, 2007; Sarà et al., 2011). Temperature is also one of the most important factors affecting the biogeographic distribution and abundance of a species (Bownes and Mcquaid, 2006; Hofmann and Todgham, 2010; Pörtner, 2010; Ezgeta Balić et al., 2011). Most marine organisms (e.g., shrimp, bivalves, and fish) are ectotherms, whose physiologies are particularly sensitive to external temperature change; this quality can be used to assess the organisms' thermal tolerances through manipulation of their external environment (Pörtner et al., 2006; Clark et al., 2008). Knowledge of an organism's thermal tolerance is crucial to the understanding of its ecology, evolution, and physiology, as these traits influence both the distribution range of the species and the effects that climate change has on the organism (Parmesan, 1996; Thomas and Lennon, 1999; Walther et al., 2002). This is especially true for aquaculture species, as thermal tolerance traits are key factors that determine survival, growth, and reproduction of these organisms when exposed to new habitats. Thermal tolerance traits can also be used to determine the magnitude of threat that thermal stress from immigration into a new environment can have on the animals (Tagliarolo and McQuaid, 2015; Chen et al., 2016; Xing et al., 2016). To date, most of the thermal tolerance studies published have focused on intertidal animals, such as porcelain crabs (*Petrolisthes*; Stillman and Somero, 1996), snails (*Tegula*; Stenseng et al., 2005) and limpets (*Cellana*; Dong and Williams, 2011). However, there exist few empirical assessments of the thermal tolerance of commercially viable subtidal species.

Traditional assessments of thermal tolerance involved determination of both the coma and lethal temperatures for the organism (Compton et al., 2007; Laing and Child, 1996; Wright et al., 1983). The index that was most commonly used in this assessment was LT_{50}, the temperature lethal to half of any given species over a certain length of

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time (Quinn et al., 1994; Dong et al., 2015). Recently, heart rate (HR) has become more widely used in thermal tolerance studies due to its sensitivity and reliability (Chen et al., 2016; Xing et al., 2016). The HR-based Arrhenius break temperature (ABT), the temperature at which the heart rate decreases dramatically during progressive heating, has also been used as an indicator of thermal tolerance in the study of marine invertebrates (Stillman, 2003; Braby and Somero, 2006; Dong and Williams, 2011; Prusina et al., 2014; Tagliarolo and McQuaid, 2015). In addition, scope for growth (SFG), which is used to predict the growth rate of an organism (Tamayo et al., 2013), is considered to be a good indicator of the fitness of an organism under different environmental stresses and conditions (Bayne et al., 1979; Widdows and Staff, 2006; Widdows et al., 1990; Smaal and Widdows, 1994). In particular, SFG can shed light on the physiological energetics involved in the growth of an organism, which can be used to better understand its thermal tolerance (Sokolova et al., 2012). Despite the availability of several indices, evaluations of the thermal tolerance of marine invertebrates have tended to employ these indices separately. In order to provide more realistic and accurate measurements of thermal tolerance, more studies need to be performed that consider all indices together.

Paphia undulata, commonly known as the “oil clam”, is an undulated surf clam with characteristics typical of a shallow sea, benthic bivalve species. It is naturally distributed along coastal areas in the Fujian, Guangdong, Guangxi, and Hainan provinces in China. Through suspension-feeding activities, this species can cycle large amounts of particulate matter within the water, convert part of them into the energy required for growth and reproduction, deposit varying amounts to the benthos, and cycle complex molecules into inorganic forms (Ward and Shumway, 2004). Due to their prized flavor, the market price of *P. undulata* is high, making it an important commercial shellfish in southern China (Zhang et al., 2000; Cui et al., 2008). However, over-exploitation over the last twenty years has depleted most of the natural populations of the bivalve, with the exception of the Beibu Bay population. In response, the fishery-dominant industry surrounding *P. undulata* has been gradually replaced by aquaculture.

Every year, millions of tons of *P. undulata* seedlings, which are 6–12 months of age and have a 2–3 cm shell length, are collected from their natural habitat in Beibu Bay for aquaculture. The seedlings are naturalized for one to two years in the wild, shallow sea habitat of the Hainan, Guangdong, and Fujian provinces until they reach maturation (Xu, 2014). The aquaculture of *P. undulata* has grown significantly over the last decade—as of 2009, the culture area measured 16,500 acres and the annual production was 100,000 tons in the Dongshan Bay of Fujian province. However, the rapid growth of the industry revealed issues that may impede further development of the aquaculture of *P. undulata*. For example, the production per acre was significantly lower for *P. undulata* than for other economically viable benthic bivalves, such as *Ruditapes philippinarum* and *Sinonovacula constricta*. This issue can be attributed in part to the limited attention paid to the ecophysiology of the organism. More in-depth studies of the thermal traits and thermal window for growth could improve the output of *P. undulata* aquaculture.

To investigate the thermal tolerance of ***P. undulata***, we used semi-lethal temperature measures, heart rate analyses, and energy budget examinations to determine how different thermal stressors affected multiple physiological performance processes. We first calculated the upper incipient lethal temperature (UILT_{50}) as a measure of extreme thermal stress. We then determined the cardiac performance of the clams at both lower and upper thermal thresholds through calculating the ABT. Finally, we examined the energy budget of the organism and calculated the SFG over a temperature range of 10 °C–34 °C.

2. Materials and methods

2.1. Clam collection and acclimation

P. undulata (shell length: mean = 40.06 mm ± 0.49 SD; weight: mean = 7.44 g ± 0.37 SD; 1.5–2 years old) were obtained from a wild aquaculture area of the Dongshan Bay (23°45′07″N, 117°26′33″E), Fujian Province, China. These individuals had been free-range cultured for 1–2 years from seedlings (6–12 months of age with a 2–3 cm shell length) collected from Beibu Bay. Changes in seawater temperature at the sea floor of Dongshan Bay were recorded every 30 min (23°47′23.64″N, 117°29′46.74″E) from February 2015 to January 2016 using a HOBO® Pendant® Temperature/Light Data Logger.

$>$ 1000 individuals were collected by diving native fishermen between June 2015 and September 2015. Clams were transported to the laboratory within 2 h of collection in aerated tanks. They were then placed into six 300-l aquaria containing approximately 15 cm of filtered sea mud to mimic the natural sea floor. Individuals were acclimated for one week at 30 psu and 24 °C before the experiments were performed. The tank water was changed every 2 days to prevent the accumulation of ammonia and nitrites.

After a one-week acclimation, 600 individuals were randomly selected for experimentation. Each clam was placed into a 140-mL glass beaker (50 mm in diameter, 80 mm high) filled with sea mud (water content approximately 45%) filtered through a #20 stainless steel sieve (mesh size: 0.83 × 0.83 mm) to remove impurities. Eighty glass beakers filled with sea mud but without clams were prepared as the control group. All of the glass beakers were then mixed and randomly placed into four 300-L polyethylene aquariums (170 glass beakers per aquarium) where the clams were acclimated for an additional week at 30 psu and 24 °C. During the acclimation period, the clams were fed daily with *Isochrysis galbana* (25,000 cells·ml^{-1}, approximately 3% of the clam dry weight), and aquaria water was continuously aerated.

2.2. Heart rate assays

Heart rates were measured following the protocol outlined by Dong and Williams (2011). To detect the heartbeat, an infrared sensor (AMP03, Newshift, Leiria, Portugal) consisting of an infrared emitter and phototransistor was fixed to the clam's shell directly over the heart using Super Glue (Alteco Ltd., Singapore). We then assisted each clam in burying itself into the sea mud contained in one 140-ml glass beaker. The clams were allowed to acclimate for seven days prior to the heart rate measurements. The variations in the light-dependent current produced by the heartbeats were amplified, filtered, and recorded using Powerlab (8/30, ADInstruments, Germany) at a rate of 200 samples per second. Data were viewed using Chart (version 5.0).

ABT is the temperature at which heart rate decreases dramatically. To measure this, eight individuals that were fixed with infrared sensors and buried in the 140-ml beakers were selected for analysis. These individuals were placed into a 4-l glass beaker filled with sea water (24 °C and 30 psu) for 30 min to allow them to recover their normal heart rate. Following recovery, the 4-l glass beaker was immersed in a water bath, allowing the seawater temperature in the 4-l glass beaker to increase at a rate of 0.2 °C per min until heartbeats were undetectable (Tagliarolo and McQuaid, 2015; Dong and Williams, 2011). During the test, the seawater in the 4-l glass beaker was saturated with oxygen through the delivery of air from a compressor via a plastic airline opening at the bottom. The thermal changes that the clams endured were recorded every 60 s by inserting the probe of a thermometer (K/L Tm903A, Lu-Tron, Taiwan) into the mud at the center of the control glass beakers. The real-time heart rates of eight individuals were recorded, and their ABTs were determined using regression analyses to generate the line of best-fit on both sides of a putative break point, following the protocol of Stillman and Somero (1996).

To investigate the impact of low temperature on the cardiac