Limpet feeding rate and the consistency of physiological response to temperature

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Abstract

Thermal reaction norms are fundamental relationships for geographic comparisons of organism response to temperature. They are shaped by an organism's environmental history and provide insights into both the global patterns of thermal sensitivity and the physiological mechanisms underlying temperature response. In this study we conducted the first measure of the thermal reaction norm for feeding, comparing the radula rasping rate of two tropical and one polar limpet species. The consistency of thermal response was tested through comparisons with limpet duration tenacity. Feeding and duration tenacity of limpets are ecologically important muscular mechanisms that rely on very different aspects of muscle physiology, repeated concentric (shortening) and isometric (fixed length) contraction of muscles, respectively. In these limpets the thermal reaction norms of feeding limpets were best described by a single break point at a maximum temperature with linear declines at higher (*S. atra*) or lower temperatures (*N. concinna* and *C. radiata*) rather than a bell shaped curve. The thermal reaction norms for duration tenacity were similar in the two tropical limpets. However, the rasping rate in Antarctic *Nacella concinna*, increased linearly with temperature up to a maximum at 12.3°C (maximal range 8.5 to 12.3°C) when feeding stopped. In contrast duration tenacity in *N. concinna*, was maximal at 1.0°C (-0.6 to 3.8°C), and linearly decreased with increasing temperature. The thermal reaction norms of muscular activity were therefore inconsistent within and between species, indicating that different mechanisms likely underlie different aspects of species sensitivities to temperature.

Key-words: Thermal reaction norm; radula; rasping rate; tropical; Antarctic; *Nacella concinna*

Introduction

Understanding how species ranges are controlled by variation in their environment, and how these ranges are likely to shift in response to future environmental conditions, is one of the major current scientific challenges (Pennisi 2005). Whilst in the oceans a range of variables such as salinity, wave exposure, primary productivity and competition are important controlling factors, temperature is arguably the most important for ectotherms as it controls the rates of all biochemical reactions within body tissues (Hochachka and Somero 2002). The magnitude and variation of temperature are important determinants of ectotherm physiological limits (Peck 2005; Gaston et al. 2009) which play a major role in limiting their distributions (e.g. Gaston et al. 2009). The predictability of temperature variation is, also, increasingly being recognised as a key factor determining both acclimatory capacity (Sinclair et al. 2006; Peck et al. 2006; Morley et al. 2012a) and thermal adaptation (Alto et al. 2013).

Unifying physiological principles such as oxygen and capacity limited thermal tolerance (Pörtner and Knust 2007; Pörtner and Farrell 2008) or the metabolic theory of ecology (Gillooly et al. 2001) provide mechanistic frameworks against which the generality of thermal response can be compared. These frameworks improve understanding of the physiological mechanisms underlying ectotherm response to temperature, which are, in turn, expected to improve predictions of future species ranges (e.g. Kearney et al. 2009; Calosi et al. 2010). However, rather than species sensitivity to temperature being caused by a single pervasive mechanism, it is increasingly argued that a combination of multiple physiological factors, which can have different thermal limits and can vary in importance through an organisms life history are likely to determine species vulnerability (Clark et al. 2013). This might explain why quite different predictions of the future range can be obtained using current realised and physiological niches based on single trait analyses (diving beetles, Sanchez-Fernandez et al. 2012). There is also evidence showing that the rate of temperature change has marked effects on measured thermal limits in both terrestrial and aquatic environments (Mora and Moya 2006; Terblanche et al. 2007; Chown et al. 2009; Peck et al. 2009; Richard et al. 2012; Nguyen et al. 2012) with indications that different mechanisms underlie temperature limits at different rates of change (Peck et al. 2009; Richard et al. 2012; Nguyen et al. 2012). Thermal limits may also vary hierarchically between different physiological measures, from high to low complexity, i.e. processes that rely on the integration of multiple organs, such as feeding and locomotion, are expected to have lower thermal limits than individual biochemical pathways (Prosser 1973; Pörtner et al. 2007). This might also be expected to vary between processes with different fitness consequences, with lethal limits being higher than those for escape responses or feeding.

Limpets and other gastropod molluscs feed using a chitinous ribbon with teeth that are hardened with iron and silicone oxides (Fig. 1, Liddiard et al 2004). The teeth of the radula rasp algae from hard substrata, which are then ingested (e.g. Boyden and Zeldis 1979). Whilst radula rasping rate is not the only factor affecting consumption rate, radula rasping is an easily quantifiable activity, which is expected to closely correlate with feeding rate. Most intertidal molluscs feed when they are awash or submerged and radula rasping rate in molluscs has generally been found to be sensitive to both temperature and body size (Boyden and Zeldis 1979; Horn 1986; Petraitis 1992; although see *Bembicium nanum*; Petraitis 1992). This study presents the first measure of the thermal reaction norm for feeding in limpets from the Antarctic, *Nacella concinna* (recent synonym *N. polaris*) and tropical Singapore, *Cellana radiata* and *Siphonaria atra*. Previous assessments of the thermal reaction norm of duration tenacity of *N. concinna* found no difference between individuals collected from the intertidal or subtidal (Morley et al. 2012a).

To investigate the mechanisms underlying thermal reaction norms, this new measure of the thermal response of feeding in all three species and new data for the thermal response of tenacity and mortality for *Siphonaria atra* were compared with previously published tenacity and mortality data for *Nacella concinna* and *Cellana radiata* (Morley et al. 2012a). Current theory predicts that thermal reaction norms will be consistent across different measures of muscular performance with consistent optimal, upper and lower thermal limits which will correlate with experienced microhabitat temperature (Angilletta 2009). This is increasingly being questioned as different physiological processes are expected to rely on different mechanisms which are likely to have different thermal responses (e.g. Morley et al. 2012; Clark et al. 2013). The study aimed to test, 1) if differences in experienced thermal regime led to differences in the thermal reaction norm of feeding in intertidal and subtidal *N. concinna*, 2) if the thermal reaction norm of rasping in one Antarctic and two tropical limpets correlated with differences in their experienced environments and 3) if the shape of the thermal reaction norm was consistent across these species and between rasping rate and duration tenacity.

Materials and methods

Cellana radiata and *Siphonaria atra* were collected from the shore at the Tropical Marine Science Institute, St John’s Island, Singapore (1° 31.2’N, 103° 51.56’E) and *Nacella concinna* were collected from both the shore and 6m depth (by SCUBA divers) at the British Antarctic Survey’s Rothera Research Station (67° 34.25’S, 68° 08.00’W). The temperature response of feeding was measured in subtidal *N. concinna* in 2006/2007 and in both subtidal and intertidal *N. concinna* in 2012/13. Singapore limpets were tested between November 2009 and March 2010.

In Rothera and Singapore, limpets were transferred after collection, in insulated seawater containers, to flow-through aquaria for at least 24 hours. They were then transferred to jacketed tanks which were heated by thermocirculators at a rate of 0.2 ± 0.1°C $hour^{-1}$ (after Morley et al. 2012a). Animals were maintained at test temperature for 24 hours (Table S1), to allow their physiology to fully adjust, before the start of feeding experiments. Feeding rate was then measured over 24 hours. Individuals were placed on a piece of overhead projector acetate sheet that had been incubated in the flow through seawater aquaria for over 2 weeks in Singapore and over 2 months in the Antarctic. This was long enough to ensure that an algal biofilm had developed that was sufficient to stimulate feeding. After 1 hour, any feeding individuals were filmed for three sequences of 6 to 10 radula rasps, with a Microsoft LifeCam Studio HD web camera, directly onto a computer hard drive. Counts started after the completion of the forward stroke and closure of the mouth. After feeding had been recorded the length of each limpet was measured and the limpet removed to the aquarium so that each limpet was only used once. The duration of each radula rasp was analysed from the time stamped videos. Trials were run at temperatures, evenly spaced over temperatures they were predicted to survive (Peck et al. 2009; Nugyen et al. 2011), in a random order, until a temperature was reached when less than 5 limpets fed within the 24 hour period (Table 1).

New data for the duration tenacity and survival of *S. atra* is also presented alongside published data for *N. concinna* and *C. radiata* (Morley et al. 2012a). The detailed method for duration tenacity is described in Morley et al. (2011, 2012), which utilised the same heating protocol to measure feeding rates. Briefly, a fine soft stainless steel cradle (Leader wire, Bass Pro Shops) was hooked underneath the shell edge, with contact at 4 points around the shell, (modified from Grenon and Walker 1981), before temperatures were changed. Animals were then held in seawater at the required experimental temperature for 24 hours before tenacity was measured following Morley et al. (2011, 2012a). Individuals were tested at one trial temperature only. During all trials any individual that did not have a measurable tenacity was tested by stimulation of the foot with a blunt seeker. Failure of the foot muscle to respond was recorded as functional mortality. This was used to determine upper 50% lethal (CT_{max}) and lower 50% lethal (CT_{min}) limits (following Peck et al. 2009). To standardise measurements, limpet shells were tapped three times to stimulate a clamping response (Grenon and Walker 1981), before the 200g (*N. concinna*) or 919g (*C. radiata* and *S. atra*) weight was attached. 20 kg mono filament fishing wire was hooked to the stainless steel cradle or cotton loop and passed over two 18KN pulleys (Petzl, France) supported by a retort stand frame. 20 seconds after the limpet shell was tapped, the weight was hooked to the line and gently lowered until the full force was supported directly above the centre of each limpet. The subsequent time taken for the limpet to be pulled from the substratum was recorded.

Microhabitat temperature for all three limpets was measured using biomimetic loggers (as described in Morley et al. 2012a). Signatrol button loggers were silicon sealed into empty limpet shells and glued onto the shore (using marine araldite) at the median height that each limpet species was found. These loggers were set to record every 30 minutes and were deployed between 16/06/10 and 02/08/10 on break waters around St. John's Island, Singapore and between 29/12/12 and 15/02/13 on East Beach, Rothera Point. Previously published logger data were also available

(Singapore *C. radiata*, Morley et al. 2012a; Rothera, *N. concinna*, Waller et al. 2006). On spring low waters foot temperatures of *N. concinna* were also recorded, using a thermocouple. As the loggers were fixed in position and limpets are mobile, movement patterns of individuals were monitored during successive low waters. The distance moved from the first recorded position was monitored over 3 low tides for *N. concinna* and *S. atra* and 19 low waters for *C. radiata*.

Data analysis

Temperatures recorded by biomimetic loggers were analysed by calculating the average number of hours in each day that each temperature was experienced (collated into 1°C bins). The temperature profiles of each replicate logger were not normally distributed and so differences between the recorded temperature distributions were tested with multiple (bonferroni corrected) Mann-Whitney tests.

The R-package strucchange (Zeileis et al. 2002) was used to find the most appropriate piecewise linear breakpoints within the relationship between temperature and either rasping rate or duration tenacity of individuals (TF_{max} and TT_{max}). After breakpoint selection, linear models were fitted to the respective sections, incorporating the endpoints in all contiguous sections. Quadratic functions were also fitted to the data. Akaike Information Criteria (AIC) were compared to determine the best overall fit to the data sets and the significance of the parameters in each model compared (modified from Angilletta 2009). 4 out 6 quadratic fits had lower AIC's (Table S2), but this was not significantly different from a 1:1 ratio ($\chi^2 = 0.67$, $P = 0.41$). However, as half of the quadratic (T^2) parameters were not significant (Table S2) and there were visible discontinuities in the thermal profiles, the piecewise linear fits were chosen as the overall best fit and biological description of the data. Post Hoc multiple Tukey test were then used to determine which temperatures were not significantly different from this breakpoint value.

Results

Microhabitat temperatures

There were no significant differences in the temperature distributions recorded by the replicate loggers (Mann-Whitney; *N. concinna*, U<122, p>0.79; *C. radiata*, U<86, P>0.07; *S. atra*, U=174, p=0.94; Fig. 2). Median logger temperature for *N. concinna* was 1°C, with a summer minimum of -1°C but also a few high temperatures to a maximum of 9°C. *S. atra* loggers also recorded a few high temperatures with a maximum over 40°C. *S atra* experienced higher maximum temperatures than those recorded by *C. radiata* loggers (maximum of 35°C). The median temperature for *S. atra* (30°C) was 1°C higher than that for *C. radiata* (29°C). The minimum logger temperature for both tropical limpets was 24°C.

The two tropical limpets moved very little (median 10cm) between successive low waters, remaining on the same rock surface, and therefore experiencing the same level of exposure to the sun during each low water (Fig. 3). *N. concinna* moved much greater distances, up to 3.4m between tides. Individuals moved to different rock surfaces and might, therefore, have experienced a wider temperature range than that recorded by loggers fixed to the rocks. However, foot temperatures of *N. concinna* were similar to those recorded by loggers (Table 1); on 31/12/12 the logger temperature was 1 to 2°C higher than the limpet foot temperature, but 1 to 2°C lower on 16/1/12. The highest foot temperature was 13.2°C, recorded on 28/12/12.

Radula rasping rate

There was no relationship between feeding rate and limpet size, or the interaction of size and temperature for any of the 3 groups of limpets: *N. concinna* ($F_{1,265}$=3.8, p=0.052; $F_{1,265}$=0.88, p=0.35); *C. radiata* ($F_{1,69}$=1.7, p=0.20; $F_{1,69}$=1.9, p=0.18) or *S. atra* ($F_{1,63}$=0.00, p=0.99; $F_{1,63}$=0.01, p=0.94).

The rasping rate of *N. concinna* increased with temperature until the temperature was too high for them to feed (Fig 4A, Table S3). The highest temperature at which individuals fed in 2007 was 6.6°C, with no feeding at 8.4°C, but 4 intertidal and 3 subtidal individuals fed at 12.3°C in 2012. Most *N. concinna* were moribund at 12.3°C, none fed at 14.4°C and only 1 intertidal limpet survived at this temperature. Despite this interannual difference in temperature limit there was no significant difference in the relationship between rasping rate and temperature in the 3 *N. concinna* trials (ANCOVA; $F_{2,265}$=0.70, p=0.50) or the interaction with temperature ($F_{2,265}$=0.29, p=0.75), so the data were combined. Tukey *posthoc* multiple comparison tests showed that the temperature over which rasping rates of *N. concinna* were fastest occurred within the range 8.5 to 12.3°C. *Cellana radiata* had a maximum rasping rate at 30.6°C and *posthoc* tests showed the optimal range was between 26.6 and 34.6°C (Fig4B). *S. atra* had a maximum rasping rate at 24.6°C with an optimal range between 24.6 and 36.6°C (Fig 4C).

Tenacity

A lower Akaike Information Criteria was obtained for a polynomial fit for tenacity of Singapore *S. atra*, rather than the breakpoint analysis (AIC= 3288 versus 3976). The polynomial fit had a maximum tenacity at 24.9°C, but tenacity was relatively constant across the temperature range 18-30°C. The individual variability in duration tenacity was high enough that *posthoc* tests could not identify significant pairwise differences between temperatures (fig 5A). *N. concinna* had a break point at 1.0°C with an optimal temperature range between -0.6 and 3.8°C (Fig S1; Morley et al. 2012a). *C. radiata* had a maximum temperature for tenacity of 27.6°C and there was a significant linear reduction in tenacity above and below this temperature.

Mortality data

The CT_{min} of *S.atra* was between 13.6 to 15.6°C and the CT_{max} between 36.6 to 37.6°C (fig 5B). The CT_{max} of Rothera *N. concinna* was 12.0°C (Morley et al. 2012a). The CT_{min} for *C. radiata* was 2.6 to 4.6°C and the CT_{max} 33.6 to 34.6°C (Morley et al. 2012a).

Discussion

Thermal reaction norm

Despite the approximately 30°C difference in environmental temperature between the seas around the Western Antarctic Peninsula and Singapore, average rasping rates of *N. concinna* and *S. atra* were similar (0.2-0.3 rasps sec^{-1}). Instead there was a bigger difference between the two tropical limpets, with *C. radiata* having three times faster average rasping rate (0.9 rasps sec^{-1}). Many factors, such as previous feeding history (Boyden and Zeldis 1979), can affect the rasping rate but there are also large differences between species (Petraitis 1992). Inter-specific comparisons were therefore focussed on the shape of the thermal reaction norms and their relationship with experienced temperature.

The concept that ectotherms will have an optimal physiological temperature range is a fundamental paradigm within thermal physiology and has led to thermal reaction norms typically being described by bell shaped curves, with an optimal range and a decline in performance either side of this range (Huey and Kingsolver 1989; Pörtner and Knust 2007, Pörtner and Farrell 2008; Angilletta 2009). These curves are useful for comparing the relative thermal response between species as temperature ranges are expected to be wider in species that experience more variable thermal environments and have optima that match their experienced environment (e.g. Huey and

Kingsolver 1989; Angilletta 2009). The thermal reaction norms for these three species were best described by a single breakpoint at a temperature where feeding rate was at a maximum, with a gradual linear reduction at temperatures below this in *N. concinna* and *C. radiata*, but a signficiant linear reduction at temperatures above this in *S. atra*. For both species the optimal temperature range over which the change in feeding rate was not significant had an upper boundary at a temperature close to that of their upper lethal limit. Previous studies of rasping rate in molluscs have also measured a linear increase with temperature, at least over a limited temperature range (Boyden and Zeldis 1979; Horn 1986; Petraitis 1992). The generally poor fit of the quadratic term meant that the expectation of a bell shaped rate temperature curve was not met. This was particularly true in *N. concinna* whose rasping rate increased linearly with temperature to a maximum that was also close to their upper lethal limit. Several recent studies have found a similar thermal response with aerobic scope only collapsing at temperatures close to upper lethal limits, contradicting one of the central tenets of the principle of oxygen and capacity limitation of thermal tolerance (see Clark et al. 2013).

Whilst the reaction norms for duration tenacity and feeding in the two tropical limpets were very similar the temperature responses were reversed, giving very different measures of thermal sensitivity in *N. concinna* (figs 5 and S1). Duration tenacity measures the time to fatigue of constant (tetanic), fixed length (isometric) contraction of muscles, as they resist a constant force (Morley et al. 2012a). Feeding, however, relies on alternate shortening (concentric contraction) of atagonistic muscles of the odontophore that scrape the radula back and forth. These are very different muscle mechanisms and are likely to have different reliance on aerobic metabolism. Behavioural responses, such as feeding, can also be governed by a hierarchical response to stimuli, where, for example, the presence of food leads to a feeding bout which overrides the stimulus of a prosobranch mollusc to right itself (Davis et al. 1974). It is therefore perhaps more surprising that the thermal reaction norms for the two tropical limpets were so similar in shape.

Microhabitat and thermal limits

In the current study there was no difference in the thermal response of rasping in intertidal and subtidal *N. concinna*, despite their vastly different experienced environments. This was the same pattern as previously measured for duration tenacity in this species (Morley et al. 2012). Intertidal temperatures in the Antarctic are highly variable and the cyclical pattern of temperature variation and the unpredictability of temperature may preclude acclimatisation to the intertidal environment (Morley et al. 2012). However, although subtidal Southern Ocean temperatures are amongst the most stable there was a large difference in the maximum temperature for feeding of subtidal *N. concinna* between 2007 (6.4°C) and 2012 (12.4°C). This corresponded to a difference in the maximum summer seawater temperature between 2007, which was a cooler year (1.0°C) and 2012, which was one of the warmest years on record (1.9°C). Differences in the upper temperature limits of Antarctic species have previously been recorded between years; with the starfish *Odontaster validus* and the clam *Laternula elliptica* having higher lethal limits in warmer years (Morley et al. 2012b).

Whilst there were no differences in the rasping rate of Rothera *N. concinna* between years, there were differences between Rothera *N. concinna* measured in this study and rasping rate of Signy Island *N. concinna* (60° 42.16'S, 45°35.45'W) measured over a reduced temperature range in 1986 (Fig. S1). Signy *N. concinna* measured in 1986, had a significantly faster rasping rate at all temperatures than Rothera *N. concinna* (ANCOVA; $F_{1,286}$=57.9, p<0.01), but rasping rate had the same slope with temperature ($F_{1,286}$=1.1, p=0.29). The consistent response of rasping rate to temperature suggests that it is a robust measure of thermal response in *N. concinna*.

Within the tropics, *S. atra* experiences higher maximum and median environmental temperatures than *C. radiata,* but approximately the same lower environmental temperature (Table 1). As predicted *S. atra* had higher upper lethal limits and a wider optimal temperature range for feeding than *C. radiata* but these ranges were centred on the same midpoint. In global latitudinal analyses, lower lethal limits of marine ectotherms are also generally correlated with latitudinal temperature gradients (e.g. Sunday et al. 2012). However, in the current study, the lower lethal limits of both tropical species were below the minimum environmental temperature experienced in Singapore. The CT_{min} of *C. radiata* was extremely low (2.6 to 4.6°C), as was the previously measured CT_{min} of the Singapore clam *Laternula truncata* (5.7°C, Lai et al. 2011) and three species of tropical oyster (1.4 to 5.0°C; Davenport and Wong 1992). There are clearly factors, other than recent thermal history, which affect the temperature tolerance of ectotherms, such as the predictability of temperature variation (Morley et al. 2012a) and evolutionary history (Bozinovic et al. 2011; Richard et al. 2012). This understanding is particularly important for intertidal species, where the complex microhabitat structure on the shore means that even small differences in climate temperature (0.75°C in 60 years) have had dramatic effects on the structure of Californian intertidal communities (Barry et al. 1995; Sagarin et al. 1999).

The thermal reaction norm for these two very different muscular activities was consistent in the two species of tropical limpet but was almost opposite in the one Antarctic species. Even measurements from these three species indicate how very different estimates of species' vulnerability to climate warming can be predicted from the thermal reaction norms measured for different muscle activity. Acute thermal tolerance will be modified by species physiological flexibility (acclimitisation capacity) and ultimately adaptation. Polar and tropical species are known to have reduced acclimation capacity (Nguyen et al. 2011; Richard et al. 2011; Peck et al. 2013) and are expected to have slower rates of adaptation (Peck 2011) than species from temperate regions. Climate warming is also a non-linear process and extreme events are also expected to increase in frequency (Karl et al. 1997). Understanding which physiological mechanisms underlie these different measures, and which will be most important to future fitness, is an important step towards improving our assessments of future vulnerability.

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References

Angilletta MJ (2009). Thermal Adaptation. Oxford University Press, New York

Alto BW, Wasik BR, Morales NM, Turner PE (2013) Stochastic temperatures impede RNA virus adaptation. Evolution 67:969-979

Barry JP, Baxter CH, Sagarin RD, Gilman SE (1995) Climate-related, long-term changes in a California intertidal community. Science 267:672-675

Boyden CR, Zeldis JR (1979) Preliminary observations using an attached microphonic sensor to study feeding behaviour of an intertidal limpet. Estuar Coast Mar Sci 9:759-769

Calosi P, Bilton DT, Spicer JI, Votier S, Atfield A (2010) What determines a species geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). J Anim Ecol 79:194–204

Chown SL, Jumban KR, Sørensen JG, Terblanche JS (2009) Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. Func Ecol 23:133-140

Clark TD, Sandblom E, Jutfelt F (2013). Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J Exp Biol 216:2771-2782

Davenport J, Wong TM (1992) Effects of temperature and aerial exposure on three tropical oyster species, *Crassostrea belcheri*, *Crassostrea iredaelei* and *Saccostrea cucullata*. J Therm Biol, 17:135–139

Davis WJ, Mpitos GJ, Siegler MVS, Pinneo JM, Davis KB (1974) Substrates of behavioural hierarchies and associative learning in pleurobranchaea. Am Zool 14:1037-1050

Gaston KJ, Chown SL, Calosi P, Bernado J, Bilton DT, Clarke A, Clusella-Trullas S, Ghalambor CK, Konarzewski M, Peck LS, Porter WP, Pörtner HO, Rezende EL, Schulte PM, Spicer JI, Stillman JH, Terblanche JS, van Kleunen M (2009) Macrophysiology: a conceptual reunification. Am Nat 174:595–612

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248-2251

Grenon, J-F, Walker G (1981) The tenacity of the limpet, Patella vulgata L.: an experimental approach. J Exp Mar Biol Ecol 54:277–308

Hochachka PW, Somero GN (2002) Biochemical adaptation: mechanism and process in physiological evolution. Oxford University Press: New York

Horn PL (1986) Energetics of Chiton pelliserpentis (Quoy & Gaimard, 1835)(Mollusca: Polyplacophera) and the importance of mucus in its energy budget. J Exp Mar Biol Ecol 101:119-141

Huey RB, Kingsolver JG (1989) Evolution of thermal sensitivity of ectotherm performance. TREE 4:131-135

Karl TR, Nicholls N, Gregory J (1997) The coming climate Sci Am ???

Kearney M, Porter WP, Williams C, Ritchie S, Hoffmann AA (2009) Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: the dengue mosquito Aedes aegypti in Australia. Funct Ecol 23:528–538

Lai C-H, Morley SA, Tan KS, Peck LS (2011) Thermal niche separation in two sympatric tropical intertidal Laternula (Bivalvia: Anomalodesmata). J Exp Mar Biol Ecol 405:68-72

Liddiard KJ, Hockridge JG, Macey DJ, Webb J, Van Bronswijk W (2004) Mineralisation in the teeth of the limpets Patelloida alticostata and Scutellastra laticostata (Mollusca : Patellogastropoda). Molluscan Res 24:21-31

Mora C, Moya MF (2006) Effect of the rate of temperature increase of the dynamic method on the heat tolerance of fishes. J Therm Biol 31:337–341

Morley SA, Lemmon V, Obermüller BE, Spicer JI, Clark MS, Peck LS (2011) Duration tenacity: a method for assessing acclimatory capacity of the Antarctic limpet, *Nacella concinna*. J Exp Mar Biol Ecol 399:39–42

Morley SA, Martin SM, Day RW, Ericson J, Lai C-H, Lamare M, Tan KS, Thorne MAS, Peck LS (2012a) Thermal reaction norm and the scale of temperature variation in intertidal Nacellid limpets to climate change. PLoS One 7:e52818

Morley SA, Martin SM, Bates AE, Clark MS, Ericson J, Lamare M, Peck LS (2012b) Spatial and temporal variation in the heat tolerance limits of two abundant Southern Ocean invertebrates. Mar Ecol Prog Ser 450:81-92

Nguyen KDT, Morley SA, Lai C-H, Clark MS, Tan KS, Bates AE, Peck LS (2011) Upper Temperature Limits of Tropical Marine Ectotherms: Global Warming Implications. PloS One 6:e29340

Peck LS (2005) Prospects for survival in the Southern Ocean: vulnerability of benthic species to temperature change. Antarctic Sci 17:497-507

Peck LS (2011) Organisms and responses to environmental change 4:237-243

Peck LS, Webb KE, Bailey D (2004) Extreme sensitivity of biological function to temperature in Antarctic marine species. Funct Ecol 18:625-630

Peck LS, Convey P, Barnes, DKA (2006) Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability. Biol Rev 81:75-109

Peck LS, Webb KE, Clark MS, Miller A, Hill T (2008) Temperature limits to activity, feeding and metabolism in the Antarctic starfish *Odontaster validus*. Mar Ecol Prog Ser 358:181−189

Peck LS, Clark MS, Morley SA, Massey A, Rossetti H (2009) Animal temperature limits and ecological relevance: effects of size, activity and rates of change. Funct Ecol 23:248–256

Peck LS, Morley SA, Richard J, Clark MS (2014) Acclimation and thermal tolerance in Antarcic marine ectotherms. J Exp Biol 217:16-22

Pennisi E (2005) What determines species diversity? Science 309:90

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science 315:95–97

Pörtner HO, Peck LS, Somero GA (2007). Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. Phil Trans Roy Soc 362:2233-2258

Pörtner HO, Farrel AP (2008) Physiology and climate change. Science 322:690-692

Prosser CL (1973) Comparative animal physiology, 3rd edn.Saunders, Philadelphia.

Richard J, Morley SA, Thorne MAS, Peck LS (2012) Estimating Long-Term Survival Temperatures at the Assemblage Level in the Marine Environment: Towards Macrophysiology. PLoS One 7:e34655

Sagarin RD, Barry JP, Gilman SE, Baxter CH (1999) Climate-related change in an intertidal community over short and long time scales. Ecol Monogr 69:465-490

Sanchez-Fernandez D, Aragón P, Bilton DT, Lobo JM (2012) Assessing the congruence of thermal niche estimations derived from distributions and physiological data. A test using diving beetles. PLoS One 7:e48163

Sinclair ELE, Thompson MB, Seebacher F (2006) Phenotypic flexibility in the metabolic response of the limpet *Cellana tramoserica* to thermally different microhabitats. J Exp Mar Biol Ecol 335:131-141

Stillman JH (2003) Acclimation capacity underlies susceptibility to climate change Science 301:65

Sunday JM, Bates AE, Dulvy NK (2011) Global analysis of thermal tolerance and latitude in ectotherms. Proc Roy Soc Lond B 278:1823−1830

Terblanche JS, Deere JA, Clusella-Trullas S, Jannion C, Chown SL (2007) Critical thermal limits depend on methodological context. Proc Roy Soc Lond B 274:2935–2942

Waller CL, Worland MR, Convey P, Barnes DKA (2006) Ecophysiological strategies of Antarctic intertidal invertebrates faced with freezing stress. Pol Biol 29:1077-1083

Zeileis A, Leisch F, Hornik K, Kleiber C (2002). Strucchange: An R Package for testing for structural change in linear regression models. J Stat Soft 7:1–38

Figure legends

Figure 1. Feeding *Nacella concinna*, showing the head and radula. Photo credit: Pete Bucktrout.

Figure 2. The average number of hours each temperature was experienced per 24 hour period was measured by replicate biomimetic loggers (different coloured bars) recording every 30 minutes. A, Rothera *Nacella concinna*, B, *Cellana radiata* and C, *Siphonaria atra*. Note logarithmic scales to emphasise rare high and low temperatures.

Figure 3. Distance moved by individual limpets, A) Rothera *Nacella concinna* at air temperatures between -1 to 9°C, B) *Cellana radiata* at air temperatures between 24 and 33°C , C) *Siphonaria atra* at air temperatures between 25 and 37°C, between successive tides. *C. radiata*, movement was averaged over 19 low tides. *S. atra* and *N.concinna*, averaged over 3 low tides. Median, 5 and 95% percentiles. Solid circles indicate outliers.

Figure 4. The rate of radula rasping at different temperatures (mean ± 1SEM). A, *Nacella concinna*, B, *Cellana radiata*, C, *Siphonaria atra*. Whilst *N. concinna* survived at 8.6°C in 2007 there was no feeding at this temperature. Dotted lines indicate non-significant linear regressions (Table S2).

Figure 5. A, Duration tenacity and B, mortality of *Siphonaria atra*.

Tables

Table 1. Rothera *Nacella concinna* foot temperatures and the 4 logger temperatures at the time of foot measurement.

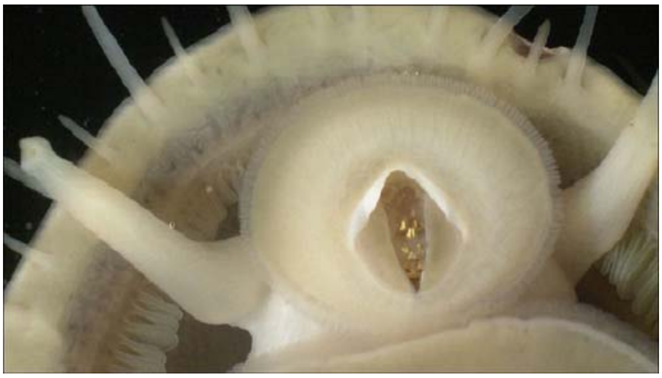
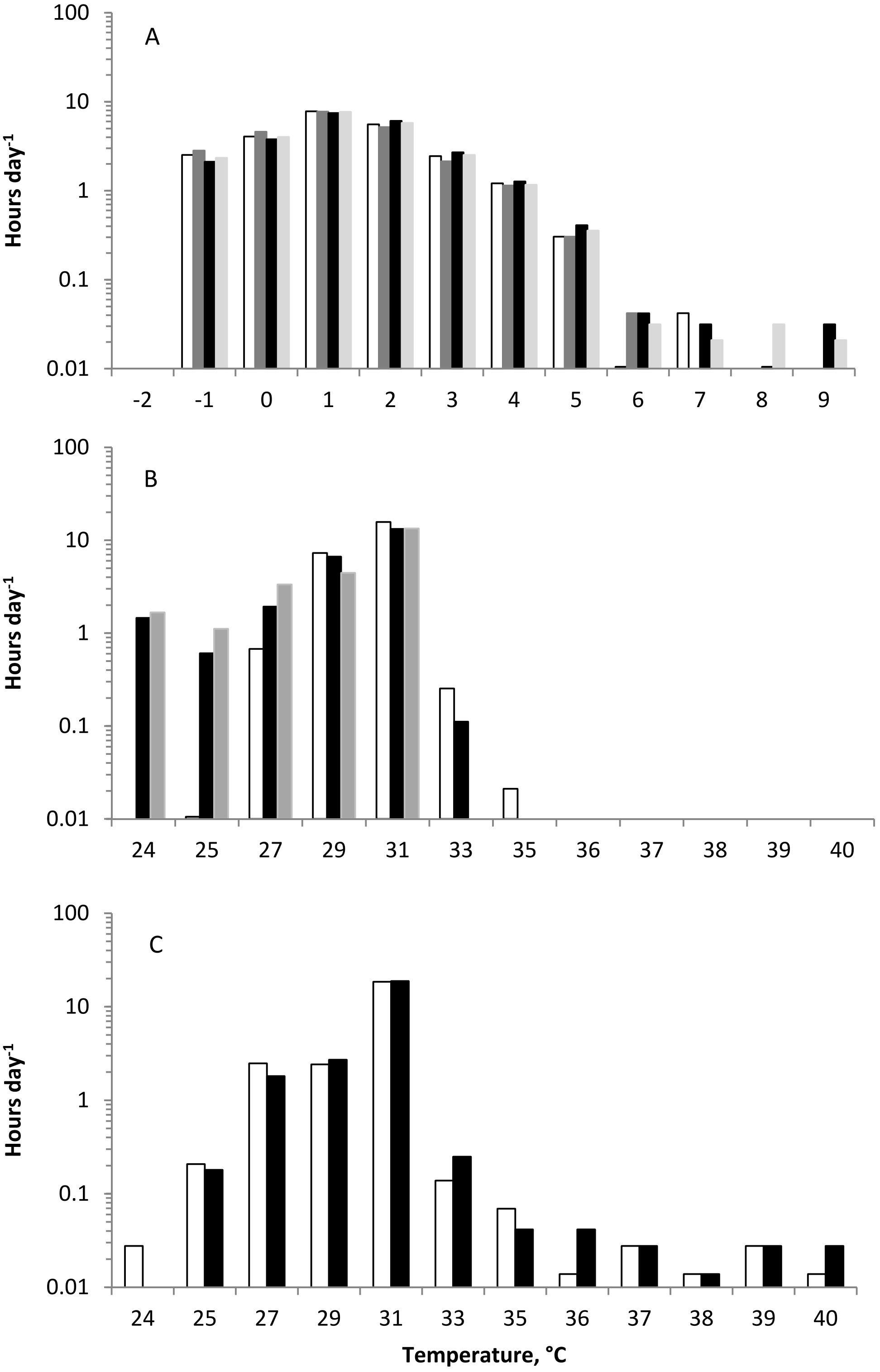


Fig. 1


A
B
C
Hours day^{-1}
100
10
1
0.1
0.01
-2
-1
0
1
2
3
4
5
6
7
8
9
24
25
27
29
31
33
35
36
37
38
39
40
Temperature, °C

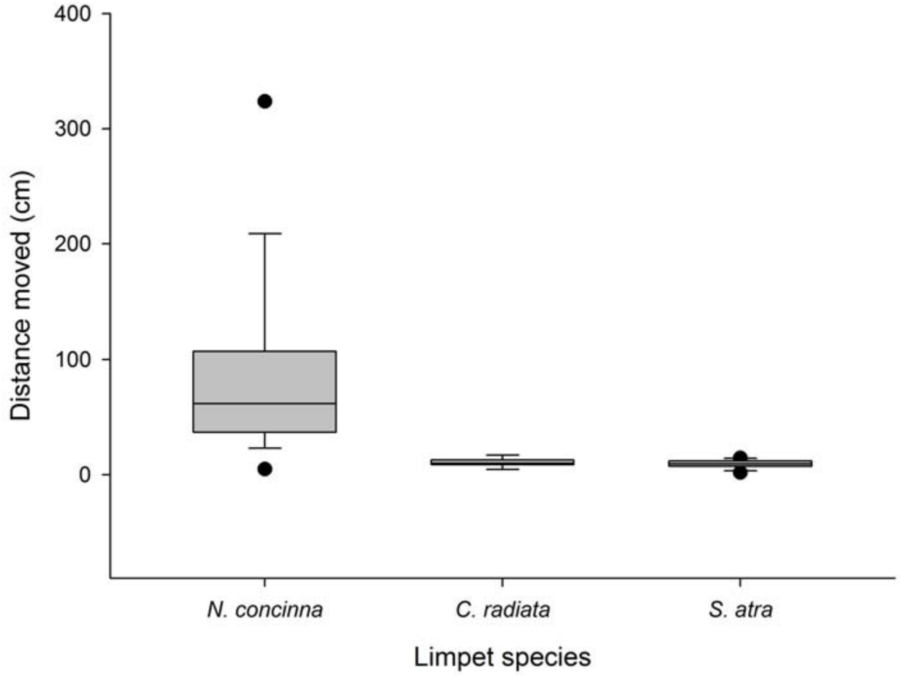
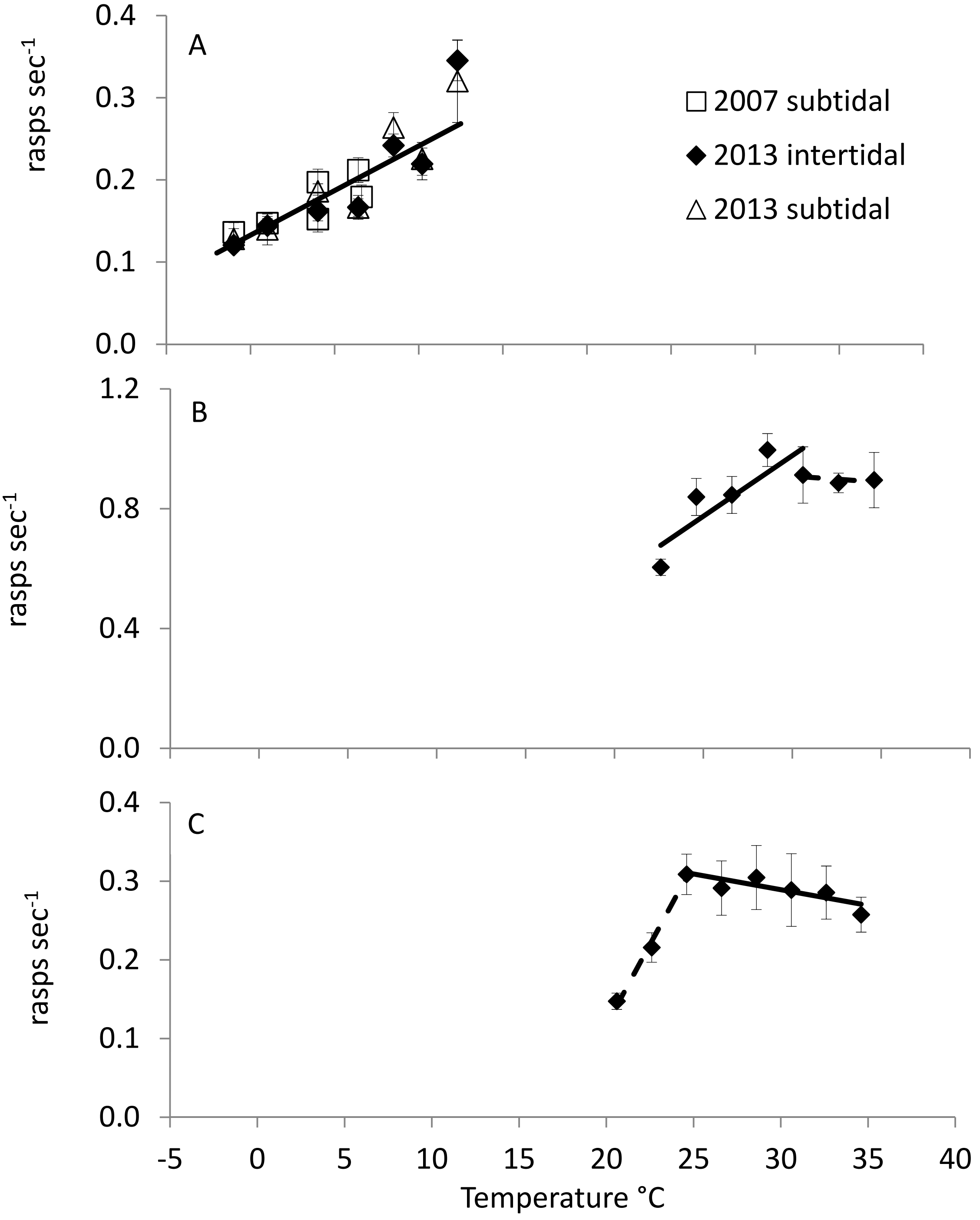

400
300
200
100
0
Distance moved (cm)
N. concinna
C. radiata
S. atra
Limpet species

Fig. 3


0.4
0.3
0.2
0.1
0.0
rasps sec^{-1}
A
□ 2007 subtidal
◆ 2013 intertidal
△ 2013 subtidal
1.2
0.8
0.4
0.0
rasps sec^{-1}
B
0.4
0.3
0.2
0.1
0.0
rasps sec^{-1}
C
-5
0
5
10
15
20
25
30
35
40
Temperature °C

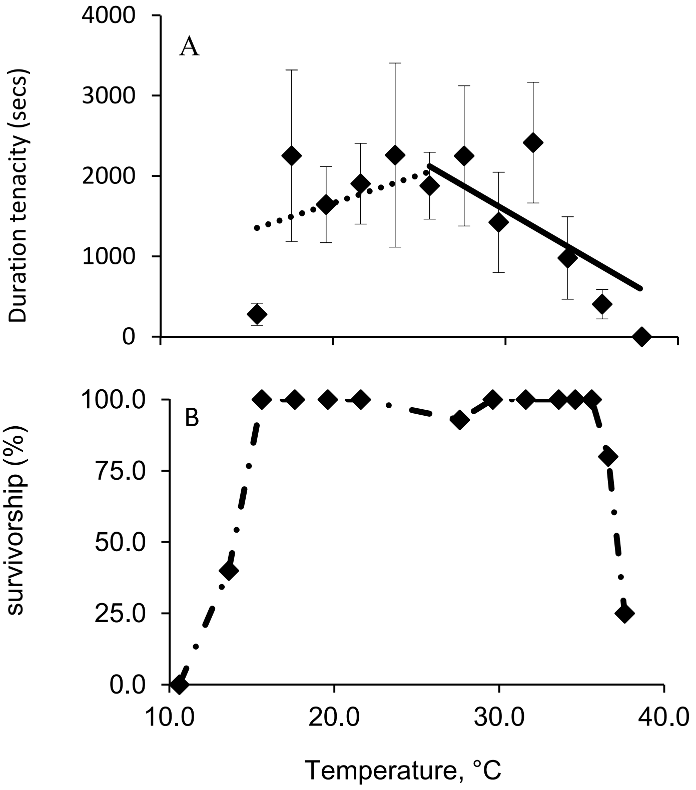
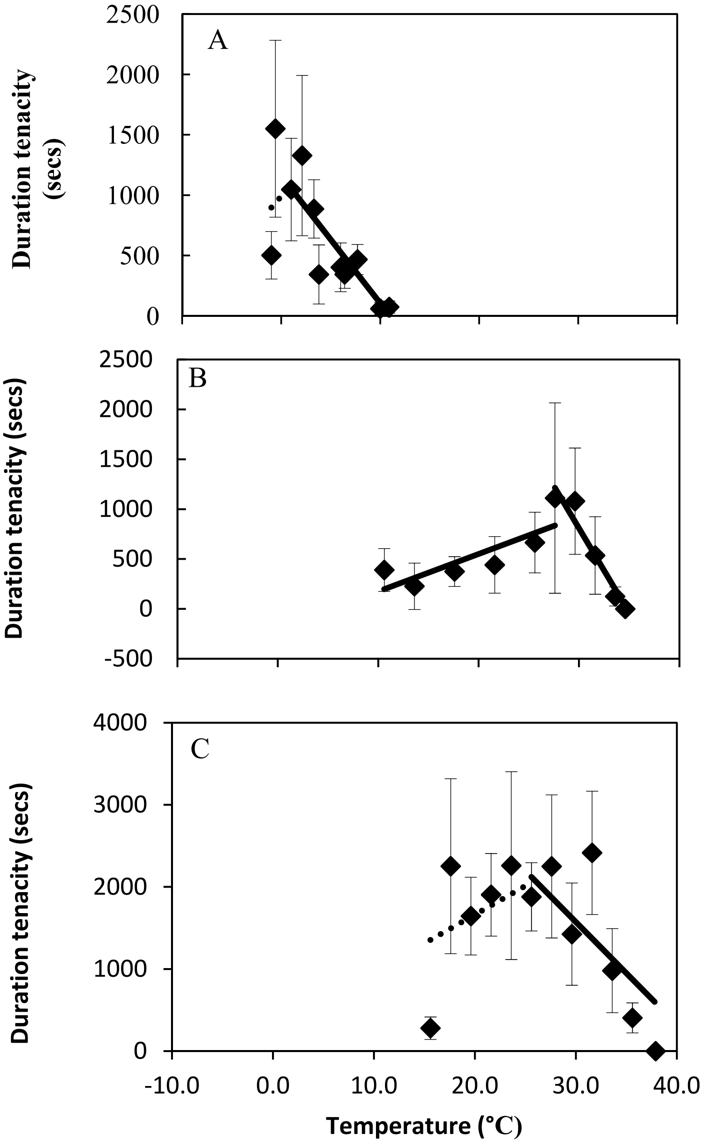

4000
3000
2000
1000
0
A
Duration tenacity (secs)
100.0
75.0
50.0
25.0
0.0
B
survivorship (%)
10.0
20.0
30.0
40.0
Temperature, °C

Table 1. Rothera *Nacella polaris* foot temperatures and the 4 logger temperatures at the time of foot measurement.

		Foot temperature °C			Logger temperature °C
Date		Mean	SEM	N	
28/12/2012	emersed	9.68	0.34	32	-
	partially immersed	8.60	0.10	2	
	Immersed‡	9.45	0.40	26	
31/12/2012	emersed	2.45	0.11	10	3.31 ,4.26, 4.30, 4.04
	immersed	2.00	0.06	3	
16/01/2012	emersed	4.45	2.55	2	2.18, 2.40, 2.23, 2.09
	immersed	2.07	0.28	15	

‡ Immersed limpets were in semi-isolated rock pools in which water could have been heated by solar radiation.


A
Duration tenacity (secs)
2500
2000
1500
1000
500
0
B
Duration tenacity (secs)
2500
2000
1500
1000
500
0
-500
C
Duration tenacity (secs)
4000
3000
2000
1000
0
-10.0
0.0
10.0
20.0
30.0
40.0
Temperature (°C)