Combined effects of thermal conditions and food availability on thermal tolerance of the marine bivalve, *Perna viridis*

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A B S T R A C T

Organisms can mitigate the effects of long term variation in environmental conditions through acclimation, which involves changes in various physiological responses. To elucidate the possible effects of temperature and food concentrations on acclimation capacity, physiological responses of the mussel, *Perna viridis*, were measured after individuals were held for six weeks under varying temperatures and food availability. Warm-acclimated mussels experiencing higher food levels had significantly greater upper thermal limits than those maintained on lower food levels. In contrast, the upper thermal limits of cold-acclimated mussels were not affected by food levels. For warm-acclimated mussels, differences in upper thermal limits were likely due to rapid depletion of energy storage as predicted by Dynamic Energy Budget model simulations for *P. viridis* exposed to lower food levels. Clearance rates of cold-acclimated mussels were significantly lower than warm-acclimated mussels, regardless of food availability. The impacts of lower food acquisition on energy storage, however, could be compensated by lower metabolic rates of the cold-acclimated mussels. The availability and the ability to acquire food are not, therefore, the main drivers differentiating between the upper thermal tolerances of cold- and warm-acclimated mussels, but these differences are driven by the past thermal history the mussels experienced. The temperature tolerance range of *P. viridis* showed a positive shift to tolerate higher temperatures after acclimation. Such flexibility in thermal tolerance implies *P. viridis* has high capacity to acclimate to novel environments, which will enhance its future success given its commercial importance as an aquaculture species.

1. Introduction

Marine environments are highly variable as a consequence of diverse environmental factors (solar radiation and precipitation etc.) operating at different spatial and temporal scales (Harley and Helmuth, 2003; Helmuth et al., 2002). Such environments are especially variable in localities which experience strong seasonal and daily variations (e.g., the west coast of North America and south coast of Japan; Denny et al., 2006; Yoshida and Shimabukuro, 2017). Being on the fringes of the tropics, the Hong Kong coastline also experiences marked seasonality, driven by changes in monsoon systems (Kaehler and Williams, 1996; Morton and Blackmore, 2001). Seawater temperatures in Hong Kong, for example, generally vary between 17 and 27 °C and primary production between 1 and 15 µg chlorophyll *a* L⁻¹ during the hot and wet season and cold and dry season (Chiu et al., 1994; Kaehler and Williams, 1996). Marine organisms respond differently to changes in such environmental conditions in terms of their physiological performance (see Boero, 1994 for review) and often have the capacity to

tolerate a range of environmental conditions, depending on the time scale and severity of these stresses, by invoking a variety of responses (Huey et al., 2012; Kassahn et al., 2009).

Changing environment can greatly challenge organisms causing physiological stress, damage, and even mortality (Huey and Bennett, 1990). When experiencing longer term, sublethal environmental variation (e.g. such as seasonal changes), organisms can mitigate such effects by acclimation (*sensu* Lagerspetz, 2006), which involves shifts in their physiological tolerance windows (Hoffmann, 1995; Hofmann and Todgham, 2010; Horowitz, 2001; Layne et al., 1985; Pörtner, 2002). Enhanced tolerance due to acclimation has been recorded in various marine taxa such as bivalves (Chapple et al., 1998; Hicks and McMahon, 2002), gastropods (Stenseng et al., 2005) and crustaceans (Stillman and Somero, 2000; Tepolt and Somero, 2014). The brown mussel, *Perna perna*, for example, survived for longer durations under high temperatures (32 °C) when acclimation temperature was increased by 15 °C (Hicks and McMahon, 2002). Acclimation, therefore, plays an important role in longer-term stress tolerance, and is one of the key

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processes proposed to enable species to survive future environmental scenarios (Somero, 2010). Such phenotypic responses are becoming of increasing interest, given the changing conditions associated with climate change (Helmuth et al., 2014; Williams et al., 2016) and the impacts this may have for ecologically and commercially important species (Cochrane et al., 2009).

The flexibility to vary tolerance limits in response to changing environmental conditions, however, involves changes in multiple physiological processes (e.g. heart, respiration, and filtration rates; Bayne and Newell, 1983; Crawshaw, 1977; Newell et al., 1977; Segal, 1961) which can disrupt the energy balance of organisms (Newell and Branch, 1980; Widdows and Bayne, 1971). Such alterations in physiological responses can involve reorganization of metabolic pathways and activation of enzymes and protein synthesis to maintain cellular function and are, therefore, energetically costly (Anestis et al., 2007, 2008, 2010; Bowler, 2005; Lagerspetz, 2006). To be able to meet these metabolic demands, organisms need to either store or rapidly assimilate energy (Sokolova et al., 2012). Understanding the energy reserves of individuals can, therefore, give a better understanding of trade-offs by which species can survive, and their subsequent performance, in variable environments. The recent development of mechanistic Dynamic Energy Budget (DEB) models (Kooijman, 2010) offers a reliable opportunity for revealing the effects of environmental changes on organisms' bioenergetics. As a main difference with net production models such as scope for growth, the DEB model includes a storage term (the 'energy reserve') which allows for examination of time/history effects. The innovative aspect of the DEB model is that it captures the processes of energy acquisition and utilization in an organism under varying environmental conditions such as temperature and food concentration and then how this energy is stored into the energy reserve for subsequent allocation for various traits such as basal maintenance, reproduction and growth (Kooijman, 2010; see Filgueira et al., 2011). DEB, therefore, offers a powerful tool to reconstruct or predict the energy reserve of an individual from past or future environmental data/projections (Kooijman, 2010; Van der Meer, 2006).

Bivalve molluscs, being widely distributed and both commercially and ecologically important species, are good candidates for assessing the effects of environmental stressors and have previously used as ecological 'sentinels' (Bayne et al., 1979; Widdows et al., 1990). The green lipped mussel, *Perna viridis*, is widely distributed throughout the Indo-Pacific region and is one of the most commercially and ecologically important bivalve species in SE Asia (Gutiérrez et al., 2003; Rajagopal et al., 2006); whilst in North America the species is invasive and competes with local species (Baker et al., 2007; Power et al., 2004). *P. viridis* has, as a consequence of its commercial and ecological importance, been increasingly studied to understand its physiological tolerances to environmental challenges such as hypoxia, salinity and heat stress (Goh and Lai, 2014; Huhn et al., 2016; Ma, 2013; McFarland et al., 2015; Wendling et al., 2013). These studies, however, only focused on the acute effects of single rather than multiple environmental factors, which limits their ability to provide a realistic picture of the ability of *P. viridis* to acclimate to changing environmental conditions (Gunderson et al., 2016; Sokolova, 2013; Todgham and Stillman, 2013). This study, therefore, investigated the potential of subtidal individuals of *P. viridis* to acclimate to seasonally variable environments by examining physiological responses of the mussel under different temperature and food conditions. The physiological responses selected included feeding and heart rate, acclimation capacity and thermal tolerance range, which are important survival metrics since they are closely associated with an individuals' energy balance, and consequently, ability to accommodate environmental stress (Schneider et al., 2010; Sokolova, 2013; Widdows, 1978).

2. Materials and methods

2.1. Mussel collection and pre-treatment

Perna viridis (4–5 cm) were collected from fish rafts (~0.5–1 m below sea surface) at Port Shelter, Hong Kong (22°43′N, 114°29′E) during the hot and wet season (August 2015, see Kaehler and Williams, 1996) when animals had a low GSI (Cheung, 1993) to avoid the possible confounding effects of gonad development during the experiment. Mussels were cleaned of attached byssus and epibionts and habituated in a closed filtered seawater (0.45 μm, ~30‰) system at 25 °C ± 0.5 °C (the same temperature of the collection site), for two weeks prior to experimentation to eliminate the handling effects due to transportation, cleaning and previous thermal history (Jost and Helmuth, 2007); and fed with a monoculture of *Isochrysis galbana* on a daily basis.

2.2. Exposure to varying acclimation conditions

To investigate the integrated effect of temperature and food availability on the physiological responses of *Perna viridis*, mussels (> 300 individuals) were allowed to acclimate to either the average seawater temperatures during the cold and dry season (16 °C; C = cold temperature) or hot and wet season (30 °C; W = warm temperature, ± 0.5–1 °C) of Hong Kong (Environmental Protection Department, HKSAR Government 2011–2013) respectively for six weeks in aquaria (25 × 25 × 25 cm (~15 L) with 18–20 mussels in 0.45 μm filtered, natural seawater, ~30‰). To achieve the desired experimental temperatures, water was either warmed or cooled at a rate of 1.5 °C per day using chillers or heaters. Within each temperature treatment, mussels were fed on either a 'high' (H) or 'low' (L) food diet. In the high food treatment mussels were fed excess (> 100,000 algal cells mL^{-1}) *Isochrysis galbana*, whereas in the low food treatment they only received ~20,000 algal cells mL^{-1} (i.e. half of the optimal food supply of *P. viridis*, = 4.9 μg chl *a* L^{-1}; Cheng, 2016). Each treatment had four replicate aquarium tanks (Σn = 2 temperature levels × 2 food levels × 4 tanks = 16).

After six-weeks acclimation, cold-acclimated mussels had lower mortality rates (~4%) as compared with warm-acclimated mussels (~34–36%) irrespective of food rations. Subsequently, individuals were randomly selected from each treatment for the determination of Arrhenius breakpoint temperatures, as a measure of upper thermal limits; clearance rates to determine feeding efficiency; and temperature tolerance range. Data for water temperature and food density were entered into a Dynamic Energy Budget model for *Perna viridis* to simulate the change in energy reserves of the mussels under the different acclimation conditions (see Cheng et al., 2018).

2.3. Arrhenius breakpoint temperatures

The Arrhenius breakpoint temperature indicates the temperature at which an individual starts to suffer from heart failure due to loss of aerobic scope (Frederich and Pörtner, 2000), and has been commonly used as a stress metric (Anttila et al., 2014; Dong and Williams, 2011; Jansen et al., 2009; Pörtner et al., 1999). Four mussels were randomly selected from each replicate of each treatment and attached with non-invasive infrared sensors on their shells above the heart (i.e. near the mid-dorsal posterior hinge; see Burnett et al., 2013; Σn = 4 treatments × 3–4 replicates = 15). Individual mussels, with sensors attached, were placed into 200 mL containers with filtered seawater (0.45 μm, ~30‰) and positioned haphazardly in a programmable water bath (Grant GP200, UK) which allowed the water to be heated up at a fixed rate (1 °C every 5 min, after Williams et al., 2011). The sensors were connected to amplifiers (Newshift, Portugal) and then to a Picoscope (Pico Technology, UK), from which heart beat traces of individuals could be recorded. Body temperatures of selected mussels were

recorded by inserting thermocouples (Omega K-type, Teflon insulated, tip diameter 0.25 mm) connected to a digital thermometer (Lutron TM-903A, Taiwan) into the mantle cavity of the mussels. Heart rates (Hz) were counted every minute (i.e. every 0.2 °C increment). Arrhenius plots were drafted, with natural-log heart rates (*ln* Hz) against the reciprocal of body temperature of each individual in Kelvin (1000/K) and fitted by segmented linear regressions using the segmented package in R (Muggeo, 2017) using the highest coefficient of determination (r^2). The intercept of these regression lines, which indicates the point at which there is a break in the increase in heart rate with increasing temperature, was defined as the Arrhenius breakpoint temperature (ABT; after Dahlhoff et al., 1991; Stillman and Somero, 1996).

2.4. Clearance rates

Four individuals of *Perna viridis* were randomly selected from each treatment and were placed into 1 L beakers of precooled (16 °C for animals which experienced cold temperatures) or preheated (30 °C; hot temperature treatment) and well-aerated filtered seawater (Whatman GF / F, ~30‰) with magnetic stirrers to mix the water to ensure stable temperature and food concentrations (Σn = 4 treatments × 3–4 replicates = 15). Water in both beakers was either heated or cooled to 25 °C with a ramping rate of 1.2 °C per hour and maintained at that temperature throughout the experiment to compare the feeding rates of mussels which had experienced different previous conditions. A fixed concentration of algal solution determined from previous trials to ensure the number of algal cells did not decrease to a very low quantity to affect the accuracy and reliability of counting (~80,000 cells mL^{-1} of *Isochrysis galbana*) was then added into each beaker. The clearance rate (CR) was measured as the volume of water cleared of suspended algal cells over time (after Widdows et al., 1981). Before the experiment commenced, 10 mL of seawater was sampled from each beaker and then every 30 min over 2 h to determine the number of algal cells remaining in the water column via counts using a haemocytometer (Neubauer, Marienfeld, Germany). For each time interval, algal cells were counted twice and the values averaged and used for subsequent calculation of clearance rate. After the experiment, mussels were dissected and their flesh was dried in an oven (Jeio Tech OF-01E, Korea) at 100 °C for 24 h and then weighed (± 0.001 g, Shinko GS223, Japan) to acquire flesh dry weights which were subsequently used to standardise CRs using the formula:

$$CR = \ln(C_1/C_2) \times V/(n \times \Delta t)$$

(Riisgård and Seerup, 2003)where C_1 and C_2 are the cell concentrations at the beginning and end of each interval, V is volume of water (i.e. 1 L) and *n* is the number of individuals used in each beaker (i.e. 1).

2.5. Dynamic energy budget model simulation

The Dynamic Energy Budget (DEB) model derived for *Perna viridis* (Cheng et al., 2018, available at https://www.bio.vu.nl/thb/deb/deblab/add_my_pet/entries_web/Perna_viridis/Perna_viridis_par.html) was used to simulate the effect of acclimation in addressing the amount of energy storage of *P. viridis* during six weeks under two different environmental temperatures (i.e. 16 °C or 30 °C) and under two food density conditions: one closer to almost unlimited food supply (function response *f* = ~1) and one closer to, according to the DEB theory (Kooijman, 2010) and based on the empirical data (Cheng, 2016) half of the optimal food condition (*f* = 0.5). For details of how the model integrates food concentration and temperature for calculation of the energy reserve refer to Sarà et al. (2013) and Kooijman (2010).

2.6. Thermal tolerance range

As there may be a deviation of mussel heart rates measured under

laboratory and field conditions (Tagliarolo and McQuaid, 2016), *Perna viridis* were maintained in a constantly submerged condition (as experienced in the natural environment) and laboratory heart rates measurements were conducted in water. To determine the thermal tolerance range of *Perna viridis*, individuals from the four different treatments were held under temperatures which *Perna viridis* may experience in the field, at 15 °C, 20 °C, 25 °C, 30 °C and 35 °C respectively for 24 h (Σn = 4 treatments × 5 temperatures × 4 replicates = 80) to eliminate the effects of feeding processes on physiological rates during heart rate measurement (Wong and Cheung, 2001). Randomly selected mussels with infrared sensors attached (see Section 2.3) were placed into 0.7 L screw-capped chambers individually filled with precooled or preheated (i.e. 15 °C or 30 °C, respectively), well-aerated and filtered seawater (0.45 μm, ~30‰). Animals were held under constant conditions for 10–15 min and then were either heated or cooled at a rate of 1 °C every 30 min as a more conservative heating rate compared to that experienced in the intertidal environmental (Braby and Somero, 2006; Lockwood et al., 2010) until the desired temperature was achieved. Heart beats were counted every minute (beat min^{-1}) for > 30 min under the different temperatures and averaged.

2.7. Statistical analysis

Two-way analysis of variance (ANOVA) was employed to investigate variation in mussel ABTs and CRs between different acclimation temperatures (16 °C and 30 °C; fixed factor) and food availability (high and low levels; fixed factor) and their interaction, using SPSS 20.0 (IBM), followed by Student-Newman-Keuls (SNK) tests to separate significant factors. Two-way ANOVA was also employed to investigate variation in mussel heart rates measured at different temperatures after 24 h among acclimation temperatures and food availability.

3. Results

3.1. Arrhenius breakpoint temperatures (ABT)

Heart rates of *Perna viridis* increased with temperature until the individuals reached their ABTs at which maximal heart rates were recorded followed by a sudden drop at the point of heart failure (see Fig. 1). There was a significant interaction (p < 0.05) between food levels and acclimation temperatures on ABTs where warm-acclimated mussels, fed on high food levels (WH), had significantly higher ABTs than those fed on low food concentrations (i.e. WL; Table 1a; Fig. 2a). Cold-acclimated mussels, in contrast, had similar ABTs at the two food concentrations (Table 1a; Fig. 2a).

3.2. Clearance rates (CRs)

Calculation of clearance rates showed good fits to There was a significant effect of acclimation temperature on mussel CRs, with the CRs of warm-acclimated *P. viridis* being significantly higher (p < 0.05) than those cold-acclimated (Table 1b; Fig. 2b). There was, however, no significant effect of food level or interaction between acclimation temperatures and food levels in mussel CRs (Table 1b), although the average CRs of WL mussels were more variable, and approximately half the rate of WH mussels (Fig. 2b).

3.3. Dynamic energy budget model simulation of energy reserves

Predicted energy reserves from warm-acclimated mussels at different food concentrations showed that mussels receiving high food levels increased their energy reserve by ~10% after the acclimation period, whereas those which received low food levels would have depleted their energy reserve by ~19% during the acclimation period (Fig. 2c). Generally, warm-acclimated mussels had much faster rates of
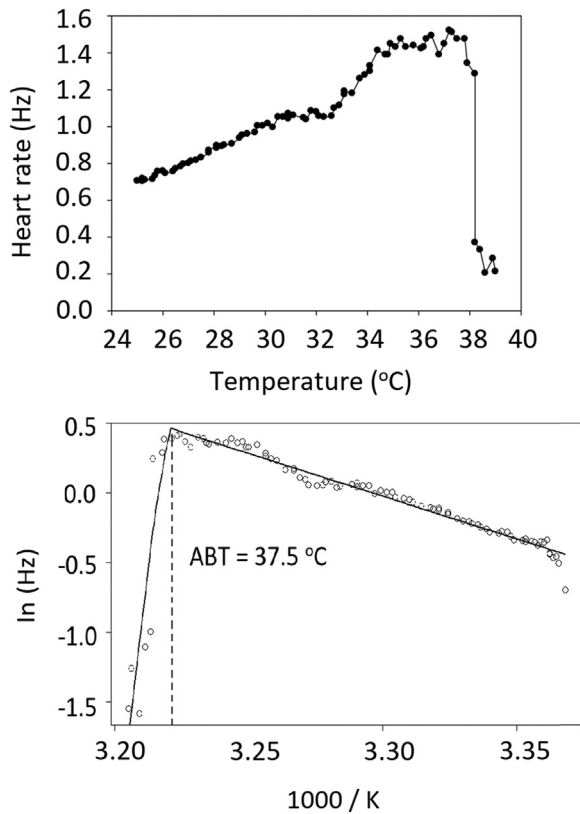
Fig. 1. Illustrative example of change in heart rates (HR) with body temperature (top) and natural-log of HR in Hertz (Hz) of a mussel individual with increasing temperature expressed as reciprocal of Kelvin times 1000 (1000/K) for determination of Arrhenius breakpoint temperatures (ABT, bottom). ABT was the point where the two regression lines intercepted (indicated by vertical dashed line).

Table 1
Two-way ANOVAs to compare variation in (a) Arrhenius breakpoint temperatures and (b) clearance rates of *Perna viridis* under different acclimation temperatures (Temp: 16 and 30 °C) and food levels (Food: High and low). Data passed tests for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test). Significant values (i.e. $p < 0.05$) are indicated in bold and SNK tests were further conducted to separate significant factors (i.e. $p < 0.05$).

Source of variation		df	MS	F	p
(a) Arrhenius breakpoint temperatures					
Temp		1	166.329	122.363	**< 0.001**
Food		1	4.572	3.363	0.094
Temp * Food		1	19.485	14.335	**0.003**
Error		11	1.359		
SNK tests	Temp	16 °C		High food = Low food	
		30 °C		High food > Low food	
	Food	High food		30 °C > 16 °C	
		Low food		30 °C > 16 °C	
(b) Clearance rates					
Temp		1	29.945	12.216	**0.005**
Food		1	7.431	3.031	0.110
Temp * Food		1	3.104	1.266	0.284
Error		11	2.451		

energy reserve gain/depletion at both high and low food levels when compared to cold-acclimated mussels (Fig. 2c).

3.4. Thermal tolerance range

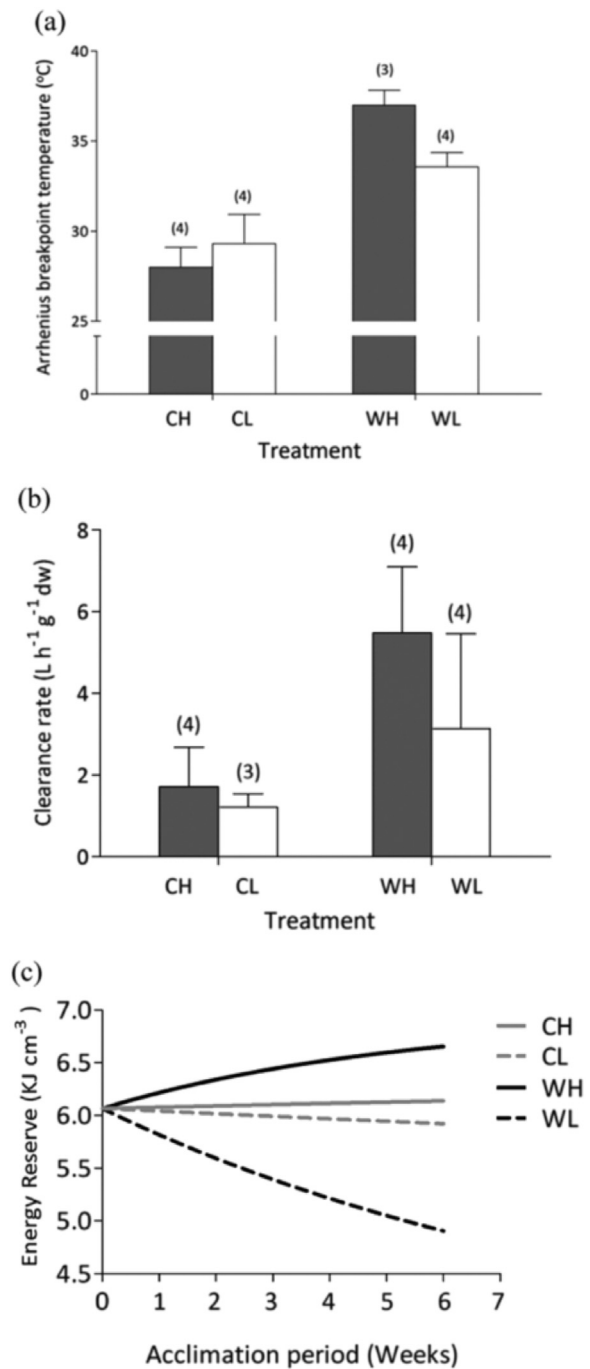At low temperature (i.e. 15 °C) cold-acclimated mussels had faster

Fig. 2. (a) Arrhenius breakpoint temperatures, (b) clearance rates and (c) energy reserve simulation from Dynamic Energy Budget models of *Perna viridis* held under different acclimation conditions. Values in parentheses indicate replicate numbers. Error bars indicate standard deviation. (CH: acclimated at 16 °C with high food levels, CL: acclimated at 16 °C with low food levels, WH: acclimated in 30 °C with high food levels and WL: acclimated at 30 °C with low food levels).

heart rates than warm-acclimated mussels (two-way ANOVA, $F_{1,12} =$ 15.8, $p < 0.005$), while warm-acclimated mussels had faster heart rates at higher temperatures (i.e. 30 °C, two-way ANOVA, $F_{1,11} = 108.7$, $p < 0.001$; Fig. 3). The temperature tolerance range of mussels under warm and cold acclimations differed, with cold-acclimated individuals having a more narrow range than those acclimated at warm temperature. WL mussels showed a trend of increased heart rate under all temperatures but exposure to high temperature (i.e. 35 °C),
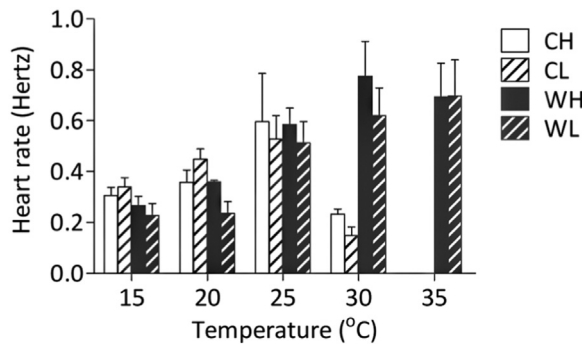
Fig. 3. Heart rates (HR) of mussel individuals from different treatments habituated under a range of water temperatures for 24 h. Error bars indicate standard deviation.

one out of four WL mussels died whereas all WH mussels survived. Within these temperature ranges, the cardiac performance of warm-acclimated *Perna viridis* could be assumed to peak at 30–35 °C; whereas cold-acclimated mussels peaked at 25 °C, irrespective of food levels.

4. Discussion

Physiological phenotypes of an organism can alter over time through acclimation to improve fitness under changing environments (Horowitz, 2001; Scheiner, 1993; Schulte et al., 2011). During acclimation, organisms undergo subcellular adjustment which increases metabolic maintenance costs (Bowler, 2005; Lagerspetz, 2006; Pörtner, 2001) at the expense of other traits such as growth and reproduction (Widdows and Bayne, 1971; Kooijman, 2010). Due to these costs, species may, therefore, suffer from increased mortality before they successfully acclimate to new environmental conditions, particularly under warmer environments (this study and Anestis et al., 2007). As an indication of successful acclimation, warm-acclimated *Perna viridis* had higher ABTs than cold-acclimated individuals (see Yu, 2014) as observed in gastropods (Sokolova and Pörtner, 2003) and crustaceans (Stillman and Somero, 1996). Warm-acclimated *P. viridis* which received high food levels (WH), however, had higher upper thermal limits (higher ABTs) than those which experienced low food levels (WL). As such shifts in thermal tolerance invoke energetic costs (Hochachka and Hayes, 1962; Hofmann and Todgham, 2010), the capacity to achieve this is assumed to be dependent on the physiological/energetic state of individuals (Newell and Kofoed, 1977). Under high temperatures, enhanced food availability has a positive influence on the physiological tolerance of organisms, particularly, during thermally stressful periods (Dahlhoff, 2004; Dahlhoff and Menge, 1996; Schneider et al., 2010). Mussels, *Mytilus* species, acclimated at high thermal stress and receiving low food rations, for example, had higher mortality rates as compared to those which received high food levels (Fitzgerald-Dehoog et al., 2012; Schneider et al., 2010) and the whelk, *Nucella ostrina*, living in an area with high food levels, was more tolerant to thermal stress than those living in a food-poor area (Dahlhoff et al., 2001).

Energy reserves of organisms are assumed to govern the ability to tolerate higher levels of stress (Sokolova, 2013). Warm-acclimated *Perna viridis* were better able to obtain energy through filter feeding than cold-acclimated individuals (as recorded in other filter feeders such as the gastropod, *Crepidula fornicata*; Newell and Kofoed, 1977). Mussels held at the same temperature, but with reduced food supply, however, had lower clearance rates, as also noted in the oyster, *Crassostrea gigas* (Honkoop et al., 2003). The reduced feeding rate combined with higher temperatures, resulted in rapid depletion of energy reserves of warm acclimated mussels at low food levels as simulated by the DEB model. Such reduction in energetic state coincided with the lower thermal tolerance in WL mussels than WH mussels. The effects of reduced food acquisition on energy reserve could, however, be

compensated by relatively lower metabolic demands of cold-acclimated mussels as compared to warm-acclimated individuals. *P. viridis* held under colder conditions have lower feeding and metabolic rates, irrespective of food availability, when compared to mussels under warmer environments (Wong and Cheung, 2001). Both studies suggest that food availability seems to be less important for thermal tolerance in cold-than warm-acclimated mussels. There is, therefore, a dynamic relationship between food availability and the ability to acquire energy which plays a critical role in the acclimation potential of *P. viridis* to heat stress. It is, however, likely that other environmental conditions, such as variation in salinity (e.g. in *Mytilus* Braby and Somero, 2006) will also influence the performance of *P. viridis* and multiple, interacting effects of environmental conditions should be considered in future studies.

Differences in phenotypic responses also involve biochemical mechanisms at subcellular levels (Hochachka, 1991; Dahlhoff and Somero, 1993; Somero, 1995). The lower thermal tolerance of cold-acclimated mussels is likely the result of the thermal history previously experienced by the organisms as, they may induce different levels of gene expression when exposed to increasing thermal stress (Giomi et al., 2016). The Pacific oyster, *Crassostrea gigas,* sampled from warm environments, for example, shows higher levels of heat shock gene expression, and subsequent higher thermal tolerance, than those from cold environments (Hamdoun et al., 2003). Composition of the mitochondrial membrane lipids of warm-acclimated abalone, *Haliotis* spp., has also been shown to differ from cold-acclimated individuals and be associated with their greater resistance to heat stress, and higher upper thermal limits (Dahlhoff and Somero, 1993).

Cardiac performance also depended on food availability as demonstrated by the fact that WL mussels had lower heart rates than WH mussels. This effect was also observed in the blue mussel, *Mytilus edulis*, which when deprived of food had reduced heart rates (Widdows, 1973). Such reduction in heart rates may be a sign of an energy conservation strategy under a food-scarce environment (Sokolova, 2013) which *P. viridis* may adopt to survive under increasing temperatures. In general, cold-acclimated mussels perform better than warm-acclimated mussels at lower temperatures until they reach their optimal temperature. Acclimation to cold environments can result in an increase in mitochondrial density to improve aerobic scope and thus ventilatory and circulatory systems under cold temperatures (Pörtner et al., 2005). Low temperatures, however, depress biochemical reaction rates and, therefore, cold-acclimated organisms have lower optimal temperatures and may perform relatively poorly at their optimal temperature as compared to warm-acclimated individuals (Angilletta et al., 2009). A similar pattern was demonstrated in the present study as, when mussels were acclimated under warm conditions, the optimal temperatures shifted to the right with increased optimal performance as compared to cold-acclimated animals. Such changes in performance support the importance of thermodynamic effects of temperature on reaction rates (Knies et al., 2009).

The strong acclimation capacity of *Perna viridis,* implies that this species can adjust its thermal window according to the environmental conditions it experiences over a relatively short time scale (weeks). As a consequence of this thermal flexibility, *P. viridis* is able to further shift its tolerance window under warmer environments with higher optimal temperatures and performance, which should be favoured by future increasing environmental temperatures (Urian et al., 2011; Tepolt and Somero, 2014). Whilst, in terms of aquaculture, increasing temperatures when associated with high food levels can enhance the performance and stress tolerance of *P. viridis,* suggesting this species may become a more important commercial species in warm-temperate and tropical waters. At the same time, warming climate also facilitates pathogen virulence (Harvell et al., 2002) exerting extra energetic costs on organisms for pathogen resistance which may impose severe repercussions on commercially cultivated species (Hawkins, 1985; Bachère et al., 2004). Identifying the acclimation potential and the pathogen

risk of such commercially and ecologically important species is, therefore, a key step in our interpretation of future aquaculture scenarios.

Acknowledgements

References

Anttila, K., Couturier, C.S., Øverli, Ø., Johnsen, A., Marthinsen, G., Nilsson, G.E., Farrell, A.P., 2014. Atlantic salmon show capability for cardiac acclimation to warm temperatures. Nat. Commun. 5, 4252.

Anestis, A., Lazou, A., Pörtner, H.O., Michaelidis, B., 2007. Behavioral, metabolic, and molecular stress responses of marine bivalve *Mytilus galloprovincialis* during long-term acclimation at increasing ambient temperature. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, 911–921.

Anestis, A., Pörtner, H.O., Lazou, A., Michaelidis, B., 2008. Metabolic and molecular stress responses of sublittoral bearded horse mussel *Modiolus barbatus* to warming sea water: implications for vertical zonation. J. Exp. Biol. 211, 2889–2898.

Anestis, A., Pörtner, H.O., Karagiannis, D., Angelidis, P., Staikou, A., Michaelidis, B., 2010. Response of *Mytilus galloprovincialis* (L.) to increasing seawater temperature and to marteliosis: metabolic and physiological parameters. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 156, 57–66.

Angilletta Jr, M.J., Huey, R.B., Frazier, M.R., 2009. Thermodynamic effects on organismal performance: is hotter better? Physiol. Biochem. Zool. 83, 197–206.

Bachère, E., Gueguen, Y., Gonzalez, M., De Lorgeril, J., Garnier, J., Romestand, B., 2004. Insights into the anti-microbial defense of marine invertebrates: the penaeid shrimps and the oyster *Crassostrea gigas*. Immunol. Rev. 198, 149–168.

Baker, P., Fajans, J.S., Arnold, W.S., Ingrao, D.A., Marelli, D.C., Baker, S.M., 2007. Range and dispersal of a tropical marine invader, the Asian green mussel, *Perna viridis*, in subtropical waters of the southeastern United States. J. Shellfish Res. 26, 345–355.

Bayne, B.L., Moore, M.N., Widdows, J., Livingstone, D.R., Salkeld, P., 1979. Measurement of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs. Philos. Trans. R. Soc. Lond. B Biol. Sci. 286, 563–581.

Bayne, B.L., Newell, R.C., 1983. Physiological energetics of marine molluscs. In: Saleuddin, A.S.M., Wilbur, K.M. (Eds.), The Mollusca 4. Academic Press, New York, pp. 407–515.

Boero, F., 1994. Fluctuations and variations in coastal marine environments. Mar. Ecol. 15, 3–25.

Bowler, K., 2005. Acclimation, heat shock and hardening. J. Therm. Biol. 30, 125–130.

Braby, C.E., Somero, G.N., 2006. Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). J. Exp. Biol. 209, 2554–2566.

Burnett, N.P., Seabra, R., Pirro, D.M., Wethey, D.S., Woodin, S.A., Helmuth, B., Zippay, M.L., Sarà, G., Monaco, C., Lima, F.P., 2013. An improved noninvasive method for measuring heartbeat intertidal animals. Limnol. Oceanogr. Methods 11, 91–100.

Chapple, J.P., Smerdon, G.R., Berry, R.J., Hawkins, A.J., 1998. Seasonal changes in stress-70 protein levels reflect thermal tolerance in the marine bivalve *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 229, 53–68.

Cheng, C.F.M., 2016. The influence of temperature acclimation and food on the physiology and dynamic energy budget of *Perna viridis* (MPhil thesis). The University of Hong Kong, Hong Kong.

Cheng, M.C.F., Tan, A.L.S., Rinaldi, A., Giacoletti, A., Sarà, G., Williams, G.A., 2018. Predicting effective aquaculture in subtropical waters: a dynamic energy budget model for the green lipped mussel, Perna viridis. Aquaculture 495, 749–756.

Cheung, S.G., 1993. Population dynamics and energy budgets of green-lipped mussel *Perna viridis* (Linnaeus) in a polluted harbour. J. Exp. Mar. Biol. Ecol. 168, 1–24.

Chiu, H.M.C., Hodgkiss, I.J., Chan, B.S.S., 1994. Ecological studies of phytoplankton in Tai Tam Bay, Hong Kong. Hydrobiologia 273, 81–94.

Cochrane, K., De Young, C., Soto, D., Bahri, T., 2009. Climate change implications for fisheries and aquaculture. FAO Fisheries and aquaculture technical paper 530, 212.

Crawshaw, L.I., 1977. Physiological and behavioral reactions of fishes to temperature change. J. Fish. J. Fish. Res. Board Can. 34, 730–734.

Dahlhoff, E., O'Brien, J., Somero, G.N., Vetter, R.D., 1991. Temperature effects on mitochondria from hydrothermal vent invertebrates: evidence for adaptation to elevated and variable habitat temperatures. Physiol. Zool. 64, 1490–1508.

Dahlhoff, E.A., Somero, G.N., 1993. Effects of temperature on mitochondria from abalone (genus *Haliotis*): adaptive plasticity and its limits. J. Exp. Biol. 185, 151–168.

Dahlhoff, E.P., Menge, B.A., 1996. Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californianus*. Mar. Ecol. Prog. Ser. 144, 97–107.

Dahlhoff, E.P., 2004. Biochemical indicators of stress and metabolism: applications for marine ecological studies. Annu. Rev. Physiol. 66, 183–1207.

Dahlhoff, E.P., Buckley, B.A., Menge, B.A., 2001. Physiology of the rocky intertidal predator *Nucella ostrina* along an environmental stress gradient. Ecology 82, 2816–2829.

Denny, M.W., Miller, L.P., Harley, C.D.G., 2006. Thermal stress on intertidal limpets: long-term hindcasts and lethal limits. J. Exp. Biol. 209, 2420–2431.

Dong, Y.W., Williams, G.A., 2011. Variations in cardiac performance and heat shock protein expression to thermal stress in two differently zoned limpets on a tropical rocky shore. Mar. Biol. 158, 1223–1231.

Filgueira, R., Rosland, R., Grant, J., 2011. A comparison of scope for growth (SFG) and dynamic energy budget (DEB) models applied to the blue mussel (*Mytilus edulis*). J. Sea Res. 66, 403–410.

Fitzgerald-Dehoog, L., Browing, J., Allen, B.J., 2012. Food and heat stress in the California mussel: evidence for an energetic trade-off between survival and growth. Biol. Bull. 223, 205–216.

Frederich, M., Pörtner, H.O., 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, 1531–1538.

Goh, B.P.L., Lai, C.H., 2014. Establishing the thermal threshold of the tropical mussel *Perna viridis* in the face of global warming. Mar. Poll. Bull. 85, 325–331.

Giomi, F., Mandaglio, C., Ganmanee, M., Han, G.D., Dong, Y.W., Williams, G.A., Sarà, G., 2016. The importance of thermal history: costs and benefits of heat exposure in a tropical, rock oyster. J. Exp. Biol. 219, 686–694.

Gunderson, A.R., Armstrong, E.J., Stillman, J.H., 2016. Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. Annu. Rev. Mar. Sci. 8, 357–378.

Gutiérrez, J.L., Clive, G., Strayer, D.L., Iribarne, O.O., 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101, 79–90.

Hamdoun, A.M., Cheney, D.P., Cherr, G.N., 2003. Phenotypic plasticity of HSP70 and HSP70 gene expression in the Pacific oyster (*Crassostrea gigas*): implications for thermal limits and induction of thermal tolerance. Biol. Bull. 205, 160–169.

Harley, C.D., Helmuth, B.S., 2003. Local-and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation. Limnol. Oceanogr. 48, 1498–1508.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D., 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296, 2158–2162.

Hawkins, A.J.S., 1985. Relationships between the synthesis and breakdown of protein, dietary absorption and turnovers of nitrogen and carbon in the blue mussel, *Mytilus edulis* L. Oecologia 66, 42–49.

Helmuth, B., Harley, C.D., Halpin, P.M., O'Donnell, M., Hofmann, G.E., Blanchette, C.A., 2002. Climate change and latitudinal patterns of intertidal thermal stress. Science 298, 1015–1017.

Helmuth, B., Russell, B.D., Connell, S.D., Dong, Y.W., Harley, C.D.G., Lima, F.P., Sarà, G., Williams, G.A., Mieszkowska, N., 2014. Beyond long-term averages: making biological sense of a rapidly changing world. Clim. Chang. Resp. 1, 6.

Hicks, D.W., McMahon, R.F., 2002. Respiratory responses to temperature and hypoxia in the nonindigenous Brown Mussel, *Perna perna* (Bivalvia: mytilidae), from the Gulf of Mexico. J. Exp. Mar. Biol. Ecol. 277, 61–78.

Hochachka, P.W., 1991. Temperature: the ectothermy option. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes Volume 1: Phylogenetic and Biochemical Perspectives. Elsevier, New York, pp. 313–322.

Hochachka, P.W., Hayes, F.R., 1962. The effect of temperature acclimation on pathways of glucose metabolism in the trout. Can. J. Zool. 40, 261–270.

Hoffmann, A.A., 1995. Acclimation: increasing survival at a cost. Trends Ecol. Evol. 10, 1–2.

Hofmann, G.E., Todgham, A.E., 2010. Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Annu. Rev. Physiol. 72, 127–145.

Honkoop, P.J.C., Bayne, B.L., Drent, J., 2003. Flexibility of size of gills and palps in the Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) and the Pacific oyster *Crassostrea gigas* (Thunberg, 1793). J. Exp. Mar. Biol. Ecol. 282, 113–133.

Horowitz, M., 2001. Heat acclimation: phenotypic plasticity and cues to the underlying molecular mechanisms. J. Therm. Biol. 26, 357–363.

Huey, R.B., Bennett, A.F., 1990. Physiological adjustments to fluctuating thermal environments: an ecological and evolutionary perspective. In: Morimoto, R.I., Tissieres, A., Georgopoulus, C. (Eds.), Stress Protein in Biology and Medicine. Cold Spring Harbour Laboratory Press, New York, pp. 37–59.

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M., Williams, S.E., 2012. Predicting organismal vulnerability to climate warming: role of behaviour, physiology and adaptation. Philos. Trans. R. Soc. Biol. Sci. 367, 1665–1679.

Huhn, M., Zamani, N.P., von Juterzenka, K., Lenz, M., 2016. Food availability in an anthropogenically impacted habitat determines tolerance to hypoxia in the Asian green mussel *Perna viridis*. Mar. Biol. 163, 1–15.

Jansen, J., Hummel, H., Bongac, S.W., 2009. The respiratory capacity of marine mussels (*Mytilus galloprovincialis*) in relation to the high temperature threshold. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 153, 399–402.

Jost, J., Helmuth, B., 2007. Morphological and ecological determinants of body temperature of *Geukensia demissa*, the Atlantic ribbed mussel, and their effects on mussel mortality. Biol. Bull. 213, 141–151.

Kaehler, S., Williams, G.A., 1996. Distribution of algae on tropical rocky shores: spatial and temporal patterns of non-coralline encrusting algae in Hong Kong. Mar. Biol. 125, 177–187.

Kassahn, K.S., Crozier, R.H., Pörtner, H.O., Caley, M.J., 2009. Animal performance and stress: responses and tolerance limits at different levels of biological organization. Biol. Rev. 84, 277–292.

Knies, J.L., Kingsolver, J.G., Burch, C.L., 2009. Hotter is better and broader: thermal sensitivity of fitness in a population of bacteriophages. Am. Nat. 173, 419–430.

Kooijman, S.A.L.M., 2010. Dynamic Energy Budget Theory For Metabolic Organization. Cambridge University Press, Cambridge.

Lagerspetz, K.Y., 2006. What is thermal acclimation? J. Therm. Biol. 31, 332–336.

Layne, J.R., Manis, M.L., Claussen, D.L., 1985. Seasonal variation in the time course of thermal acclimation in the crayfish *Orconectes rusticus*. Freshw. Invertebr. Biol. 4, 98–104.

Lockwood, B.L., Sanders, J.G., Somero, G.N., 2010. Transcriptomic responses to heat stress in invasive and native blue mussels (genus Mytilus): molecular correlates of invasive success. J. Exp. Biol. 213, 3548–3558.

Ma, W.L., 2013. The Role of Stress Tolerance on Marine Invasive Mussels (MPhil Thesis). The University of Hong Kong, Hong Kong.

McFarland, K., Baker, S., Baker, P., Rybovich, M., Volety, A.K., 2015. Temperature, salinity, and aerial exposure tolerance of the invasive mussel, *Perna viridis*, in estuarine habitats: implications for spread and competition with native oysters, *Crassostrea virginica*. Estuar. Coasts 38, 1619–1628.

Morton, B., Blackmore, G., 2001. South China Sea. Mar. Poll. Bull. 42, 1236–1263.

Muggeo, V.M.R., 2017. Segmented: Regression Models with Break-Points / Change-Points Estimation. R package ver. 0.5-2.2. ⟨https://CRAN.R-project.org/package=segmented/⟩ (accessed 25 September 2017).

Newell, R.C., Branch, G.M., 1980. The influence of temperature on the maintenance of metabolic energy balance in marine invertebrates. Adv. Mar. Biol. 17, 329–396.

Newell, R.C., Johnson, L.G., Kofoed, L.H., 1977. Adjustment of the components of energy balance in response to temperature change in Ostrea edulis. Oecologia 30, 97–110.

Newell, R.C., Kofoed, L.H., 1977. Adjustment of the components of energy balance in the gastropod *Crepidula fornicata* in response to thermal acclimation. Mar. Biol. 44, 275–286.

Pörtner, H.O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88, 137–146.

Pörtner, H.O., 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 132, 739–761.

Pörtner, H.O., Hardewig, I., Peck, L.S., 1999. Mitochondrial function and critical temperature in the Antarctic bivalve, *Laternula elliptica*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 124, 179–189.

Pörtner, H.O., Lucassen, M., Storch, D., 2005. Metabolic biochemistry: its role in thermal tolerance and in the capacities of physiological and ecological function. Fish. Physiol. 22, 79–154.

Power, A.J., Walker, R.L., Payne, K., Hurley, D., 2004. First occurrence of the non-indigenous green mussel, *Perna viridis* in coastal Georgia, United States. J. Shellfish Res. 23, 741–744.

Rajagopal, S., Venugopalan, V.P., van der Velde, G., Jenner, H.A., 2006. Greening of the coasts: a review of the *Perna viridis* success story. Aquat. Ecol. 40, 273–297.

Riisgård, H.U., Seerup, D.F., 2003. Filtration rates in the soft clam *Mya arenaria*: effects of temperature and body size. Sarsia 88, 416–428.

Sarà, G., Palmeri, V., Montalto, V., Rinaldi, A., Widdows, J., 2013. Parameterisation of bivalve functional traits for mechanistic eco-physiological dynamic energy budget (DEB) models. Mar. Ecol. Prog. Ser. 480, 99–117.

Scheiner, S.M., 1993. Plasticity as a selectable trait: reply to Via. Am. Nat. 142, 371–373.

Schneider, K.R., Van Thiel, L.E., Helmuth, B., 2010. Interactive effects of food availability and aerial body temperature on the survival of two intertidal *Mytilus* species. J. Therm. Biol. 35, 161–166.

Schulte, P.M., Healy, T.M., Fangue, N.A., 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr. Comp. Biol. 51, 691–702.

Segal, E., 1961. Acclimation in molluscs. Am. Zool. 1, 235–244.

Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. Mar. Environ. Res. 79, 1–15.

Sokolova, I.M., Pörtner, H.O., 2003. Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda: littorinidae) from different latitudes. J. Exp. Biol. 206, 195–207.

Sokolova, I.M., 2013. Energy-limited tolerance to stress as conceptual framework to integrate the effects of multiple stressors. Integr. Comp. Biol. 53, 597–608.

Somero, G.N., 1995. Proteins and temperature. Annu. Rev. Physiol. 57, 43–68.

Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J. Exp. Biol. 213, 912–920.

Stenseng, E., Braby, C.E., Somero, G.N., 2005. Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (genus *Tegula*): implications for vertical zonation. Biol. Bull. 208, 138–144.

Stillman, J.H., Somero, G.N., 1996. Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): correlation of physiology, biochemistry and morphology with vertical distribution. J. Exp. Biol. 199, 1845–1855.

Stillman, J.H., Somero, G.N., 2000. A comparative analysis of the upper thermal tolerance limits of eastern pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. Physiol. Biochem. Zool. 73, 200–208.

Tagliarolo, M., McQuaid, C.D., 2016. Field measurements indicate unexpected, serious underestimation of mussel heart rates and thermal tolerance by laboratory studies. PloS One 11, e0146341.

Tepolt, C.K., Somero, G.N., 2014. Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, *Carcinus maenas*. J. Exp. Biol. 217, 1129–1138.

Todgham, A.E., Stillman, J.H., 2013. Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. Integr. Comp. Biol. 53, 539–544.

Urian, A.G., Hatle, J.D., Gilg, M.R., 2011. Thermal constraints for range expansion of the invasive green mussel, *Perna viridis*, in the southeastern United States. J. Exp. Zool. A Ecol. Genet. Physiol. 315, 12–21.

Van der Meer, J., 2006. An introduction to Dynamic energy budget (DEB) models with special emphasis on parameter estimation. J. Sea Res. 56, 85–102.

Wendling, C.C., Huhn, M., Ayu, N., Bachtiar, R., Von Juterzenka, K., Lenz, M., 2013. Habitat degradation correlates with tolerance to climate-change related stressors in the green mussel *Perna viridis* from West Java, Indonesia. Mar. Poll. Bull. 71, 222–229.

Widdows, J., Bayne, B.L., 1971. Temperature acclimation of *Mytilus edulis* with reference to its energy budget. J. Mar. Biol. Assoc. UK 51, 827–843.

Widdows, J., Bayne, B.L., Donkin, P., Livingstone, D.R., Lowe, D.M., Moore, M.N., Salkeld, P.N., 1981. Measurement of the responses of mussels to environmental stress and pollution in Sullom Voe: a base-line study. Proc. R. Soc. Edin. B Biol. Sci. 80, 323–338.

Widdows, J., 1973. Effect of temperature and food on the heart beat, ventilation rate and oxygen uptake of *Mytilus edulis*. Mar. Biol. 20, 269–276.

Widdows, J., 1978. Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis*. J. Mar. Biol. Assoc. UK 58, 109–124.

Widdows, J., Burns, K.A., Menon, N.R., Page, D.S., Soria, S., 1990. Measurement of physiological energetics (scope for growth) and chemical contaminants in mussels (*Acra zebra*) transplanted along a contamination gradient in Bermuda. J. Exp. Mar. Biol. Ecol. 138, 99–117.

Williams, G.A., De Pirro, M., Cartwright, S., Khangura, K., Ng, W.C., Leung, P.T.Y., Morritt, D., 2011. Come rain or shine: the combined effects of physical stresses on physiological and protein-level responses of an intertidal limpet in the monsoonal tropics. Funct. Ecol. 25, 101–110.

Williams, G.A., Helmuth, B., Russell, B.D., Dong, Y.W., Thiyagarajan, V., Seuront, L., 2016. Meeting the climate change challenge: pressing issues in southern China and SE Asian coastal ecosystems. Reg. Stud. Mar. Sci. 8, 373–381.

Wong, W.H., Cheung, S.G., 2001. Feeding rates and scope for growth of green mussels, *Perna viridis* (L.) and their relationship with food availability in Kat O, Hong Kong. Aquaculture 193, 123–137.

Yoshida, G., Shimabukuro, H., 2017. Seasonal population dynamics of *Sargassum fusiforme* (Fucales, Phaeophyta), Suo-Oshima Is., Seto Inland Sea, Japan—development processes of a stand characterized by high density and productivity. J. Appl. Phycol. 29, 639–648.

Yu, T.N., 2014. The Thermal Tolerance of the Bivalves *Septifer virgatus* and *Perna viridis*, and Implications for Marine Coastal Management. Master of Science Dissertation. The University of Hong Kong, Hong Kong.