Phenotypic plasticity of upper thermal tolerance in marine invertebrates at several hierarchical and geographical scales

by
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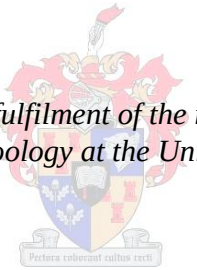

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Abstract

To predict the influence of temperature increases on organisms, and their capacity to respond to climate change, information on the upper thermal tolerance of organisms and its plasticity is required. However, various factors, such as rate of temperature change, may influence basal thermal tolerance and plastic responses, and consequently the vulnerability of organisms to temperature change. Although slower rates of temperature change might be more ecologically relevant, the majority of thermal tolerance studies feature rates of temperature change that are faster than those experienced by organisms in nature. Additionally, studies using slower rates of temperature change have been criticized as factors such as changes in body condition and accumulation of stress over time may confound results. This study determined the influence of fast and slow rates of temperature change and plasticity, induced by acclimation to different temperature conditions for 5 – 15 days, on the upper thermal tolerance of marine amphipod and isopod species from sub-Antarctic Marion Island and South Africa. Using congeners, intra- and inter-specific comparisons of the upper thermal tolerance and plasticity of these organisms were made across geographical regions (South Africa vs. Marion Island), across sites within regions (West coast vs. South coast of South Africa) and between tidal zones. Overall, lower rates of temperature change were found to be associated with lower values of upper thermal tolerance. At fast rates of temperature change, acclimation at high temperatures was associated with an increase in thermal tolerance, whereas at slow rates, acclimation to higher temperatures either had no effect or caused a decrease in thermal tolerance. Furthermore, microclimate recordings suggest that these organisms likely experience rates of temperature increase that are intermediate between the fast and slow rates employed in this study. Thus, in nature these marine invertebrates have upper thermal tolerances that are higher than mean environmental temperature and can likely mount plastic responses during short-term temperature variation. At slow rates of temperature change, however, the upper thermal tolerance of these organisms approximates environmental temperature and plasticity is reduced, likely increasing vulnerability to high temperatures. At the intra-specific level, upper thermal tolerance and plasticity response varied based on mass and sex, however, these effects were species-specific. Isopods inhabiting cooler but more variable microsites on the West coast of South Africa had a higher upper thermal tolerance, but similar magnitude of plasticity, than a population of the same species from the warmer, less variable South coast. Generally, Marion Island species had a lower upper thermal tolerance but higher magnitude of plasticity than South African species. The variability reported here at different hierarchical and geographical scales could be linked to the distinct thermal environments experienced, and the differing physiological and behavioural responses

of populations and species to their thermal environments. This variation in thermal tolerance might be critical during environmental change and suggests that species composition may be altered in the future.

Opsomming

Om die invloed van temperatuur verhogings op organismes, en hul vermoë om te reageer op hierdie verandering, te voorspel, word inligting oor hoë temperatuur verdraagsaamheid van organismes en die plastisiteit hiervan, benodig. Verskeie faktore, soos die tempo van verandering, kan egter basale termiese verdraagsaamheid en plastiese reaksies beïnvloed. Dus, mag dit die tasbaarheid vir temperatuur verandering beïnvloed. Alhoewel stadiger tempo van verandering meer ekologies relevant kan wees, fokus die meerderheid van warm verdraagsaamheid studies op temperatuur veranderinge wat vinniger gebeur as wat ervaar word deur organismes in die natuur. Boonop word studies wat fokus op stadige veranderinge in temperatuur, gekritiseer omdat faktore soos ‘n verandering in liggaamstoestand en die opeenhoping van stres, potentieël die resultate kan belemmer. Hierdie studie ondersoek die invloed van vinnige en stadige temperatuur veranderinge en die plastisiteit, geïnduseer deur akklimasie, met betrekking tot verskeie temperature vir 5-15 dae. Ons fokus spesifiek op die hoë temperatuur verdraagsaamheid van mariene amphipod- en isopod spesies van sub-Antarktiese Marion Island en Suid Afrika. Deur gebruik te maak van spesies wat aan dieselfde genus behoort, is vergelykings getrek tussen intra- en inter-spesies verbande met betrekking tot hul termiese verdraagsaamheid en plastisiteit. Die studie is oor geografiese streke (Suid Afrika vs. Marion Island), tussen areas binne ‘n geografiese streek (Weskus vs. Suidkus van Suid Afrika) en tussen gety sones voltooi. Oor die algemeen was stadige temperatuur veranderinge geassosieër met ‘n laer termiese verdraagsaamheid vir hoë temperature. Met vinnige veranderinge in klimaat, was akklimasie by hoë temperature geassosieër met ‘n hoër temperatuur limiet, maar by stadige temperatuur veranderinge het akklimasie by hoë temperature geen effek gehad nie, of het ‘n afname in termiese verdraagsaamheid veroorsaak. Verder het mikroklimaat opnames aangedui dat hierdie organismes waarskynlik temperatuur verhogings ondervind in hul natuurlike habitat, wat intermediêre is van die vinnige en stadige veranderinge wat in hierdie studie gebruik is. Dus, in die natuur, het hierdie mariene invertebrate `n boonste termiese toleransies wat hoër is as die gemiddelde omgewingstemperatuur en kan hulle waarskynlik van platiese reaksies gebruik maak tydens kort-termyn temperatuur variasie. Gedurende stadige temperatuur veranderinge toon hulle alhoewel hoë termiese verdraagsaamheid teenoor die omgewingstemperature en plastisiteit is verminder, wat heel waarskynlik toenemende kwesbaarheid vir hoë temperature tot gevolg het. Op die intra-spesifieke vlak was wisseling in hoë termiese verdraagsaamheid gebaseer op liggaams massa en geslag, maar hierdie veskille was spesie-spesifiek. Isopoda wat koeler areas bewoon, met meer variasie in hul mikroklimaat, soos ondervind in die Weskus van Suid Afrika, het `n hoër termiese

verdraagsaamheid. Maar, soortgelyke mate van plastisiteit, as 'n populasie van dieselfde spesie van die warmer, minder veranderlike Suidkus. Oor die algemeen het Marion-eiland spesies 'n laer termiese verdraagsaamheid, maar hoër grootte van plastisiteit as Suid-Afrikaanse spesies. Die veranderlikheid wat hier geraporteer is, kan op verskeie hiërargiese en geografiese vlakke gekoppel wees aan die unieke termiese omgewings wat hierdie organismes ervaar en aan die verskillende fisiologiese- en gedrags reaksies van populasies en spesies tot hulle termiese omgewings. Die variasie in termiese verdraagsaamheid kan krities wees tydens omgewingsverandering en dui daarop dat spesie-samestelling kan verander in die toekoms.

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Chapter 1

General Introduction

Organisms are expected to respond to climate change in three ways, by dispersing to favourable habitats, adapting to new conditions through evolutionary processes, and/or tolerating new conditions through phenotypic plasticity (Chevin et al., 2010; Hofmann and Todgham, 2010). As the changes to climate are expected to continue and possibly escalate in the future, predictions of how organisms will be influenced and respond to these changes are needed (Helmuth et al., 2005; Solomon et al., 2007; Helmuth, 2009). Currently, the majority of such predictions are made using correlative models that relate large-scale environmental data and species presence to predict geographical distribution shifts through the use of climate projections (Peterson et al., 2002; Thuiller et al., 2005; Huntley et al., 2008; Wiens et al., 2009; Ackerly et al., 2010). However, mechanistic models that can include information on phenotypic traits (e.g. morphology, physiology, phenology and behaviour) and the sensitivity of organisms to on-going change have more recently been developed (Helmuth et al., 2005; Kearney, 2006; Helmuth, 2009; Chevin et al., 2010). Species distributions modelled using a mechanistic approach can be estimated with more confidence when predicting novel circumstances than those modelled using correlative methods (Duncan et al., 2009; Kearney and Porter, 2009; Buckley et al., 2010).

As land and sea surface temperatures are expected to continue to increase in the future (Solomon et al., 2007), studies on the sensitivity, plasticity and adaptability of organisms to increased temperature are essential, and need to be incorporated into these predictive models. This study aimed to evaluate the upper thermal tolerance of several marine invertebrate species from South Africa and Marion Island. Furthermore, the sensitivity of these organisms to temperature change as well as their capacity to respond through phenotypic plasticity was assessed. As these factors may be influenced by rate of temperature change (Terblanche et al., 2007; Chown et al., 2009; Peck et al., 2009; Mitchell and Hoffmann, 2010), the effects of fast and slow rates were evaluated. Moreover, as variation between populations and species may be essential to the persistence of organisms through the predicted change (Chown et al., 2010b; Somero, 2010), thermal tolerance and plasticity responses were assessed at various geographical and hierarchical scales. The information gained from this study will be valuable, as marine invertebrates may be particularly vulnerable to increases in temperature, and as the thermal tolerances of these organisms have been little studied in the Southern Hemisphere (Feder and Hofmann, 1999; Stillman, 2003).

This chapter reviews the relevant background literature for this study. First, the mechanisms underlying thermal tolerance limits and the response of organisms to temperature

change are discussed briefly. Second, the methodologies used to investigate such mechanisms, as well as the various confounding factors that need to be accounted for are examined. Third, thermal tolerance patterns that have been identified within and among species in other studies are discussed. Finally, background information on the climate and oceanographic patterns for this study's collection sites is provided.

Mechanisms and limiting factors of upper thermal tolerance

All physiological systems function optimally within a specific range of temperatures. However, some systems are more thermally sensitive or constrained than others, and thus play an important role in setting thermal limits (Hochachka and Somero, 2002). Cellular membranes are particularly sensitive to temperature changes (alterations to membrane permeability, enzymatic activity, and the capacities for exo- and endocytosis) and some membrane properties, e.g. synaptic transmission, are generally more sensitive than others (Hochachka and Somero, 2002; Hofmann and Todgham, 2010). For example, synaptic failure has been found to contribute to the heat death of Antarctic Notothenioid fish, which cannot survive at temperatures higher than 4°C (Hochachka and Somero, 2002).

The membranes of intracellular organelles are involved in the transport of macromolecules and are, therefore, thought to be particularly thermally unstable (Pörtner, 2001; 2002). The cells of single-celled eukaryotes and multi-cellular organisms are both characterised by the presence of intracellular organelles (Pörtner, 2001; 2002). However, multi-cellular organisms have narrower thermal tolerance windows than single-celled eukaryotes (Pörtner, 2001; 2002). It has thus been argued that membrane stability does not determine thermal tolerance limits (Pörtner, 2001; 2002). Instead, the greater functional and organisational complexities of multi-cellular organisms are associated with increases in metabolic rate and organism performance, which in turn increase thermal sensitivity. Pörtner (2001; 2002) argued that thermal limits are set at the highest functional level of the organism: for eukaryotes, limits are set at the level of the organelle, while for multi-cellular organisms limits are set by ventilatory and circulatory systems. This hierarchy is also demonstrated within multi-cellular organisms, and the thermal sensitivity of cellular and molecular functioning is less than that of the organism as a whole (Pörtner, 2002).

Data obtained on water and air breathing marine ectotherms suggest that oxygen supply failure may be an underlying mechanism that sets upper and lower thermal tolerance limits (the 'oxygen limitation hypothesis'; Pörtner, 2002; Peck, 2005; Pörtner and Farrell, 2008).

Warming causes the metabolic rate of marine ectotherms to increase, and leads to a rise in oxygen demand (Peck, 2005; Pörtner et al., 2006). As warming causes a decrease in oxygen solubility and negatively affects the efficiency of oxygen supply systems (i.e. limits set by the maximum functioning rate of circulatory systems), the oxygen demand may not be met (Peck, 2005; Boutet et al., 2009). Symmorphosis is the concept that the structural systems within an organism are designed in proportion with functional requirements, and are regulated to satisfy but not exceed requirements (Taylor and Weibel, 1981). The declining efficiency of oxygen supply systems may thus be due to their design, which satisfies functional requirements within an optimal thermal range, but does not exceed them (Pörtner, 2002).

Various thresholds have been identified in this process, and the temperature range at which oxygen supply to tissues begins to decline and body fluids start to become hypoxic, has been named the 'pejus temperature' (Pörtner, 2001; Pörtner et al., 2006). 'Critical temperatures' (T_c) represent the temperature threshold at which mitochondria, due to decreased oxygen supply to the tissues, receive less oxygen than is required (Pörtner, 2001). The organism as a whole will then demonstrate a decreased aerobic scope (the range between the minimum and maximum oxygen consumption levels) and a transition from aerobic to anaerobic metabolism will occur (Pörtner, 2001; 2002; Peck, 2005; Pörtner et al., 2006). When T_cs are reached, cellular energy levels become progressively insufficient and the bi-products of anaerobic metabolism (e.g. succinate) build up in the tissues. Anaerobiosis cannot be tolerated indefinitely, and survival becomes time limited; continued temperature increases will cause the ventilatory and circulatory systems to collapse (Pörtner, 2002; Pörtner et al., 2006; Peck et al., 2007). 'Critical temperatures' differ across populations and species (Pörtner, 2001). An inter-specific comparison showed that a population of the Antarctic eelpout *Pachycara brachycephalum* had an upper T_c of 9°C while that of the North Sea eelpout *Zoarces viviparus* was 23°C (van Dijk et al., 1999). Different species inhabiting the same geographical region also showed variation. The upper limit of the Antarctic bivalve *Limopsis marionensis* was significantly lower than that reported for the Antarctic eelpout *P. brachycephalum* (Pörtner, 2001). Therefore, variation in critical temperatures may be related to the environmental temperatures experienced by organisms (microsites) as well as phylogenetic relationships. Moreover, as environmental temperatures vary temporally (e.g. seasonally) and spatially (e.g. latitudinally), the T_c of populations and species may covary with these temperature patterns.

Peck et al. (2007) argued that the oxygen limitation hypothesis is supported if an increase in oxygen concentration is paralleled with an increase in lethal or critical temperatures. This

result was observed in a study on the bivalve *Laternula elliptica*, which indicated that increased oxygen concentrations resulted in a higher proportion of individuals reburying in sediment at high temperatures (Peck et al., 2007). By contrast, the upper and lower thermal limits of the terrestrial beetle *Tenebrio molitor* and isopod *Porcellio scaber* were not affected by ambient oxygen concentrations (Stevens et al., 2010). Additionally, hypoxia did not cause a reduction in the thermal range of these organisms (Stevens et al., 2010). These differing results indicate that oxygen limitation may not set the thermal limits of all organisms.

Ectotherms have various compensatory mechanisms that enhance survival under natural temperature fluctuations, e.g. tidal regimes for inter-tidal species (Gourgou et al., 2010). These mechanisms include behavioural adjustments and physiological responses such as metabolic compensation and the expression of cytoprotective proteins (i.e. heat shock proteins) (Clark et al., 2008; Gourgou et al., 2010). The cell damage caused by temperature extremes can in some instances be counteracted or mitigated by these molecular mechanisms, and thus increase the chance of organism survival (Clark et al., 2008; Gourgou et al., 2010).

Heat shock proteins

The main molecular response to extreme temperature conditions is the increased production of stress proteins, e.g. heat shock proteins (HSP's) (Bowler, 2005; Clark et al., 2008). Some HSPs, but not all, act as molecular chaperones that aid in the stabilisation and refolding of denatured proteins, and prevent the formation of cytotoxic aggregates (Feder and Hofmann, 1999; Bowler, 2005; Clark et al., 2008). As heat shock causes the unfolding or the incomplete/improper folding of proteins, the functioning of molecular chaperones after a sub-lethal heat exposure can cause an increase in the resistance of cells to heat stress (Schlesinge, 1990; Feder and Hofmann, 1999; Dong et al., 2010). HSPs, based on their molecular weight and sequence homology, have been designated into families (Feder and Hofmann, 1999; Bowler, 2005). Although proteins from the most common HSP family, HSP70, are frequently expressed in response to increased temperature, HSPs can also be induced by a variety of other stress factors (e.g. cellular energy depletion, extreme concentrations of ions, other osmolytes, gases and various toxic substances) (Schlesinge, 1990; Feder and Hofmann, 1999; Bowler, 2005; Clark et al., 2008).

HSPs, and the genes that code for them, have been found in all major phylogenetic lineages (Clark et al., 2008; Dong et al., 2010). As HSP expression reflects the evolutionary histories of species and the recent thermal conditions experienced by individuals, their

induction and expression are variable (Clark and Peck, 2009; Dong et al., 2010). Intra- and inter-specific differences as well as temporal and spatial patterns have been demonstrated (Feder and Hofmann, 1999; Fangue et al., 2006). Inter-specific differences in HSP response have been demonstrated in Antarctic organisms. For example, Antarctic Notothenioid fish permanently express HSPs but due to mutations do not demonstrate a heat-shock response (Clark and Peck, 2009). By contrast, the Antarctic marine molluscs *Nacella concinna* and *Laternula elliptica* do demonstrate HSP responses, but HSP up-regulation temperatures differ between the species (Clark et al., 2008). Intra-specific differences in HSP response are evident between populations of the European flounder *Platichthys flesus* (Hemmer-Hansen et al., 2007), as well as the colour variants of the sea cucumber *Apostichopus japonicus* (Dong et al., 2010). Furthermore, HSP expression is highly costly (HSP degradation and synthesis may occur to the detriment of cell energy and nutrient stores) and at high concentrations cytogenic effects may interfere with cell functions and processes (Feder and Hofmann, 1999; Clark et al., 2008; Clark and Peck, 2009). Small increases in HSP70 expression, for example, cause increased tolerance in *Drosophila* flies, while large increases cause tolerance to decline (Feder and Hofmann, 1999). Variation in HSP expression may thus be due to the trade-offs under which this response to temperature extremes evolved (Clark and Peck, 2009).

Metabolic alteration

Metabolic depression is an alternative mechanism that may ensure survival when organisms are exposed to extreme temperatures (Anestis et al., 2010). Changes to metabolism may occur through alterations to the density or functional properties of mitochondria (molecular and membrane levels), which in turn affect the functioning of cells, tissues and central control systems, e.g. nervous, circulation and respiration systems (Pörtner, 2002). At the northern limit of its distributional range (South Georgia), *Nacella concinna* has fewer but more efficient mitochondria than further south (Morley et al., 2009). Although evidence exists for this mechanism to maintain system stability under extreme environmental temperatures, changes to mitochondrial levels are energetically expensive, and trade-offs may negatively influence growth, fecundity and recruitment (Pörtner, 2002).

As temperature also affects metabolic enzyme activities, ectotherms may alter their metabolism by making qualitative or quantitative changes to enzyme functioning (Hochachka and Somero, 2002; Doucet-Beaupré et al., 2010). These changes include alterations to the rate of transcription and enzyme concentrations, which may be altered through the expression of allozymes and isozymes with differing thermal sensitivities (Doucet-Beaupré et al., 2010).

Changes to enzyme activity may allow for seasonal acclimatization, and thus lead to increased thermal tolerance (Fitt et al., 2001). For example, the enzyme activities of the freshwater mussels *Pyganodon fragilis* and *P. grandis*, and the marine mussel *Mytilus galloprovincialis* are positively related to temperature (Doucet-Beaupré et al., 2010). Enzyme replacement (isozymes) or protection has also been recorded in organisms (e.g. corals) and may enable normal functioning during seasonal temperature fluctuations (Fitt et al., 2001). Although evidence exists that the alteration of metabolic functioning plays a role in balancing temperature-induced increases in energy demand, the energetic costs associated with such changes may cause a decrease in the aerobic scope for activity (Anestis et al., 2010).

Tests and evaluation criteria of upper thermal limits

Various criteria may be employed to determine the upper thermal tolerances of organisms (Hoffmann et al., 2003; Chown and Nicolson, 2004; Terblanche et al., 2007). Lethal temperature is typically determined by exposing organisms to different temperature treatments for a set period of time (Beitinger et al., 2000; Hoffmann et al., 2003; Chown and Nicolson, 2004; Terblanche et al., 2007). These tests (ULTs) provide survival response curves from which the upper lethal temperature limit of a given percentage of the population can be determined (e.g. 50% for ULT_{50}) (Beitinger et al., 2000). By contrast, the temperature at which an organism loses the ability to respond in an ecologically relevant manner (e.g. locomotion ability, retraction of appendages), is determined by heating individuals at a specific rate until pre-determined responses are recorded (Beitinger et al., 2000; Chown and Nicolson, 2004; Terblanche et al., 2007). Generally, the temperature at which an organism's locomotory activity becomes disorganised, e.g. a loss of coordinated movement or righting response, is designated the 'critical thermal maximum' (CT_{max}) (Cowles and Bogert, 1944; Lutterschmidt and Hutchison, 1997; Beitinger et al., 2000; Terblanche et al., 2007). This end-point generally represents the temperature at which organisms can no longer escape a predator or behaviourally thermoregulate, and thus affects organism fitness (Cowles and Bogert, 1944; Lutterschmidt and Hutchison, 1997; Terblanche et al., 2007). Although both ULTs and CT_{max} are used to assess thermal tolerance limits, as the protocols (static versus ramping) and resulting end-points generally differ, the results obtained likely reflect differences in genetic and physiological mechanisms that underlie organism response (Chown and Nicolson, 2004; Sørensen et al., 2005; Sgrò et al., 2010).

In ULT_{50} tests, organisms can be directly (e.g. Marsden, 1985; Urban, 1994; Dong et al., 2010) or gradually (e.g. Buchanan et al., 1988; Gaston and Spicer, 1998; Miller et al., 2009)

exposed to pre-determined test temperatures. These protocols are respectively known as static and dynamic ULT's and the protocol employed may influence results. For example, the upper lethal tolerance of *Daphnia magna* is higher if a continuous heating protocol is followed than after a 24 hour constant temperature exposure (Kivivuori and Lahdes, 1996). By contrast, the freshwater isopod *Lirceus brachyurus* had similar LT_{50} results under both direct and gradual exposures (Cheper, 1980). The period of exposure may also influence results and longer exposure periods generally result in a decline in LT_{50} (Chown and Nicolson, 2004; Peck et al., 2004). This result was demonstrated in South American freshwater invertebrates (Quinn et al., 1994) and South African bivalve and gastropod species (Ansell and McLachlan, 1980). In the literature, period of exposure varies greatly from study to study. Two hour exposures were employed in tests of the sea cucumber *Apostichopus japonicus* (Dong et al., 2010) and the limpets *Nacella concinna* and *Kerguelenella lateralis* (Davenport, 1997), while a 24 hour exposure was utilised in tests of various South American bivalves (Urban, 1994).

In CT_{max} tests, the criteria for performance failure must be pre-determined and may vary between species and studies, e.g. loss of righting response (see Mundahl, 1989; McGaw, 2003; Re et al., 2005; 2006) or the onset of muscle spasms (see Zakhartsev et al., 2003). In the freshwater isopod *Asellus aquaticus*, differing temperature limits were recorded for the failure of righting response and the cessation of pleopod respiratory movements (Korhonen and Lagerspetz, 1996). Experimental protocol may also influence results (Chown and Nicolson, 2004; Terblanche et al., 2007). The experimental start temperature and rate of temperature change may have a direct or, through influences on exposure time, an indirect effect on critical limits (Terblanche et al., 2007). Slow rates, or a start temperature that deviates greatly from CT_{max}, could increase exposure time and either cause a stress-induced decrease in thermal tolerance or, through acclimation, an increase in thermal limits (Terblanche et al., 2007). For example, rate of temperature change was positively correlated with thermal tolerance in *Drosophila melanogaster* (Chown et al., 2009), the tsetse fly *Glossina pallidipes* (Terblanche et al., 2007), the argentine ant *Linepithema humile* (Chown et al., 2009) and various Antarctic marine invertebrate species (Peck et al., 2009). By contrast, in other studies on *Drosophila melanogaster* (Kelty and Lee, 1999; Overgaard et al., 2006) and the aphid *Sitobion avenae* (Powell and Bale, 2006), a decrease in rate of temperature change was associated with an increase in thermal tolerance. The effect of rate of change may thus reflect a trade-off between the amount of time available for acclimation and the period spent at near lethal temperatures (Cocking, 1959; Beitinger et al., 2000; Peck et al., 2009). However, it has been argued that the collinearity of various factors (e.g. captivity period and

energy expenditure) with temperature at slow-rates of change may confound CT_{max} results (Rezende et al., 2011). For example, the starvation resistance of *Drosophila melanogaster* was negatively related to temperature, and thus the CT_{max} results obtained from slow-rate trials may be due to a decrease in the organism's physical condition, and not exclusively to temperature effects (Rezende et al., 2011). However, factors such as desiccation and starvation resistance are highly context- and species dependent (e.g. food deprivation during trials, species body size; Terblanche et al., 2011). Despite the recent concerns raised by Rezende and colleagues, studies employing slow rates of change are still highly valuable given the large impact rate of change may have on CT_{max}, and the potential implications this impact may have on the predictions of species response to environmental change. Furthermore, these potential confounding factors may be controlled for, and taken into account during statistical analyses.

Thermal performance curves

Performance curves indicate the relationship between the environment and a demographic parameter or trait closely related to this parameter (Huey and Kingsolver, 1989; Chown et al., 2010a). For example, locomotion performance is evaluated and plotted over a broad range of temperatures. Thermal performance curves provide estimates of the 'optimal temperature' T_o (the temperature at which an organism performance is optimized), 'performance breadth' (the temperature range in which an organism performs above a certain level) and 'tolerance zone' (the temperatures that bound performance or life) of an organism (Huey and Kingsolver, 1989; Angilletta et al., 2002). Thermal performance curves generally rise gradually with increasing temperature from the 'critical thermal minimum' (CT_{min}) to T_o, and then decrease rapidly to CT_{max} (Huey and Kingsolver, 1989). The form of thermal performance curves is related to environmental variability both within and between generations (Angilletta et al., 2002; Chown et al., 2010a). From a theoretical view (i.e. modelling performance traits that affect survivorship), organisms living in highly variable environments should have broader thermal tolerance curves than those inhabiting areas with less thermal variability (Angilletta, 2009). However, in some organisms the predictability of the environmental change may play a greater role than the magnitude of temperature variation experienced (Chown et al., 2010a).

Thermal performance curves can be used to predict the influence of increased temperature on organism fitness (Deutsch et al., 2008). The impact of warming will depend on a) the breadth of the curve, b) the position of the performance curve in relation to mean climate, and c) the amount of local temperature variation (Deutsch et al., 2008). An organism's 'warming

tolerance' is the difference between CT_{max} and current environmental temperature (T_{hab}), and is an estimation of the amount of warming an organism can experience before performance decreases to fatal levels (Deutsch et al., 2008). An organism's 'thermal safety margin', is the difference between T_o and T_{hab} (Deutsch et al., 2008). Organisms living in environments with temperatures close to their physiological temperature optima have small 'thermal safety margins', and small increases in temperature may negatively affect their performance (Deutsch et al., 2008).

The influence of environmental temperature variation on the shape of thermal performance curves is evident in latitudinal trends. Tropical species that live in relatively thermally stable environments have, in general, a narrower temperature tolerance than temperate species that live in more variable environments (e.g. Huey and Kingsolver, 1989; Addo-Bediako et al., 2000). Tropical insects generally have a lower 'warming tolerance' and smaller 'thermal safety margin' than those from mid- and high latitude regions (Deutsch et al., 2008; Tewksbury et al., 2008). Deutsch et al. (2008), therefore, predicted that tropical insects should be negatively affected by small amounts of warming, while mid- and high latitude species may initially be positively influenced by warming. It must be noted, however, that this study did not account for phenotypic plasticity in the form of acclimation responses. Moreover, when climate variables such as precipitation and temperature variation were used to predict the preferred body temperature and the CT_{max} of squamate reptiles, the 'thermal safety margin' of these organisms at mid-latitudes was smaller than that of tropical species, making the former more vulnerable to warming (Clusella-Trullas et al., 2011).

Phenotypic plasticity

Phenotypic plasticity is the ability of a genotype to produce various phenotypes when exposed to different environmental conditions (Miner et al., 2005; Garland and Kelly, 2006; Ghalambor et al., 2007; Reed et al., 2010). This variation can occur in response to various types of environmental stress (e.g. temperature and oxygen tension) and may result in changes to behaviour, physiology, morphology, life history, growth and demography (Schmidt-Nielsen, 2004; Gabriel et al., 2005; Miner et al., 2005; Chevin et al., 2010). This process can act within an individual's lifetime and may be heritable (Garland and Kelly, 2006). Phenotypic plasticity may be adaptive and increase an organism's fitness under new environmental conditions, or may have negative or negligible effects on fitness and thus be maladaptive or neutral (Hughes et al., 2003; Garland and Kelly, 2006; Ghalambor et al., 2007). Adaptive plasticity may cause organism performance to be close to optimum levels in

new environmental conditions, and thereby slow or constrain adaptive divergence between the original population and that in the new environment (Ghalambor et al., 2007; Chown et al., 2010a). However, if adaptive plasticity produces a phenotype that comes close but does not meet optimum requirements, organisms may persist and adaptation may occur through strong directional selection (Ghalambor et al., 2007; Chown et al., 2010a). Moreover, non-adaptive plasticity may produce a phenotype that is far from optimum or add substantial variance to the mean (Ghalambor et al., 2007; Chown et al., 2010a). Theoretical models have shown that adaptive plasticity will be present if certain criteria are met: a) sufficient genetic variation is present, b) organisms live in variable environments, c) environments produce reliable cues, d) selection favours different phenotypes in each environment and e) no one phenotype has superior fitness in all environments (Chown and Terblanche, 2007; Ghalambor et al., 2007). Fitness costs associated with the production and maintenance of plasticity may prevent its development, and in stressful environments there may be a trade-off between having a genotype that produces one phenotype ('canalisation'), or one that produces many, possibly adaptive phenotypes (Hughes et al., 2003; Ghalambor et al., 2007). Patterns of plasticity variation have been recognised at the intra- and inter-specific levels (e.g. Garland and Kelly, 2006; Chown and Terblanche, 2007; Ghalambor et al., 2007; Angilletta, 2009). In general, phenotypic plasticity is reduced in populations that have high gene flow and in organisms that demonstrate behavioural responses to change (Ghalambor et al., 2007; Chown et al., 2010a). For example, tropical and polar species that live in relatively stable environmental conditions demonstrate less plasticity than mid-latitude species, while those that have wide tolerance ranges may also exhibit reduced plasticity (Stillman, 2003; Tewksbury et al., 2008; Chown et al., 2010a).

Acclimation and acclimatization are forms of phenotypic plasticity which reflect physiological variation resulting from pre-exposures (relatively long as opposed to acute) to a set of environmental conditions. Acclimation is a response that occurs in controlled laboratory experiments, in which only the variable of interest is altered (Hochachka and Somero, 2002; Schmidt-Nielsen, 2004). For example, in the case of thermal acclimation, responses after exposure to several temperature regimes are assessed. Acclimatization occurs in the natural environment in which several variables, including that of interest, may change (Hochachka and Somero, 2002; Schmidt-Nielsen, 2004).

Acclimatization

Field-fresh organisms have demonstrated acclimatization to daily and seasonal temperature changes (Mundahl, 1989; Schmidt-Nielsen, 2004; Hopkin et al., 2006). The thermal tolerance of the catfish *Ictalurus [Ameiurus] nebulosus* (Schmidt-Nielsen, 2004) and the crayfish *Orconectes rusticus* (Mundahl, 1989) increased with an elevation in seasonal temperature. Juvenile *O. rusticus* demonstrated cyclic, diel variation in their upper thermal tolerance (Mundahl, 1989). As this response lagged behind daily temperature change it is likely that this species does not anticipate temperature variation, but relies on rapid acclimatization (Mundahl, 1989).

Acclimation

Although acclimation effects assessed in the laboratory may differ from those found in the natural environment, these experiments provide information on the direction and magnitude of the acclimation response and are suitable for comparative purposes (across populations and species) (Peck et al., 2008). Evidently, a closer matching of acclimation treatments with natural conditions, and the consideration of confounding factors (e.g. phylogeny, nutrition status) result in more ecologically-relevant data. Acclimation causes changes to the ability of organisms to persist through temperature change (Bowler, 2005). In general, organisms acclimated to higher temperatures will have higher thermal tolerances than those acclimated to cooler temperatures (Bowler, 2005). This result has been found in organisms such as the crab *Hemigrapsus nudus* (McGaw, 2003), the beachflea *Orchestia gammarellus* (Gaston and Spicer, 1998), the shrimp *Litopenaeus stylirostris* (Re et al., 2006) and the isopod *Asellus aquaticus* (Korhonen and Lagerspetz, 1996). In addition, a specific period of time is required for full acclimation to occur. The required period depends on the temperature of acclimation and varies both within and between species (Buchanan et al., 1988; Schmidt-Nielsen, 2004). Higher acclimation temperatures are generally associated with higher acclimation rates, which has led to the identification of metabolic enzyme changes as a possible underlying mechanism of acclimation (Schmidt-Nielsen, 2004). For example, the catfish *Ictalurus nebulosus* requires 24 hours to fully acclimate to 28 ºC when moved from 20 ºC (Schmidt-Nielsen, 2004). In the isopod *Asellus aquaticus* (Lagerspetz and Bowler, 1993) and the fresh water amphipod *Paramelita nigroculus* (Buchanan et al., 1988) a longer acclimation period was associated with an increase in thermal limits. In *Asellus aquaticus*, acclimation effects were evident after 24 hours but increased further for 4 to 7 days, when a new steady level was attained (Lagerspetz and Bowler, 1993; Korhonen and Lagerspetz, 1996).

Heat hardening

Exposure to sub-lethal temperatures for short periods of time or 'heat shock' can cause a transitory increase in an organism's thermal tolerance (Chown and Nicolson, 2004; Bowler, 2005). This response, called 'heat hardening' is evident in the responses of the isopod *Asellus aquaticus* (Korhonen and Lagerspetz, 1996) and the sea cucumber *Apostichopus japonicus* to extreme temperatures (Dong et al., 2010). 'Heat hardening' differs from the longer term effects of acclimation/acclimatization through its time course and underlying cellular mechanisms (Lagerspetz, 2003; Hopkin et al., 2006). For example, a 'heat hardening' response was evident in *A. aquaticus* one hour after heat shock, but acclimation effects in this species are only apparent after 24 hours (Korhonen and Lagerspetz, 1996). Heat hardening may be associated with the production of HSPs, but may also involve other cellular changes (Bowler, 2005).

Latitudinal trends in temperature tolerance

Due to large-scale environmental variation, such as latitudinal temperature patterns, populations and species living in different geographical regions may demonstrate physiological differences that allow them to function in distinct environments (Osovitz and Hofmann, 2007). In general, in marine species, higher thermal tolerance is found at lower latitudes (Spicer and Gaston, 1999; Stillman, 2003). For example, higher latitude populations of the horseshoe crab *Limulus polyphemus* and the fiddler crab *Uca rapax* have lower upper thermal tolerances than lower latitude populations (Spicer and Gaston, 1999). Similarly, *Petrolisthes gracilis* from the Northern Gulf of California has a higher upper thermal tolerance than its congener *P. cinctipes*, which inhabits the cold-temperate zone of the northeastern Pacific (Stillman, 2003). If differences in thermal tolerance across species and populations do not disappear when acclimated to common temperatures in laboratories, irreversible, genetic differences or 'local genetic adaptations' may be evident (Spicer and Gaston, 1999). Such divergence has been identified in the bivalve *Tellina* sp and amphipod *Orchestia gammarellus* (Gaston and Spicer, 1998; Spicer and Gaston, 1999; Morritt and Ingólfsson, 2000). Not all geographically separated populations or species have differing thermal tolerances, e.g. the eelpout *Zoarces viviparus* (Zakhartsev et al., 2003), and differences may not always be due to genetic adaptation but rather local acclimatization, e.g. an Icelandic *O. gammarellus* population (Spicer and Gaston, 1999; Morritt and Ingólfsson, 2000).

The thermal history of an individual will influence its physiology, and organisms living in stable thermal environments (e.g. low latitudes and poles) may have thermal tolerances set closer to habitat temperature, and/or have a lower acclimation capacity than those from more thermally variable regions (e.g. mid-latitudes) (Spicer and Gaston, 1999; Stillman, 2003; Deutsch et al., 2008; Tewksbury et al., 2008). For example, tropical crab species of the genus *Petrolisthes* have a lower acclimation capacity and an upper thermal tolerance set closer to maximum habitat temperature than their mid-latitude congeners (Stillman, 2003). Furthermore, Antarctic marine species that are exposed to a narrow temperature range (between -1.9 to 1°C) demonstrate poor acclimation ability (Pörtner, 2002; Peck et al., 2009; 2010). Additionally, the predictability of thermal variation may influence the capacity of organisms to respond to change through acclimation (Chown et al., 2010b). Organisms living in highly unpredictable environments may have a reduced acclimation capacity in comparison to those inhabiting more predictably variable environments (Chown et al., 2010b). For example, a lack of acclimation response in the mites *Halozetes fulvus* and *Podacarus auberti* that inhabit sub-Antarctic Marion Island was attributed the island's unpredictable environmental temperatures (Deere and Chown, 2006).

Microclimates

At a point in time and space, an organism will only experience its immediate environmental vicinity and may not be influenced by the large-scale temperature data which are often used to make predictions (Spicer and Gaston, 1999; Helmuth, 2009). The temperature tolerance of species or populations may instead be dependent on the availability of microclimates (Spicer and Gaston, 1999; Helmuth, 2009). Inter-tidal zones exhibit large variations in temperature over small spatial scales (Spicer and Gaston, 1999). Organisms living higher up on the shore are exposed for longer periods to air temperature, and thus may experience greater thermal extremes than those living lower on the shore (Spicer and Gaston, 1999). Therefore, upper inter-tidal organisms may have higher thermal tolerances than lower inter-tidal or sub-tidal species (Spicer and Gaston, 1999). This trend has been supported by intra- and inter-specific studies on a variety of marine invertebrates, including the dogwhelk *Nucella lapillus* (Davenport and Davenport, 2005), porcelain crabs of the genus *Petrolisthes* (Stillman, 2003) and the limpets *Nacella concinna* and *Kerguelenella lateralis* (Davenport, 1997). By contrast, the upper thermal tolerance of the periwinkle *Littorina littorea* did not differ between individuals living at different tidal heights (Davenport and Davenport, 2005).

Various factors interact with temperature to determine the thermal environment experienced by an organism (Helmuth et al., 2005; Broitman et al., 2009). The colour, size and shape of an individual affects the amount of solar radiation received, the heat fluxes to and from the organism and the degree of temperature buffering by sea water and thus, the organism's body temperature (Helmuth et al., 2005; Broitman et al., 2009; Harley et al., 2009; Miller et al., 2009). For example, the shape and colour of corals can cause body temperature to differ from that of the surrounding water by several degrees (Helmuth, 2009). Also, phenotypic or evolutionary responses to microclimate may have caused intra- and inter-specific shell morphology differences in the limpets *Lottia gigantea*, *Patella vulgata* and *Siphonaria gigas* (Harley et al., 2009). Other factors that may influence an organism's body temperature include: direction of slope and radiation received (Evans, 1948; Miller et al., 2009) as well as the timing and duration of exposure (Helmuth et al., 2002).

Organisms may employ thermoregulatory behaviour to buffer environmental variation and thus reduce the need to adapt or acclimate to new conditions (Spicer and Gaston, 1999). To avoid high water temperature, individuals of the crayfish *Orconectes rusticus* either move out of pools or burrow into the sand below rocks (Mundahl, 1989). Limpets avoid high air temperature by migrating with the tide, occupying crevices or mushrooming to decrease exposure to warm rock surfaces (Harley et al., 2009). It must be noted, however, that additional costs may be associated with behavioural thermoregulation (Huey and Slatkin, 1976; Kearney et al., 2009). For example, organisms will expend energy when moving between shaded and sunny areas, and thermoregulatory behaviour may negatively influence predator avoidance, feeding and social behaviours (Huey and Slatkin, 1976; Kearney et al., 2009).

Confounding factors

Age

Most marine invertebrates have complex life-cycle stages that differ in their anatomy and body size (Vernberg and Vernberg, 1969). Physiological tolerances may vary depending on the life-cycle stage and as specific thermal conditions are often required for development, juveniles are, generally, more likely to be negatively influenced by environmental change than adults (Vernberg and Vernberg, 1969; Spicer and Gaston, 1999). Not all studies have demonstrated this relationship, and in some organisms thermal tolerance decreases with increased growth (Spicer and Gaston, 1999). For example, juveniles of the marine bivalve *Donax serra* have lower thermal tolerances than adults (Ansell and MacLachlan, 1980), but

the upper thermal tolerance of the starfish *Asterias vulgaris* decreases with increased growth (Spicer and Gaston, 1999). These differences may originate from differences in physiology, behaviour, or the use of contrasting thermal environments across life-stages. Including individuals of different ages in an analysis of thermal tolerance complicates results (unless they are evaluated and accounted for), and in most cases, studies focus on one age group (e.g. Kivivuori and Lahdes, 1996; Kohonen and Lagerspetz, 1996; Lagerspetz, 2003).

Sex

The sex of individuals has been found to influence the thermal tolerance of some organisms (Bradley, 1978; Winne and Keck, 2005). However, sex did not affect the upper temperature tolerance of the amphipod *Paramelita nigroculus* (Buchanan et al., 1988), the crayfish *Orchonectes rusticus* (Mundahl, 1989), or the fruit flies *Ceratitis capitata* and *Ceratitis rosa* (Nyamukondiwa and Terblanche, 2009). If the determination of sex is possible, including sex as a covariate in statistical analyses is the best approach.

Phylogeny

Species may have similar physiologies due to their phylogenetic relatedness (Spicer and Gaston, 1999). Relatedness can confound intra- and inter-specific comparisons as some species or populations will be more closely related than others, and traits studied will represent non-independent data points (Garland and Adolph, 1994; Spicer and Gaston, 1999). Closely related species or subspecies may not have similar thermal tolerances due to divergent evolution, while convergent evolution may make the tolerances of distantly related species similar (Spicer and Gaston, 1999). If the phylogeny of the group investigated is available, analyses that incorporate species' phylogenetic relationships can adjust for the species' relatedness (Garland and Adolph, 1994; Kellermann et al., 2009). If information on phylogeny is not available, comparing congeners or closely related species may allow for phylogeny and adaptation effects to be distinguished from one another (Hochachka and Somero, 2002; Somero, 2010).

Reproductive status

The reproductive status of individuals can affect their thermal tolerance. Investing energy in reproduction may decrease the energy available for other physiological functions, and thus influence thermal tolerance (Peck et al., 2007; Pörtner and Farrell, 2008). In order to control for the influence of reproductive status, organisms may be studied during periods when reproductive activity is reduced or after long-term acclimations that synchronize the

reproductive cycle of individuals. In organisms where reproductive status may be determined, including this factor as a covariate in statistical analysis is adequate.

Size

Studies have found differing relationships between thermal tolerance and the size of individuals, and while some studies have found that thermal tolerance may increase or decrease with size, others have found no relationship. The upper temperature tolerance of the amphipod *Chroestia lota* (Marsden, 1985), the bivalve *Laternula elliptica* (Peck et al., 2007), and various Antarctic marine invertebrate species (Peck et al., 2009) are negatively correlated with size. By contrast, the upper thermal tolerance of the amphipod *Orchestia gammarellus* is positively correlated with body size (Gaston and Spicer, 1998; Morritt and Ingólfsson, 2000). No relationship between size and upper thermal tolerance was observed in the sea star *Ophionotus victoriae* (Peck et al., 2008) and the isopod *Lirceus brachyurus* (Cheper, 1980). To account for differences in the body size of two *Orchestia gammarellus* populations, Gaston and Spicer (1998) only compared the upper thermal tolerances of organisms of a similar size. This factor may also be accounted for by including body size as a covariate in the analyses.

Nutrition status

The nutrition status of an organism and the amount of energy available for functioning may affect thermal tolerance. A decreased thermal tolerance in starved organisms has been found, but exceptions have also been noted. In the mussel *Mytilus edulis* (Read and Cumming, 1967) and the fruit flies *Ceratitis capitata* and *C. rosa* (Nyamukondiwa and Terblanche, 2009) low feeding condition was associated with a lower thermal tolerance. By contrast, fed and non-fed individuals of the amphipod *Paramelita nigroculus* had a similar CT_{max} (Buchanan et al., 1988). Feeding status can be accounted for in experiments, as individuals may be fed standardized amounts of food or starved equally before experimental trials.

Study regions

Continental South Africa: West and Southern shores

South Africa (29° S; 24° E) has contrasting sea temperature conditions along its West and South-East coasts (Branch et al., 2007). The Agulhas current which flows along the East coast brings warm water from the subtropics, and at East London (33° S; 27° E) the current moves further off shore and eventually retroflects and flows eastwards (Fig. 1; Branch et al., 2007; Demarcq et al., 2011). By contrast, winds along the West coast blow surface water offshore

and cause the upwelling of cold, nutrient-rich water (Branch et al., 2007; Demarcq et al., 2011). Between 2000 and 2009, mean sea surface temperature on the West coast was 13.6°C ± 1.0 SD and 16.9°C ± 1.1 SD on the South coast (South African Weather Service). Sea surface temperature variability is high both on the West and South coasts, but areas of extreme upwelling (e.g. Lüderitz, Namibia) have low variability (Demarcq et al., 2011). Differences in mean sea surface temperature along the coast of South Africa are associated with distinct biogeographical provinces (Branch et al., 2007). On the cold West coast, the 'Namaqua Province' runs from Lüderitz to Cape Point, and the warm-temperate 'Agulhas Province' runs from Cape Point to northern Transkei (Branch et al., 2007). The distribution of continental South Africa's marine fauna is also affected by tidal regimes (Branch et al., 2007). As tides rise and fall twice a day, sessile (or slow) organisms living high on the shore are intermittently submerged, while those low on the shore are almost continually submerged (Branch et al., 2007). This inter-tidal stress gradient results in the development of horizontal bands of organisms, each dominated by differing species (Branch et al., 2007).

Marion Island

Marion Island (46° 54' S; 37° 45' E) is a small (290 km^2), South African island in the southern Indian Ocean (de Villiers, 1976; Smith, 2002). The island is found in the path of the easterly flowing Antarctic Circumpolar Current, between the sub-Antarctic and Polar Fronts (Pakhomov and Froneman, 1999). Marion Island has an oceanic, thermally stable (low variation) but unpredictable climate, with high humidity and frequent cloud cover (de Villiers, 1976; Smith, 2002; Chown and Froneman, 2008). Mean air temperature varies seasonally by 3.6°C, and daily by 1.9°C (Smith, 2002). Between 1999 and 2008, mean air temperature was 6.1°C ± 3.0 SD and mean sea surface temperature was 6.0°C ± 1.7 SD (South African Weather Service). The island's coastal topography is characterised by steep cliffs and boulder beaches (de Villiers, 1976). Although the island has a small tidal range (~ 21 - 71 cm), large swells (up to 3 m) and violent storms (de Villiers, 1976) can have a large influence on sea water levels on the shore. Boulder rotation and variable wave action make the littoral zone of the island highly unstable (de Villiers, 1976). This instability, as well as the island's small size and young age (~ 450,000 years before present), mean that the littoral biota is characterised by low species diversity and low organism density (de Villiers, 1976; Smith, 2002; Chown and Froneman, 2008). The island's littoral species are thought to comprise a few species that have survived extinction during previous glacial phases and new migrants (de Villiers, 1976). Marion Island shares many common littoral species with the sub-Antarctic region, and to a lesser degree Antarctica (de Villiers, 1976). In particular, the broad

geographical ranges of amphipod and isopod species in the Southern Ocean may be due to long distance dispersal via rafting on detached kelp (Leese et al., 2010; Nikula et al., 2010). The island also shares congeneric amphipod and isopod species with continental South Africa.

Climate change on Marion Island and South Africa

Climate change has had an effect on the surface temperatures of continental South Africa and Marion Island. The annual mean temperature of continental South Africa has increased by 0.13°C per decade from 1960 to 2003 (Kruger and Shongwe, 2004). From 1982 to 2009 the sea surface temperatures, from January to August, on the West and South coasts of South Africa have decreased by up to 0.55 and 0.35°C per decade, respectively (Rouault et al., 2010). During this same period (1982 to 2009), no change in the sea surface temperatures of these regions were recorded from September to December (Rouault et al., 2010). By contrast, from 1982 to 2009, the Agulhas current, on the East coast of South Africa, has warmed by up to 0.55°C per decade, (Rouault et al., 2010). On Marion island an increase in air temperature of 1.2°C between 1949 and 1999, and an increase in sea surface temperature of 0.9°C between 1967 and 1985 have been recorded (Smith and Steenkamp, 1990; Smith, 2002).

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Chapter 2

Effect of rate of change and thermal acclimation on the upper thermal tolerance of marine organisms: patterns at several hierarchical and geographical scales

Introduction

Organisms function optimally within a specific range of body temperatures (Huey and Stevenson, 1979; Hochachka and Somero, 2002). Within this range, the functioning at the systemic, cellular and molecular levels of organisation is optimised (Pörtner and Farrell, 2008). If temperatures exceed these limits organism functioning will rapidly decline (Pörtner, 2002; Pörtner and Farrell, 2008). These temperature thresholds are not fixed and an organism can respond to temperature change within its lifetime through phenotypic plasticity, or over generations through evolutionary adaptation (Angilletta et al., 2002; Chown and Terblanche, 2007). Phenotypic plasticity, the ability of a single genotype to produce various phenotypes if exposed to different environments (Miner et al., 2005; Garland and Kelly, 2006; Ghalambor et al., 2007; Reed et al., 2010), includes morphological, behavioural and physiological changes (Chevin et al., 2010).

The capacity of an organism to respond to temperature change though phenotypic plasticity or evolutionary adaptation may also be influenced by other factors. Rate of temperature change may affect the mean and variance of thermal tolerance traits, as well as an organism's acclimation response (Terblanche et al., 2007; Chown et al., 2009; Peck et al., 2009; Mitchell and Hoffmann, 2010). As rate of temperature change may influence acclimation responses and cause changes to trait variance, heritability of thermal tolerance may be affected. Mitchell and Hoffmann (2010) showed that under slow ramping conditions, the heritability of thermal resistance in *Drosophila melanogaster* was low compared to when flies were treated to a static stress, and Chown et al. (2009) suggested that incorrect estimates of variance can also result in spurious estimates of heritability. Therefore, the rate of temperature change could influence the potential of organisms to respond to environmental change through both phenotypic plasticity and evolutionary adaptation.

The majority of thermal tolerance studies employing a dynamic method, feature standard or fast rates of change (e.g. Hicks and McMahon, 2002; McGaw, 2003). As these rates may be faster than most temperature variations in nature, and as rate of change may influence thermal limits, studies using slow or more ecologically relevant rates have begun to appear more frequently in the literature (e.g. Mora and Maya, 2006; Terblanche et al., 2007; Chown et al., 2009; Peck et al., 2009; Sgrò et al., 2010). Although a decrease in rate of change has been associated with a decline in thermal tolerance (see Mora and Maya, 2006; Terblanche et al., 2007; Chown et al., 2009; Peck et al., 2009; Mitchell and Hoffmann, 2010), this result has

not been consistent and the opposite has also been found (see Kelty and Lee, 1999; Overgaard et al., 2006; Powell and Bale, 2006). These contrasting results may originate from a trade-off between the period of exposure to near lethal temperatures, and the amount of time available for acclimation to experimental temperatures (Cocking, 1959; Beitinger et al., 2000; Peck et al., 2009). Various other factors, however, may influence the results obtained in studies featuring slow rates of change (Rezende et al., 2011). Increasing stress levels associated with captivity time as well as nutrition status, for example, may also influence thermal tolerance (Read and Cumming, 1967; Mora and Maya, 2006; Nyamukondiwa and Terblanche, 2009). Furthermore, as increasing temperature is associated with an increase in metabolic rate, and thus energetic demands, body condition during slow-rate experiments may decline even when animals are well fed (Mora and Maya, 2006; Rezende et al., 2011). In light of these contrasting effects and the current debate regarding the most adequate rate of change to use in thermal tolerance protocols (Rezende et al., 2011; Terblanche et al., 2011), there is an urgency to explore the effects of diverse rates of change on the thermal tolerance of a broad range of taxonomic groups.

Basal thermal tolerance and an organism's capacity to respond to temperature change through phenotypic plasticity are also influenced by mean environmental temperature, and the degree of temperature variability experienced in the past and present by an organism (Spicer and Gaston, 1999; Chown and Terblanche, 2007; Chown et al., 2010a). Due to spatial variation in temperature, the thermal tolerance thresholds and acclimation ability of individuals, populations, and species may vary (Spicer and Gaston, 1999). Marine species and populations living at relatively high environmental temperatures (e.g. tropics, upper inter-tidal zone) generally have a higher basal thermal tolerance than those living in cooler areas (e.g. mid-latitudes, sub-tidal zone) (Gaston and Spicer, 1998; Stillman, 2002; 2003; Sorte and Hofmann, 2005; Somero, 2010). Theoretical models indicate that organisms living in predictably variable environments should have greater plasticity responses than those in thermally stable or unpredictably variable environments (Chown and Terblanche, 2007). In accordance with these predictions, organisms in the thermally stable tropics and poles generally have little or no acclimation capacity (Stillman, 2002; Peck et al., 2010, but see Sgrò et al., 2010). Similarly, the 'warming tolerance' (the difference between the critical thermal maximum (CT_{max}) and mean environmental temperature) of an organism may be related to the temperature range experienced (Deutsch et al., 2008; Tewksbury et al., 2008). Organisms living in areas with a small thermal range (e.g. tropics) may have a lower 'warming tolerance' than those experiencing a wider range of temperatures (e.g. mid-

latitudes) (Deutsch et al., 2008; Tewksbury et al., 2008). By contrast, marine crustaceans and molluscs living in the thermally variable upper inter-tidal zone, have been found to have reduced acclimation responses and an upper thermal tolerance closer to maximum habitat temperature than organisms inhabiting the less thermally variable sub-tidal zone (Tomanek and Somero, 1999; Stillman and Somero, 2000; Stillman, 2002; 2003; Somero, 2010). As the thermal environment experienced by an organism is determined by microclimate temperatures as well as various interacting factors (e.g. extreme events, cloud cover, wave splash, timing of tides), the thermal tolerance and plasticity responses of organisms may not always follow large scale latitudinal patterns (see e.g. Helmuth et al., 2002; Clusella-Trullas et al., 2011). Therefore, investigating the direction and magnitude of intra- and inter-specific variation in critical thermal limits has important consequences for predicting long-term responses of organisms to temperature change. Individuals that have higher upper thermal tolerances, greater plasticity responses and higher 'warming tolerances', may facilitate the persistence of populations and species during environmental change (Chown et al., 2010b; Somero, 2010).

The world's oceans have demonstrated widespread warming over the last 50 years, with the rate of warming increasing over the period 1993 – 2003 (Solomon et al., 2007). Although at small spatial scales, water environments are more thermally homogenous than terrestrial environments, behavioural or avoidance responses of sub-tidal or near-shore marine organisms may not be effective in counteracting warming effects (Feder and Hofmann, 1999). Inter-tidal organisms, which are exposed to a highly variable environment, are often living close to their upper thermal limits and are thus potentially more vulnerable to change (Stillman, 2002; Hofmann and Todgham, 2010). Furthermore, research into the upper thermal tolerances of marine invertebrates has largely been limited to the study of Northern Hemisphere species. Studies on Southern Hemisphere organisms have mostly been restricted to Antarctic (e.g. Davenport, 1997; Peck et al., 2008; 2009) and South American species (e.g. Urban, 1994), with a few exceptions (e.g. studies on molluscs in southern Africa; Ansell and McLachlan, 1980; Zardi et al., 2011).

Therefore, this study aimed to examine the upper thermal tolerances and acclimation responses of near-shore marine invertebrates, and specifically compare congeners of amphipods and isopods that inhabit contrasting temperature regimes within continental South Africa, and between South Africa and sub-Antarctic Marion Island. These localities and the heterogeneity of coastal shores (tidal heights) coupled with the occurrence of congeneric species of amphipods and isopods across regions, provided a unique system to undertake

intra- and inter-specific comparisons of thermal tolerance and its plasticity at various geographic scales.

Species and Study Regions

Species of the amphipod genus *Hyale* and isopod *Exosphaeroma* were chosen as model organisms as they are common on the coastlines of Marion Island and South Africa (Day, 1969; Griffiths, 1976; Kensley, 1978; Branch et al., 1991). Furthermore, several *Exosphaeroma* congeners in South Africa inhabit different tidal heights (*E. antikraussi* inhabits the upper inter-tidal and *E. laeviusculum* the inter-tidal to infra-tidal zone), and have disparate biogeographical distributions along the South African coastline (Day, 1969; Kensley, 1978). The species *Parisocladus stimpsoni*, an upper inter-tidal isopod, was included in the study to further examine the upper thermal tolerances of organisms inhabiting distinct tidal zones. Moreover, given that the life history of brooding organisms such as amphipods and isopods does not include a dispersive planktonic larval stage, reduced gene flow between populations could promote local adaptation (Teske et al., 2007; Sherman et al., 2008; Sanford and Worth, 2010). A disparity in the upper thermal tolerances and plasticity responses of populations living in different thermal environments may thus be expected (Gaston and Spicer, 1998).

Marion Island, in the sub-Antarctic, has an oceanic, thermally stable but unpredictable climate and from 1999-2008 a mean sea surface temperature of ~ 6.0°C (de Villiers, 1976; Smith, 2002; Chown and Froneman, 2008). By contrast, continental South Africa has mean sea surface temperatures of ~ 14°C and ~ 17°C on the West and South coasts, respectively (South African Weather Service). Despite the cold upwelling that is associated with the West coast of South Africa, both the West and South coasts exhibit high sea surface temperature variability (Demarcq et al., 2011). Additionally, semi-diurnal tides on the South African coastline cause upper inter-tidal organisms to be periodically exposed to air temperatures, while those in the lower inter-tidal or sub-tidal zones are either continually submerged or only exposed during low spring-tides (Branch et al., 2007). Despite a small tidal regime, frequent storms and recurring wave splash cause Marion Island to have a dynamic coastline.

Research questions

1. What is the direction and magnitude of plasticity of thermal tolerance between congeneric species from South Africa and Marion Island? This question will also be investigated between populations of one isopod species inhabiting the West and South coasts of South Africa.
2. What is the effect of rate of change on the upper thermal tolerance of these species?
3. Is there an interaction effect between the rate of change and acclimation on upper thermal tolerance?
4. What is the amount of variation in 'warming tolerance' across species and sites?

Research Predictions

The following research predictions are based on the reviewed literature:

1. Individuals acclimated at higher temperatures should demonstrate increased upper thermal tolerance. Across biogeographical regions, species in environments with high thermal variability will demonstrate a greater magnitude of plasticity than those in more thermally stable areas. Across inter-tidal zones, a trade-off between improved basal thermal tolerance and acclimation response (Gause macrophysiological 'rule', Gaston et al., 2009) may cause upper inter-tidal species to demonstrate a reduced magnitude of plasticity in comparison to lower inter-tidal organisms.
2. A decrease in rate of change will be associated with a decline in upper thermal tolerance.
3. There will be a significant interaction between rate of change and acclimation, with a decreased acclimation response at slower rates of change.
4. At all spatial scales investigated, 'warming tolerance' will vary greatly between species and populations. Species and populations in more thermally variable environments will have a higher 'warming tolerance' than those exposed to more stable environmental temperatures.

Materials and Methods

Animal collection and transportation

Study species and respective collection localities are provided in Table 1 and Figure 1 (p. 85, 69). All individuals were collected haphazardly by hand from the inter-tidal zone. Amphipods (*Hyale* spp.) were collected from amongst seaweeds in South Africa and from rotting kelp on Marion Island, while isopods from all localities were collected from the underside of easily lifted boulders. Boulders varied slightly in size, with those at Trypot Beach (Marion Island) and Hangklip (South Africa) being generally smaller than those at Lamberts Bay (South Africa). Collections occurred on Marion Island in April and May 2010, and in South Africa between January and July 2011.

The majority of experiments (Table 1) were undertaken at the Marine Research Aquarium in Seapoint, Cape Town, South Africa. Fast-rate CT_{max} experiments on *H. hirtipalma* were performed at the South African research base on Marion Island, but due to time constraints on the island, the remainder of the experiments on Marion Island species were performed in Cape Town. For the experiments performed in Cape Town, animals were transported to South Africa on the South African Antarctic Programme research vessel, the SA Agulhas. During the 9 day journey, animals were maintained in plastic tanks containing aerated sea water. The tanks were housed in a chest freezer (DMF333, Defy, Durban, South Africa) and sea water temperature was maintained within the temperature range experienced by the animals on Marion Island (5.9 ± 1.6°C), and recorded using I-button data loggers (see details below). During transportation animals were provided with food (the kelp *Durvillaea antarctica* collected from the shore of Marion Island) and refuge (algae and rocks). Animals were then transported to laboratory facilities, where they were placed in recovery and acclimation treatments. For South African species, individuals were transported to the laboratory facilities within two days of collection using plastic tanks containing aerated sea water and maintained in coolers at the sea water temperature of the site of collection.

Recovery period

In the laboratory, animals were placed in plastic tanks containing aerated, filtered sea water maintained at mean sea surface temperatures resembling the locality of origin: 6.5 ± 0.4°C and 15.1 ± 0.3°C for Marion Island and South Africa, respectively. Fast-rate CT_{max} experiment individuals were maintained in these conditions for 1 to 3 days. As animal collections could not occur at all sites on the same day, animals collected for slow-rate CT_{max} experiments were maintained in these conditions until all animals for the trials were collected.

The recovery period for slow-rate CT_{max} animals thus varied between 2 and 11 days. During this recovery period any mortality was noted and individuals were identified to species level using taxonomic keys (Day, 1969; Griffiths, 1976; Kensley, 1978; Branch et al., 1991) and later confirmed with the help of taxonomic experts.

Acclimation

After the recovery period, animals were divided into three acclimation treatments. For Marion Island species, these were 3.2 ± 0.5°C, 6.6 ± 0.3°C and 11.6 ± 0.9°C and for South African species 11.2 ± 0.2°C, 15.0 ± 0.3°C and 19.2 ± 0.4°C. These temperatures represent low, mean and high sea water temperatures, and are within the range of sea surface temperature experienced at the collection sites (for experimental design, see Table 1). Fast-rate CT_{max} experiments began on the 6^{th} day of acclimation and continued for 7 days. Fast-rate CT_{max} individuals were thus acclimated for 5 to 12 days. Due to logistical constraints, slow-rate CT_{max} experiment individuals were acclimated for 13 (South African species) and 15 days (Marion Island species).

Experimental maintenance

Throughout recovery periods, acclimation treatments and experiments, water temperature was monitored using thermocouples (type T, gauge 24) and a handheld thermometer CHY 507 Thermometer, Taiwan) and recorded with data loggers (I-buttons DS1922L, accuracy ± 0.5°C, Dallas Semiconductor Maxim, USA) protected in silicone enclosures (SL-ACC06, Signatrol, Tewkesbury, UK). All sea water was aerated and filtered for standardization. Sea water used for fast-rate CT_{max} experiments on Marion Island species was filtered through filter paper (8 - 12 μm, Munktell, Falun, Sweden). For all other experiments, filtered sea water (0.22 μm) was supplied by the Marine Research Aquarium. Every three days, fresh sea water was supplied to all organisms in recovery, acclimation, and slow-rate CT_{max} experiment and control tanks. During the provision of fresh sea water, part of the sea water was removed and the same amount of filtered sea water, at the required temperature, was added. Fresh sea water was cooled or heated to the required temperature using incubators (MIR – 153, Sanyo, Osaka, Japan) and electronic aquarium heaters (ART A-761, Tronic Aquarium Heater, Rolf C. Hagan, Yorkshire, UK). Both before and after the provision of fresh sea water, the salinity (Marion Island experiments: 35.9 ± 2.9 ppt; South Africa experiments: 36.5 ± 2.2 ppt) and dissolved oxygen content (Marion Island experiments: 9.9 ± 1.8 mg l^{-1}; South Africa experiments: 9.7 ± 1.3 mg l^{-1}) of the sea water was measured using salinity and oxygen meters (YSI 30 Salinity Conductivity and Temperature Meter, Ohio, USA; DO-5510 Lutron

Dissolved Oxygen Meter, Taipei, Taiwan). Organisms in recovery, acclimation and slow-rate CT_{max} experiments and controls were provided with seaweed (*Ulva* spp.) or kelp (*Durvillaea antarctica* for experiments on Marion Island, and *Ecklonia maxima* for experiments in South Africa) as a form of food and refuge. To prevent differences in feeding status, animals in acclimation and recovery tanks, as well as containers in the slow-rate CT_{max} experiments and controls, were provided with a standardized amount of food (kelp: ± 100 g for tanks and ± 20 g for vials; seaweed: ± 30 g for tanks and ± 10 g for vials). All animals in recovery and acclimation, as well as slow-rate CT_{max} experiments and controls were maintained at a photoperiod of 12L:12D.

Fast-rate CT_{max}

Fast-rate CT_{max} experiments were performed on both field fresh (within two days of collection) and acclimated individuals (Table 1). Once recovered and acclimated, five individuals were placed individually in mesh vials housed in a container filled with filtered, aerated sea water and immersed in a waterbath (Grant Instruments GP 200-R4, Cambridge, UK). This set up allowed the responses of five individuals to be monitored at once and sea water to move freely between the vials. The start temperature of all experiments was 7°C (Marion Island species) or 15°C (South African species), which was maintained for 30 minutes to allow for equilibration and checked before the beginning of trials using a digital handheld thermometer. The temperature of the sea water was then increased at a rate of either 0.1 or 0.5°C min^{-1}. Throughout the trials, organism response to mechanical stimuli (gentle prodding) was noted. The temperatures at which an organism's response first slowed down, and at which active response ceased but small movements were still visible were recorded. To later allow for the comparison of data obtained in these trials with that obtained in slow-rate CT_{max} trials, the experiments were continued until all signs of movement ceased (CT_{max}). At the end of each trial, animals were placed in recovery at 7°C (Marion Island species) or 15°C (South African species), and their response checked after 10 minutes and one hour. For acclimated individuals, 10 to 15 animals were tested at each acclimation x rate of change combination; 10 to 30 individuals of field fresh organisms were tested at each rate of temperature change (Table 1).

Slow-rate CT_{max}

After recovery, organisms were divided into four groups, three acclimation treatment groups (see acclimation section) and a control group [comprised of individuals acclimated at 7°C (Marion Island species), 15°C (*E. laeviusculum*) and 11°C (*H. grandicornis*), see below].

Due to space constraints within experimental tanks, slow-rate CT_{max} experiments were not performed on *E. antikraussi* and *P. stimpsoni*.

After acclimation, individuals were tested at one of three slow rates of change: 1°C/day (0.0007°C min^{-1}), 1°C/3 days (0.0002°C min^{-1}) and 1°C/6 days (0.0001°C min^{-1}). The start temperature of all trials was 7°C for Marion Island species or 15°C for South African species, which was maintained for a 24 hour equilibration period before the ramping initiated. As high mortality occurred during the 19°C acclimation treatment of *H. grandicornis*, this acclimation group was only included in the 0.0007°C min^{-1} trial. The control animals of the South African isopods were divided into three groups, thus ensuring a control group for each experimental trial. For Marion Island species (*H. hirtipalma* and *E. gigas*) and *H. grandicornis*, one control group per species served for all the experimental trials. Marion Island and South African controls were maintained for the duration of the experiments at 6.8 ± 0.3°C and 14.7 ± 0.2°C, respectively. Due to their high mortality rates, *H. grandicornis* controls were maintained at 11.4 ± 0.2°C.

Each rate of change trial occurred within a jacketed perspex tank which contained three sections divided by mesh partitions. An acclimation group was randomly assigned to each section, with each trial containing the same arrangement. Due to difficulties associated with checking the responses of organisms swimming freely within the tanks, and to distinguish between *E. laeviusculum* individuals from different locations, individuals (including controls) were placed in containers (honey jars with mesh sides). Within each trial, a container existed for each acclimation x species/population combination, with ± 15 - 30 individuals in each container. These containers allowed the responses of individuals to be checked while not preventing the flow-through of sea water. The density of the organisms in the containers was presumed to have no negative effect, as these species tend to aggregate in very high densities in the natural environment (personal observation; see other congener's behavioural patterns in Lancellotti and Trucco, 1993; Henninger et al., 2008).

The sea water temperature in each trial was maintained using water baths (Grant Instruments GP 200-R4, Cambridge, UK). Fresh water, at the required temperature, was pumped and circulated from the water bath into the outer wall (jacket) of the tank, thereby heating the sea water in the inner part of the tank. The temperature of each trial was increased every day at 09h30. Water temperature was monitored throughout the day using a digital thermometer and temperature was further increased if required. The response of individuals

(including controls) to mechanical stimuli was checked daily, and the temperature at which organisms were found to be lethargic or dead was noted. Lethargic individuals were placed back in the trials and their response checked the following day. Unresponsive individuals were removed, placed at 7 (Marion Island species) or 15°C (South African species), and their response checked after one hour. In these trials no gradual change in response (such as those seen in the fast-rate CT_{max} experiments) was noted at pre-lethal temperatures, and thus the temperature at which death occurred was deemed CT_{max}.

CT_{max} of controls

Within two to three days after the end of each slow-rate CT_{max} trial on *E. laeviusculum*, fast-rate CT_{max} experiments were performed on the control animals. For *H grandicornis* (1 control group only), fast-rate CT_{max} of controls was determined at the end the slowest rate of change trial. The CT_{max} of 15 control individuals was determined at a rate of 0.5°C min^{-1} (see fast-rate CT_{max} section). Results were then compared to the CT_{max} of field fresh organisms, and an indication of whether captivity period influenced CT_{max} results was thus obtained. As an insufficient number of individuals could be transported from Marion Island to South Africa, these experiments were only performed on the controls of South African species.

Nutrition status

To determine whether nutrition status may have affected the results of the slow-rate CT_{max} experiments, a lipid content analysis was performed on the remaining control individuals for each trial, and compared to field fresh individuals. For this analysis a random sample of ± 50 - 200 field fresh individuals was collected. A chloroform-methanol (1:2) lipid dissolving method was used to determine lipid content (Clarke, 1977; Clarke and Holmes, 1986; Terblanche et al., 2006; Lease and Wolf, 2011). The wet mass of each individual (control and field fresh) was obtained (AE163, Mettler, Greifensee, Switzerland and Sartorius Analytic balance, Göttingen, Germany; ± 0.0001 g). Animals were then dried for two to three days at 60°C until the mass of each individual stabilised, dry mass then recorded (Avery Berkel balance FA304T, Fairmont, USA; ± 0.0001 g). As the hard exoskeletons of crustaceans could potentially influence the effectiveness of fat extraction, individuals were cut into several pieces before being placed individually in vials containing the chloroform-methanol solution. Individuals were maintained in the solution for three days and the solution was changed every 24 hours. Animals were then dried at 60°C for two to three days, until mass stabilized. Lipid content was determined by subtracting the dry mass of individuals after lipid extraction from

the dry mass prior to lipid extraction. Due to a scarcity of *E. gigas* individuals during collection, lipid content analysis was not performed on this group.

Confounding factors

Mass and Sex

At the end of all CT_{max} experiments, dead individuals were weighed and a wet mass obtained (AE163, Mettler, Greifensee, Switzerland and Sartorius Analytic balance, Göttingen, Germany; ± 0.0001 g). To remove excess water before weighing, individuals were dabbed with paper towelling. Organisms were then preserved in 99.9 % ethanol and stored individually in vials. The sex of each individual was determined under a dissecting microscope (Stemi 2000-C, Zeiss, New Jersey, USA) and following Griffiths, 1976; Branch et al., 1991; Wilson, 1991; Branch et al., 2007.

Climate data

Sea surface temperature data obtained from the South African Weather Service were used to determine the temperature of acclimation treatments. To obtain microsite temperature data, I-button data loggers (I-buttons DS1922L, accuracy ± 0.5°C, Dallas Semiconductor Maxim, USA) inside waterproof capsules (SL-ACC06, Signatrol, Tewkesbury, UK) were deployed at exposed, semi-exposed and submerged sites at Trypot Beach, Hangklip and Lamberts Bay. Microsite temperature data were collected over the periods of May 2009 to September 2010 for Trypot Beach, and December 2010 to August 2011 for Hangklip and Lamberts Bay. Due to I-button loss, data for the semi-exposed site at Trypot Beach were not available, and only data from December 2010 to April 2011 were available for the submerged site at Lamberts Bay. Environmental data were also collected during fieldwork, and included: sea water temperature, sea water salinity and dissolved oxygen content, shaded air temperature, and the temperature at the site of animal collection.

'Warming tolerance'

To calculate the 'warming tolerance' (CT_{max} minus mean environmental temperature) of each species or population, information on the tidal heights inhabited by each of the species was obtained from the literature (Day, 1969; de Villiers, 1976; Kensley, 1978; Branch et al., 1991). 'Warming tolerance' calculations were made using the microsite data that would most closely represent the temperatures experienced by the study species (Table 1). For organisms exposed to conditions at more than one tidal height (e.g. inter- to infra-tidal species), 'warming tolerance' was calculated for all positions on the shore likely experienced (Table 1).

As microsite temperature data were not available for Muizenberg (South coast) and given its relatively close proximity Hangklip, the 'warming tolerance' of *H. grandicornis* was calculated using data collected from Hangklip. The 'warming tolerance' of South African species and populations were calculated for the period in which microsite data were available for all sites (i.e. December 2010 to April 2011). For Marion Island species, 'warming tolerance' was calculated using all data collected (i.e. May 2009 to September 2010).

Statistical Analysis

As five individuals were assessed at once in fast-rate CT_{max} experiments, the effect of replicate in each rate of change x acclimation combination was assessed. Replicate was only found in a few cases to have a significant effect, and in such cases a consistent pattern was not evident. The effect of replicate was thus not further considered in analyses. Mortality of possibly weak/old individuals was noted in both controls and at low temperatures in slow-rate CT_{max} experiments. To correct for this mortality, data 1 SD from the mean were removed from all acclimation x rate of change combinations in slow-rate CT_{max} experiments. Prior to statistical analysis, data were checked for normality and homogeneity of variances using plotting techniques as recommended in Faraway (2005) and Crawley (2007). For intra- and inter-specific analyses, general linear models (GLM) were used to assess the effect of rate, acclimation, mass, sex and, rate x acclimation, rate x mass, rate x sex, acclimation x mass, acclimation x sex and mass x sex interactions on CT_{max}. The assumption of normality failed in some cases as data followed a uniform (short-tailed) distribution. However, for short-tailed distributions, the consequences of non-normality in GLM are not serious and can be discounted (Faraway, 2005). To determine whether the period of captivity influenced the CT_{max} of slow-rate experimental individuals, a GLM was used to determine the effect of treatment (control and field fresh groups), mass, sex and, treatment x mass, treatment x sex and mass x sex interactions on CT_{max}. A GLM was used to assess the effect of treatment (controls versus field fresh), dry mass and a treatment x dry mass interaction on lipid content. For comparative analyses, GLMs were used to determine the effect of species or population (species/population), acclimation, mass, sex and, species/population x acclimation, species/ population x mass and species/population x sex interactions on CT_{max} at fast rates of change. For these analyses only species from the same genus were compared. As *E. laeviusculum* populations were found to have significantly different thermal tolerances, these populations were included separately in the analysis of *Exosphaeroma* species. In cases where mass and lipid content were not normally distributed these variables were $\log_{10}$ transformed. The technique of model simplification (Crawley, 2007) was used for all analyses, and the results

of the minimum adequate models are presented in the results section. Differences between factor levels were further investigated using treatment contrasts and aggregating non-significant factor levels (Crawley, 2007). Statistical and graphical analyses were implemented in R version 2.10.1 (R Development Core Team, 2009).

The mean, minimum and maximum temperatures, as well as the coefficient of variation were calculated for exposed, semi-exposed and submerged positions using the microsite temperature data from each site. Minimum and maximum temperatures provided an indication of the range of temperatures experienced, and the coefficient of variation an idea of the thermal variability of habitats. Additionally the mean and maximum rate of temperature increase at each exposure was calculated at each site. Rare instances of I-button exposure to air temperatures were noted in data recorded at submerged positions at Hangklip and Lamberts Bay, and therefore, in instances when temperatures were higher than those recorded at semi-exposed positions, the temperature peaks were removed from data sets before calculations (0.8 % for Hangklip and 1.2 % for Lamberts Bay). For South African collection sites, calculations were made using microsite data available for all sites (i.e. December 2010 to April 2011). For Marion Island, all data were used in calculations (May 2009 – September 2010).

Results

The CT_{max} of field fresh individuals differed slightly (0 - 1.4°C) from that of organisms acclimated to average sea water temperature (Table 2). These differences were not always significant, and varied within species, among species (Fig. 2 - 5), and between rates of change (Fig. 2 - 5). Within rates of change, significant differences between the CT_{max} of field fresh and average sea water temperature treatment groups occurred mostly at 0.5°C min^{-1} (5 out of 8 comparisons), whereas for 0.1°C min^{-1}, only 1 out of 8 comparisons were significant.

In the majority of the species (except *E. antikraussi*) and both *E. laeviusculum* populations, rate of temperature change had a large, significant effect on CT_{max} (Table 3). In general, slower rates of change were associated with a decrease in CT_{max} (Fig. 2 - 5). Significant rate x acclimation interactions (except in *H. grandicornis* and *P. stimpsoni*) indicated complex acclimation effects that varied depending on rate (Table 3; Fig. 2 - 5). At fast rates of change, acclimation at higher temperatures was, generally, associated with an increase in CT_{max}, however, this difference was not always significant (Fig. 2 - 5). At slow rates of change, CT_{max} across acclimation groups was similar or acclimation at higher temperatures caused a decline in CT_{max} (Fig. 2 - 5). Mass and sex did not have a significant effect on the CT_{max} of the majority of the studied groups (Table 3). In the *Hyale* species, larger individuals had a lower CT_{max} than smaller animals, but in *E. antikraussi* larger individuals had a higher CT_{max} than smaller individuals (Table 3). In *E. laeviusculum* from Lamberts Bay, males (n = 221; linear model estimate ± se: 23.83 ± 0.51) had a higher CT_{max} than females (n= 29; 19.80 ± 0.99), however, at 0.0002°C min^{-1} the opposite was found (significant Rate x Sex interaction; Table 3). *E. antikraussi* females (n = 5; 37.98 ± 0.66) had a higher CT_{max} than males (n = 72; 36.33 ± 0.27; Table 3). In *H. hirtipalma* sex did not have an overall effect, but at fast rates of change males had a higher CT_{max} than females, whereas at slow rates the opposite was found (significant Rate x Sex interaction; Table 3). In the Hangklip population of *E. laeviusculum*, males and females demonstrated differing magnitudes of plasticity response accross acclimation treatments, for example, from 11 to 15°C, females increased CT_{max} by 4.4°C, while male's CT_{max} increased by 1.8°C (significant Acclimation x Sex interaction; Table 3).

In *H. grandicornis*, the CT_{max} of slow-rate controls was not significantly different from the CT_{max} of field fresh individuals (Fig. 6). In this species, larger individuals had a lower CT_{max} than smaller animals (Table 4). In both *E. laeviusculum* populations there were significant differences among the CT_{max} of slow-rate controls and field fresh individuals (Table 4; Fig. 6). In these populations, the mean CT_{max} of the 0.0001°C min^{-1} controls (the longest

treatments) differed most from that of field fresh individuals, however, this difference was within 1°C (Table 4; Fig. 6). Large individuals of *E. laeviusculum* from Lamberts Bay had higher CT_{max} than small individuals (Table 4).

The slow-rate CT_{max} controls of *H. hirtipalma* and *H. grandicornis* had higher lipid content than field fresh individuals (Table 5; Fig. 7). In the *E. laeviusculum* populations, the lipid content of slow-rate CT_{max} controls and field fresh individuals differed significantly, however, these differences were small (Table 5; Fig. 8). In the *Hyale* species and *E. laeviusculum* populations, there was a significant positive relationship between lipid content and dry mass (Table 5). In *E. laeviusculum* from Lamberts Bay, the increase in lipid content with dry mass was lower in field fresh individuals (slope = 0.54 ± 0.12) than in the 0.0001 (0.61 ± 0.12), 0.0002 (0.86 ± 0.13), and 0.0007°C min^{-1} (0.80 ± 0.13) controls (significant Treatment x Dry mass interaction; Table 5).

In all acclimation treatments tested at fast rates of change, *H. grandicornis* had a consistently higher CT_{max} than *H. hirtipalma* (Table 6; Fig. 9). In both *Hyale* species, CT_{max} was higher in individuals acclimated at average sea water temperature than those acclimated at low temperature. At 0.5°C min^{-1}, the pattern of acclimation response differed between the two species (significant Species x Acclimation interaction; Fig. 10). For example, the CT_{max} of *H. hirtipalma* increased by 1.99°C from low to average acclimation treatments, whereas the CT_{max} of *H. grandicornis* increased by 0.18°C (Fig. 10). *H. hirtipalma* thus has a greater magnitude of acclimation response than *H. grandicornis* (Fig. 10).

In the majority of the acclimation treatments tested at fast rates of change, the *E. laeviusculum* population from Lamberts Bay had a higher CT_{max} than the Hangklip population (5 out of 8 comparisons; Table 6; Fig. 11). In both *E. laeviusculum* populations at 0.5°C min^{-1}, an increase in acclimation temperature was associated with an increase in CT_{max} and the magnitude of acclimation response in the two populations was similar (Fig. 12). However, at 0.1°C min^{-1}, the patterns of acclimation effect on CT_{max} varied between the two populations (significant Population x Acclimation interaction; Fig. 12). For example, the CT_{max} of *E. laeviusculum* from Lamberts Bay increased by 0.43°C from low to average acclimation treatments, whereas the population from Hangklip remained constant (Fig. 12).

In all acclimation treatments tested at fast rates of change, *E. laeviusculum* populations had a significantly higher CT_{max} than *E. antikraussi* and *E. gigas* (Table 6; Fig. 13). Additionally,

E. antikraussi had a consistently higher CT_{max} than *E. gigas* (Table 6; Fig. 13). In the *Exosphaeroma* species and populations, higher acclimation temperatures were, generally, associated with an increase in CT_{max} (Fig. 14). CT_{max} across acclimation treatments varied between species (significant Species x Acclimation interaction; Fig. 14). For example, at 0.5°C min^{-1}, the CT_{max} of *E antikraussi* and the two *E. laeviusculum* populations changed by 0.39 - 0.46°C from average to high acclimation treatments while *E. gigas*' CT_{max} increased by 3.92°C. *E. gigas* thus had a higher magnitude of acclimation response than *E. antikraussi* and the *E. laeviusculum* populations. In addition, *E. antikraussi* had a slightly higher magnitude of acclimation response than *E. laeviusculum* from Hangklip (Fig. 14). At 0.5°C min^{-1}, larger *Exosphaeroma* individuals had a higher CT_{max} than smaller animals (Table 6). At 0.1°C min^{-1}, the CT_{max} of *E. antikraussi* increased with an increase in mass (slope = 0.16 ± 0.25), whereas in *E. gigas* and *E. laeviusculum* populations from Hangklip and Lamberts Bay, the opposite was found (- 0.76 ± 0.32, - 0.22 ± 0.42, and -0.07 ± 0.35, respectively; significant Species/Population x Mass interaction; Table 6).

At fast rates of change, *H. hirtipalma*, which inhabits exposed and submerged shore positions on Marion Island, had a consistently higher 'warming tolerance' than *H. grandicornis* from semi-exposed sites in South Africa (Table 7). The 'warming tolerance' of the Lamberts Bay population of *E. laeviusculum* was consistently higher than that of the Hangklip population (semi-exposed and submerged microsites for both populations; Table 7). *E. gigas*, which is a typically submerged species, had a consistently higher 'warming tolerance' than *E. antikraussi* and the *E. laeviusculum* populations (Table 7). In comparison to the other studied groups (including the Hangklip population of *E. laeviusculum*, that inhabits semi-exposed and submerged microsites), *E. antikraussi* that inhabits semi-exposed sites at Hangklip, consistently has the lowest 'warming tolerance' (Table 7).

The majority of the studied groups are likely to experience a mean and maximum rate of temperature increase that is between the fast and slow-rates of change featured in this study (Table 8). Only those species in semi-exposed positions at Hangklip and Lamberts Bay will experience maximum rates of temperature increase that are equal or close to the fast rates of change employed (Table 8). Marion Island species experience a lower mean, minimum and maximum temperature than South African congeners (Table 8). At an exposed position on the shore, Marion Island species experience a much lower range of temperatures than South African congeners, but at a submerged position, temperature range on Marion Island is only slightly lower than that in South Africa (Table 8; Fig.15). The degree of thermal variation to

which Marion Island species are exposed is greater or similar to that experienced by South African organisms, especially for species occurring in exposed microsites (Table 8). At semi-exposed and submerged positions, the mean and maximum temperatures experienced by organisms at Hangklip are higher than at Lamberts Bay, however, organisms at the latter site are exposed to a slightly greater thermal range (Table 8). The degree of thermal variation experienced by organisms at semi-exposed and exposed positions at Lamberts Bay is higher or similar to that at Hangklip (Table 8). At Hangklip and Lamberts Bay, organisms at the semi-exposed positions experience a greater mean and maximum temperature, as well as a greater thermal range and a higher degree of thermal variability than those that are submerged (Table 8).

Discussion

Effects of rate of change on CT_{max} and acclimation response

In general, a decrease in rate of temperature change is associated with a decline in the thermal tolerance of ectotherms (Mora and Maya, 2006; Terblanche et al., 2007; Chown et al., 2009; Mitchell and Hoffmann, 2010). The results of this study on South African and sub-Antarctic marine invertebrates are in keeping with these findings, as well as those of a study on Antarctic marine invertebrates by Peck et al. (2009). However, in insects and terrestrial arthropods, slow rates of change have also been associated with an increase in thermal tolerance (Kelty and Lee, 1999; Overgaard et al., 2006; Powell and Bale, 2006). Improved thermal tolerance at slow rates has been attributed to the increased period of time available for hardening (Kelty and Lee, 1999; Overgaard et al., 2006; Powell and Bale, 2006; Sgrò et al., 2010). By contrast, Peck et al. (2009) speculated that decreased thermal tolerance at slower rates may be attributed to the importance of differing survival mechanisms at different rates of change. Resistance mechanisms (e.g. heat hardening) may be important at fast rates, but at slow rates acclimation or evolutionary adaptation may be essential to survival (Peck et al., 2009). The acclimation response of the upper thermal tolerance of the ant *Linepithema humile* was found to vary based on rate of change (Chown et al., 2009). In this species, fast rates of change were associated with small acclimation responses, while the opposite was found at slow rates (Chown et al., 2009). Most marine invertebrates in this study demonstrated acclimation responses that varied based on rate of change. However, in contrast to the findings of Chown et al. (2009), acclimation at high temperatures in marine invertebrates tended to improve thermal tolerance at fast rates, whereas at slow rates, acclimation at high temperatures either worsened thermal tolerance or had no effect. At slow rates of change, organisms may begin to lose pre-experiment acclimation effects and may acclimate to slowly increasing experimental temperatures (Cocking, 1959). Indeed, at slow rates of change a trade-off between the amount of time required for full acclimation and the period of exposure to near lethal temperatures may influence thermal tolerances (Cocking, 1959). Marine invertebrates in this study may have lost pre-acclimation effects and acclimated during slow rates of change, but acclimation effects may have been overshadowed by time spent at near lethal temperatures (Cocking, 1959; Beitinger et al., 2000). Therefore, contrasting results from different studies may reflect differences in the acclimation periods and rates of change used relative to the study organism, as differences in the time course of acclimation and the period of the acclimation treatment relative to the organism's life span may affect various mechanisms that influence thermal tolerance and the trade-offs discussed above (Rezende et al., 2011; Terblanche et al., 2011).

Energy reserves or body condition in slow-rate experiments may, however, interact with captivity period and affect thermal limits (Mora and Maya, 2006; Rezende et al., 2011). This study showed that there was no difference between the CT_{max} of slow-rate CT_{max} experiment controls and field fresh individuals of *H. grandicornis*, and only small differences (< 1°C) were found in *E. laeviusculum* populations. Therefore, the large decline in CT_{max} at slow rates of temperature change (by 20°C for *H. grandicornis*, 18 and 19°C for *E. laeviusculum* from Hangklip and Lamberts Bay, respectively) is unlikely to have been caused by the effect of captivity period on thermal tolerance. Furthermore, fat content analysis of control and field fresh organisms demonstrated that control organisms kept in captivity either had a higher or slightly lower fat content in comparison to field fresh individuals. Based on these results, the body condition of experimental animals may have improved or been unaffected during slow-rate experimental trials. However, given that increased temperature causes an increase in metabolic rate and thus energetic demands, it is questionable whether the body condition of experimental individuals was affected in the same way as controls which were maintained at a constant temperature (Mora and Maya, 2006; Rezende et al., 2011). Despite a constant food supply, a decline in the body condition of organisms in slow-rate temperature trials has been noted in other studies. For example, even though slow-rate trial and control individuals of the fish *Acantemblemaria hancocki* received a steady supply of food, after only a few days within experimental trials, experimental fish were more voracious and bonier than control individuals (Mora and Maya, 2006). It is not certain whether the organisms in this study demonstrated a similar temperature related decline in body condition. However, given the data obtained for lipid content and the CT_{max} of control and field fresh individuals, it is unlikely that the outcomes of the slow-rate CT_{max} trials are due to body condition alone.

As in studies on other organisms (e.g. Terblanche et al., 2007; Mitchell and Hoffmann, 2010), ecologically relevant rates of change for the marine invertebrates of Marion Island and South Africa are, generally, slower than the standard rates employed in typical thermal tolerance studies. Given the data collected at the microsites relevant for each species, the organisms in this study are exposed to rates of change intermediate of the fast and slow rates featured in this study, and fast rates will only be experienced in extreme circumstances by the South African species restricted to the upper inter-tidal zone. Thus in nature, the upper thermal tolerance of these organisms, as well as acclimation responses are likely to be intermediate of those recorded at the fastest and slowest rates of change. At such intermediate rates of change, upper thermal tolerance is likely to be higher than the mean and maximum

temperatures experienced by these organisms in the field. The functioning of these marine invertebrates will thus only be negatively affected during extreme events, and in such instances resistance mechanisms (e.g. heat hardening) or acclimation may be important to survival. Although acclimation responses may be slightly reduced from those at fast rates of change, in nature these organisms may still be able to respond to change though phenotypic plasticity.

It has been argued that studies featuring trials with slow rates of change are relevant for investigating organism response to climate warming (Peck et al., 2009). At the slowest rate of change featured in this study (1°C/6 days), upper thermal tolerance declined to the extent to which it approximated habitat temperature. At such rates, the amount of warming these marine invertebrates can withstand thus decreases, which may lead to consequences for survival that are unlikely to be alleviated through acclimation responses. However, in such instances other responses such as evolutionary adaptation or movement to favourable habitats may allow for survival (Parmesan, 2006). Furthermore, at slow rates of change a decrease in the heritability of thermal tolerance in *Drosophila melanogaster* has been demonstrated, and thus organisms experiencing slow rates of change may struggle to mount an evolutionary response (Mitchell and Hoffmann, 2010). However, these results must be taken with caution, as the influence of rate of change on heritability might be species-specific (Chown et al., 2009).

Thermal tolerance and plasticity response at various hierarchical and geographical scales

Marine invertebrates living in warm environments (e.g. tropics), generally, have higher upper thermal tolerances and reduced acclimation responses in comparison to organisms living in cooler areas (e.g. mid-latitudes) (Stillman, 2003; Sorte and Hofmann, 2005; Somero, 2010). At large spatial scales and at fast rates of change, the marine invertebrates featured in this study corroborate with this pattern. South African species experience higher mean and maximum temperatures and have a higher upper thermal tolerance, but lower magnitude of plasticity than congeners from Marion Island (Fig. 16). The costs associated with maintaining a high upper thermal tolerance may lead to trade-offs, and species of the crab genus *Petrolisthes* that have evolved the highest upper thermal tolerances have done so at the expense of plasticity responses (Stillman, 2002; Hoffmann et al., 2003; Stillman, 2003). However, the differing plasticity responses of Marion Island and South African congeners may also be attributed to the degree of environmental thermal variation experienced. In comparison to those inhabiting thermally variable regions (e.g. mid-latitudes), marine

invertebrates living in thermally stable environments (e.g. tropics and polar regions) demonstrate little or no plasticity in upper thermal tolerance (Stillman, 2003; Peck et al., 2010). Despite the generally thermally stable climate of Marion Island (Smith, 2002; Chown and Froneman, 2008), microclimate data in the intertidal zone indicated that Marion Island marine invertebrates experience a higher or similar degree of thermal variability than South African congeners, and thus the plasticity responses of Marion Island species may be expected.

As brooding organisms do not have a planktonic larval stage that may promote long distance dispersal, local adaptation of basal thermal tolerance and variation in the degree of plasticity response may be more prominent in populations living in distinct thermal environments (Gaston and Spicer, 1998; Sherman et al., 2008; Sanford and Worth, 2010). For example, the upper thermal tolerance of the direct developing limpet *Nucella lapillus* varies over relatively small spatial scales (Davenport and Davenport, 2005). Additionally, populations of marine invertebrates (e.g. the amphipod *Orchestia gammarellus* and the limpet *Nucella lapillus*) living at higher environmental temperatures, generally, have higher upper thermal tolerances than those living in cooler areas (Gaston and Spicer, 1999; Davenport and Davenport, 2005; Sorte et al., 2011). Surprisingly, the upper thermal tolerance of an *E. laeviusculum* population living on the cold West coast (Lamberts Bay) of South Africa was higher than that of a warmer South coast population (Hangklip). In addition, these populations demonstrate similar magnitudes of plasticity (Fig. 16). In accordance with macroclimate temperature data, microclimate data indicated that the mean and maximum temperatures experienced by *E. laeviusculum* on the West coast are lower than on the South coast. By contrast, while macroclimate temperature data show that the West and South coasts of South Africa demonstrate a similar degree of sea surface temperature variability (Demarcq et al., 2011), microclimate data from this study, indicated that infra-tidal organisms on the West coast will be exposed to a greater thermal range and, at submerged positions, to a higher degree of thermal variability than populations on the South coast. Therefore, organisms on the West coast may have responded to the more thermally variable environment through the broadening of the thermal tolerance range and maintenance of plastic responses (Angilletta, 2009). For example, *Drosophila buzzatii* populations that experience more variable environments have a wider thermal tolerance range than those experiencing higher temperatures, but lower degrees of thermal variation (Sørensen et al., 2001). Additionally, rocky shore marine invertebrates with the highest upper thermal tolerances tend to also demonstrate the lowest minimum thermal tolerances (Davenport and Davenport, 2005). The

West and South coast populations of *E. laeviusculum* may thus be exhibiting local adaptation. This result is not surprising as although isopod species may raft great distances on seaweeds and kelps (Leese et al., 2010; Nikula et al., 2010), *E. hylecoetes*, a congeneric South African species, exhibits little gene flow between populations (Teske et al., 2007). It must, however, be noted that the suggestion of local adaptation made here must be taken with caution, as genetic information for this species is not available, and thus the degree of differentiation between populations is not known at present.

In the intertidal zone, organisms inhabiting high shore positions that are exposed more often to extreme, high air temperatures, have in general higher upper thermal tolerances and lower acclimation capacities than low shore organisms (Tomanek and Somero, 1999; Stillman and Somero, 2000; Stillman, 2003; Davenport and Davenport, 2005; Somero, 2010). By contrast, *E. antikraussi* from the upper inter-tidal zone at Hangklip has a lower upper thermal tolerance but slightly higher magnitude of plasticity than the inter- to infra-tidal species *E. laeviusculum* (Fig. 16). As *E. laeviusculum* is not strictly a sub-tidal species, this organism may at times be exposed to the higher microclimate temperatures experienced by *E. antikraussi* in the upper inter-tidal zone. Furthermore, *E. antikraussi* may be exhibiting behavioural thermoregulation, and during low tides is found in the shaded, moist areas under rocks which, due in part to evaporative cooling, may offer a degree of thermal buffering (Stillman and Somero, 1996). *E. antikraussi* thus may experience lower temperatures than *E. laeviusculum* which, during low tide, is found on rocks in open shallow pools. Thermal buffering by rocks may play a role in reducing the temperatures experienced by the crab *Petrolisthes eriomerus*, which is exposed to air temperatures during spring low tides but still maintains a lower upper thermal tolerance than the upper inter-tidal species *P. cinctipes* (Stillman and Somero, 1996). Additionally, during temperature extremes the crayfish *Orchinectes rusticus* burrows underneath rocks where temperatures are lower than in shallow pools (Mundahl, 1989). As *E. laeviusculum* is found in potentially warmer pools at low tide, and has a higher upper thermal tolerance than *E. antikraussi*, this species may have developed a higher upper thermal tolerance at the expense of plasticity responses. This pattern is evident in various marine invertebrate species that inhabit the inter-tidal zone and, for example, species of the marine snail *Tegula* that have high upper thermal tolerances also have reduced plasticity responses (Tomanek and Somero, 1999; Stillman, 2003; Bedulina et al., 2010). Additionally, the upper inter-tidal species *P. stimpsoni* exhibits no acclimation response. This species is found under dry rocks at low tide, which potentially offer less buffering from temperature extremes than the moist rocks under which *E. antikraussi* is found. Like *E.*

laeviusculum, *P. stimpsoni* may have a high upper thermal tolerance at the expense of plasticity responses. It must be noted, however, that these differences may also be due to physiological differences that reflect phylogenetic relationships (Garland and Adolph, 1994; Spicer and Gaston, 1999).

This study showed that the amphipod *H. grandicornis* was particularly sensitive to increases in temperature, and had the lowest temperature tolerance among the South African species examined. Behavioural responses may be the principal mechanism by which this upper intertidal species withstands extreme temperatures. All slow-rate experiments on *H. grandicornis* concluded within days of each other (~ 2 - 6 days), and at the slowest rates of change (1°C/6 days and 1°C/3 days), the CT_{max} of these organisms drastically decreased (from ~ 30°C at 1°C/day to 18.5 and 20.3°C at 1°C/6 days and 1°C/3 days, respectively), with no significant difference between 1°C/3 days and 1°C/6 days. Furthermore, high mortality was observed in these organisms when acclimated at 19°C for 13 days. Despite a significant acclimation effect on CT_{max} at the fastest rate of change, *H. grandicornis* has an inability to tolerate long periods at a constant temperature above 15°C, and showed limited plasticity. The mean (17.5 ± 2.7°C) and maximum (33.2°C) microclimate temperatures recorded for the upper inter-tidal zone on the South coast of South Africa exceed 15°C, and during low tides, *H. grandicornis* is found in seaweeds that may act as thermal refuges (Griffin et al., 1999; Branch et al., 2007). Additionally, on the south-western shores of South Africa, McQuaid and Branch (1984) reported that *H. grandicornis* prefers cold water beaches, and thus these organisms may move to colder waters when environmental temperatures are unsuitable. The congener *H. media* also demonstrates behavioural responses to environmental extremes and, to avoid desiccation, seeks refuge under limpets (Underwood and Verstegen, 1988).

Inter-individual variation in upper thermal tolerance and plasticity responses was also evident in this study, and species specific variation based on sex and body size was noted. Previous work has shown differing relationships or no relationship between these factors (sex and body size) and thermal tolerance (see Bradley, 1978; Gaston and Spicer, 1998; Sørensen et al., 2001; Winne and Keck, 2005; Peck et al., 2008; Nyamukondiwa and Terblanche, 2009; Peck et al., 2009). This study showed that in a few cases, mass had an effect on CT_{max}, and in the amphipod species larger individuals were more tolerant than small individuals. The opposite trend was, however, found for the one isopod species in which a mass effect was found. While the mass effects were fairly straightforward, sex effects within species were more complex. In *E. laeviusculum* from Lamberts Bay, males were generally more tolerant

than females, but in *E. antikraussi* the opposite was found. Interactions between rate of change and sex, and acclimation and sex indicated that trade-offs may exist between the amount of energy allocated to the potentially different overall maintenance costs (possibly due to differences in reproduction costs) of the sexes and acclimation responses (to both pre-experimental acclimation treatments and to increasing temperatures in slow-rate trials) (Clarke, 2003). Similarly, the temperature at which a heat hardening response is expressed in *Drosophila buzzatii* males, is lower than in females (Sørensen et al., 2001).

The upper thermal tolerances of *Hyale* and *Exosphaeroma* species thus demonstrate intra- and inter-specific variation at various geographical scales. In the face of climate change, such variation may be important as the vulnerability of populations or species to increased temperature will also vary. At large spatial scales, the 'warming tolerance' and magnitude of plasticity of Marion Island species is greater than that of South African congeners, and thus Marion Island species may be less vulnerable to future increases in temperature. At smaller spatial scales and at semi-exposed and submerged positions on the shore, the West coast population of *E. laeviusculum* has a higher 'warming tolerance' than the South coast population, and thus may be less vulnerable to increases in temperature. Furthermore, within the inter-tidal zone, *E. laeviusculum* has a higher 'warming tolerance' than *E. antikraussi*, but as *E. antikraussi* has a higher magnitude of plasticity, this species may mount acclimation responses that will enable persistence through temperature change. The intra- and inter-specific variation identified in this study may be critical in times of change, and less vulnerable species or populations (e.g. Marion Island species and West coast *E. laeviusculum* population) may act as a buffer and ensure the survival of species or genera (Chown et al., 2010b; Somero, 2010).

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Chapter 3

General Conclusion

Studies featuring slow rates of change have, generally, demonstrated that thermal tolerance declines with a decrease in rate of change (Mora and Maya, 2006; Terblanche et al., 2007; Chown et al., 2009; Peck et al., 2009; Mitchell and Hoffmann, 2010). Corroborating these studies, the marine invertebrates in this study demonstrated declines in thermal tolerance and plasticity responses with a decrease in rate of change. The large impact of rate of change on these factors may consequently influence the vulnerability of organisms to increases in temperature. If the vulnerability and potential response of organisms to temperature change are predicted based on data obtained at fast-rates of change, vulnerability may be underestimated and incorrect predictions may be made. As climate change involves slow rates of temperature change, in order to better understand and predict the influence of these changes, more studies such as this and that by Peck et al. (2009), that employ very slow rates of change are required. Such studies, however, involve long periods of data collection, and thus to be more effective, the organisms studied should be carefully selected given their ecological importance, and sites investigated should include regions likely to be impacted by changes to climate.

Methodology may have a large effect on the results of thermal tolerance studies (Beitinger et al., 2000; Chown and Nicolson, 2004; Terblanche et al., 2007; Chown et al., 2009). As in *Glossina pallidipes* and various other organisms, the marine invertebrates of Marion Island and South Africa are exposed to rates of temperature change that are slower than those generally used in thermal tolerance studies (Terblanche et al., 2007; Mitchell and Hoffmann, 2010). Due to the large influence of rate of change on results, this factor must be considered when developing study protocols, and either ecologically relevant rates must be employed, or the rates utilized must be pertinent to the questions at hand (Beitinger et al., 2000; Terblanche et al., 2007; Chown et al., 2009). Methodology must also be taken into account when intra- or inter-specific comparisons are made, and in such instances it may be preferred to utilize results obtained at the same rate of change (Chown et al., 2009). It must be noted, however, that in large scale comparisons, the variability introduced by methodology may be small in relation to inter-specific differences, and can be accounted for in statistical analyses (Sunday et al., 2011).

Although slow rates of change may be more ecologically relevant, studies featuring slow rates have been criticized as various factors (e.g. nutrition status and captivity period) may confound results (Mora and Maya, 2006; Rezende et al., 2011). In this study, marine organisms were kept in controlled conditions, in which the temperature and dissolved oxygen

and salinity content of the sea water were closely monitored. Furthermore, the effects of captivity period and nutrition status were controlled for, and were found to have had little or no effect on thermal tolerance.

The thermal tolerances and acclimation responses of organisms are influenced by the thermal environment experienced, and thus may vary spatially with temperature patterns (Spicer and Gaston, 1999; Chown and Terblanche, 2007; Chown et al., 2010a). The thermal tolerances, plasticity responses and 'warming tolerances' of the marine invertebrates in this study were found to vary over various geographical scales. This variation, however, did not always follow the patterns predicted based on macroclimate temperature data. As organisms only experience their immediate environment, it is unlikely that they are exposed to the temperatures observed at large spatial scales, and thus the thermal tolerances of many species do not follow the large scale predicted patterns (Spicer and Gaston, 1999; Helmuth et al., 2005; Helmuth, 2009). Microclimate data provide a better indication of the thermal environment experienced by organisms (Helmuth et al., 2002; 2010). Indeed, in this study microsite temperature better matched the thermal tolerance and plasticity responses of the species studied despite the likely buffering of environmental variation via behavioural thermoregulation and the occurrence of rare weather or oceanic events, e.g. greatly influencing the duration and timing of exposure to temperature variation. (Spicer and Gaston, 1999; Helmuth et al., 2002; 2005; Tewksbury et al., 2008; Broitman et al., 2009; Somero, 2010).

The genera studied also exhibited intra- and inter-specific variation in upper thermal tolerance, plasticity response and 'warming tolerance'. This variation may be extremely important to the survival of genera and species during times of temperature change, and thus must be studied and considered in mechanistic predictions (Chown et al., 2010b; Somero, 2010; Denny et al., 2011). Species and population comparisons may be confounded by the relatedness of the studied groups, and are greatly improved when more than two species or populations are compared (Garland and Adolph, 1994; Spicer and Gaston, 1999). This study would thus have benefitted from the inclusion of additional species and populations and the incorporation of the relevant phylogeny to account for species/populations relatedness (Garland and Adolph, 1994). Currently very little genetic work has been done on the marine amphipods and isopods of South Africa, and morphological descriptions are used for species identifications. Despite the availability of taxonomic guides on the South African isopods and amphipods (see Griffiths, 1976; Kensley, 1978), it is also likely that many species have been

overlooked. Further work requires improved information on taxonomy and biogeographical distributions, with the aim to enhance predictions on these organisms' responses to climate change at local and global scales.

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Figures

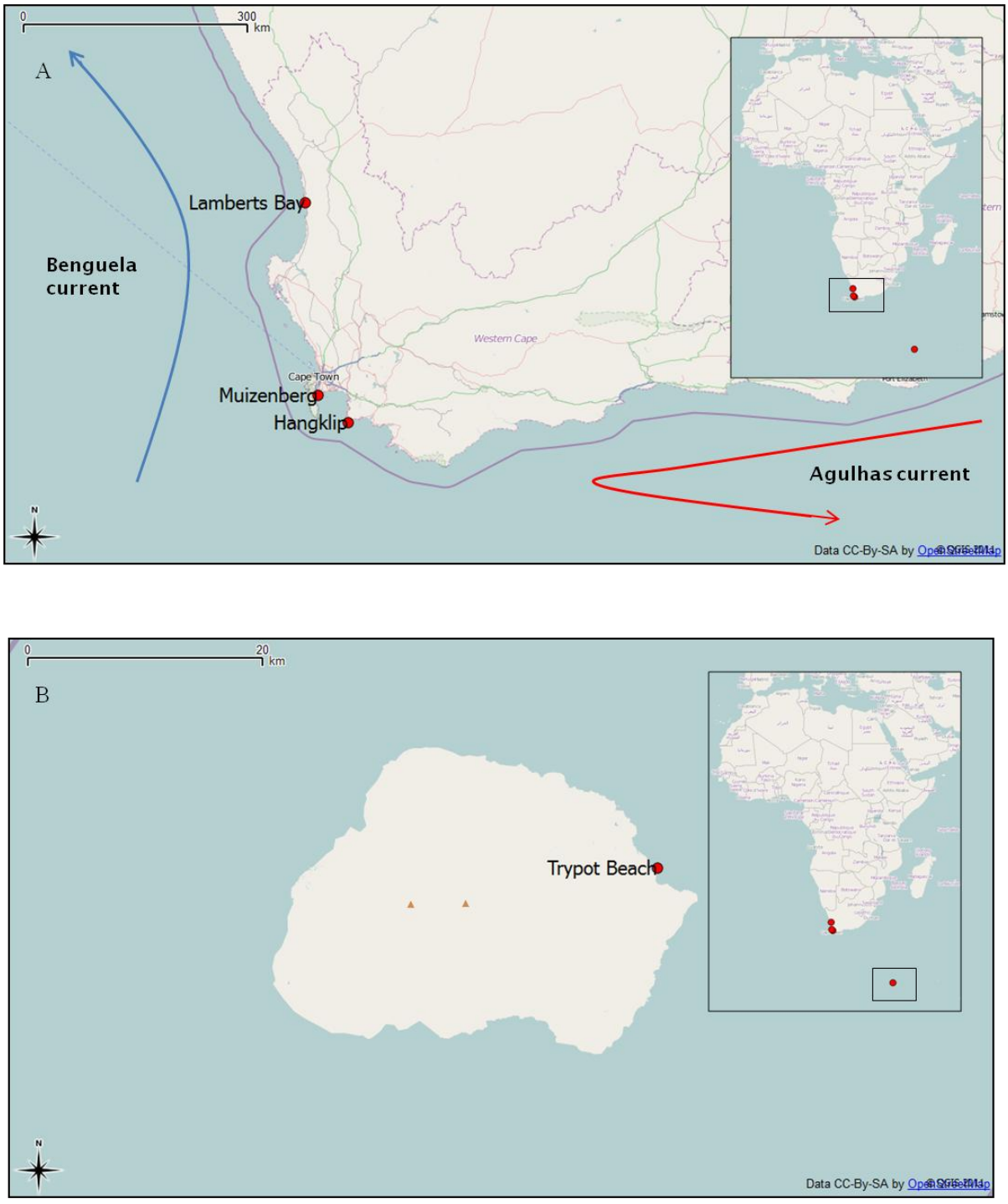

Fig. 1. Maps of South Africa (A) and Marion Island (B) indicating the study collection sites (Quantum GIS 1.7.1; QGIS Development Team).

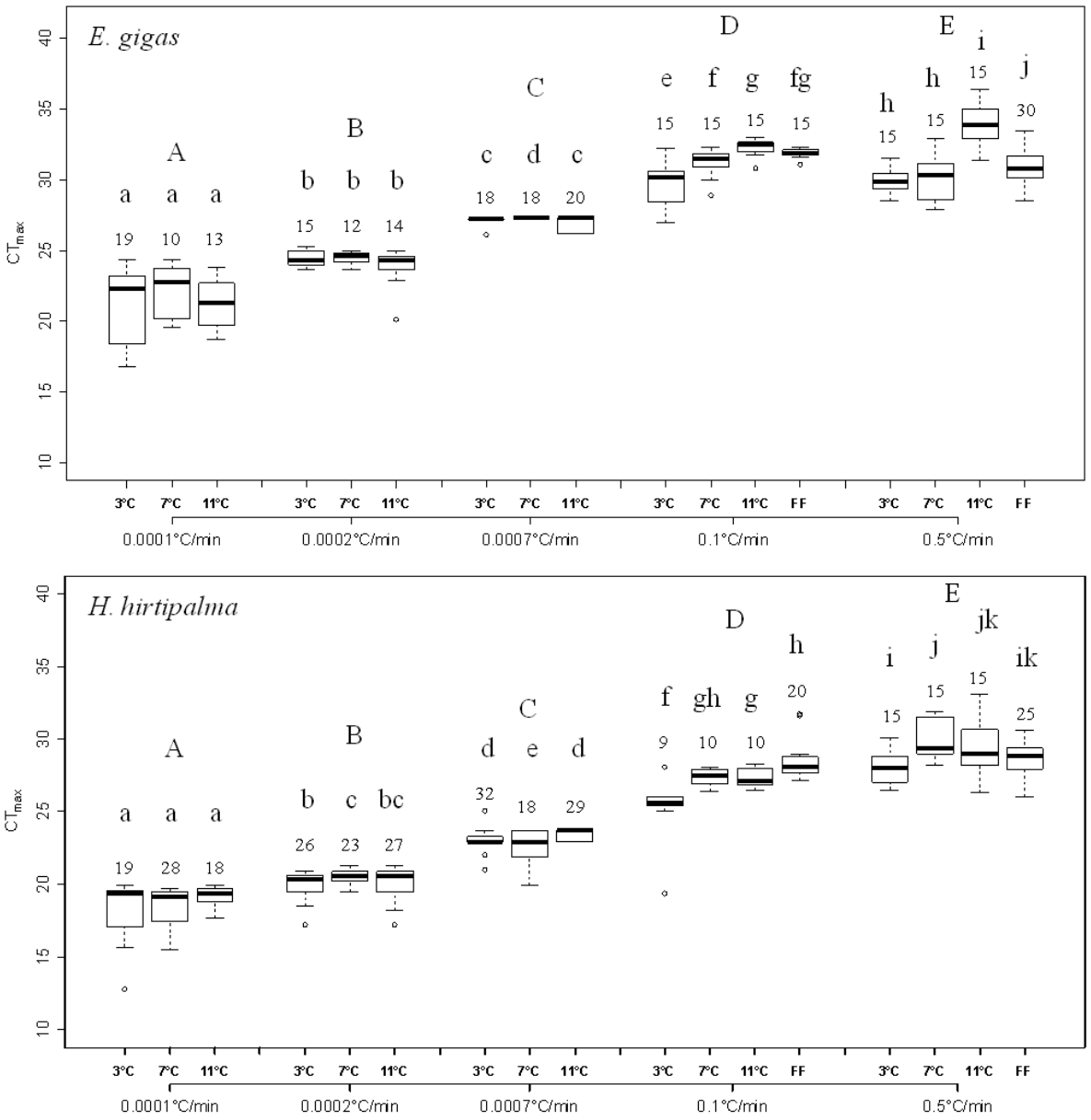

Fig. 2. Effect of rate of temperature change and acclimation on the CT_{max} (median ± 25th and 75th percentiles) of Marion Island species, *Exosphaeroma gigas* and *Hyale hirtipalma*. FF denotes field fresh individuals. Numbers indicate sample sizes within each treatment. Differing upper and lower case letters indicate significant differences among rates of change and among acclimations within each rate of change, respectively (significance set at $p < 0.05$; see methods p. 42).

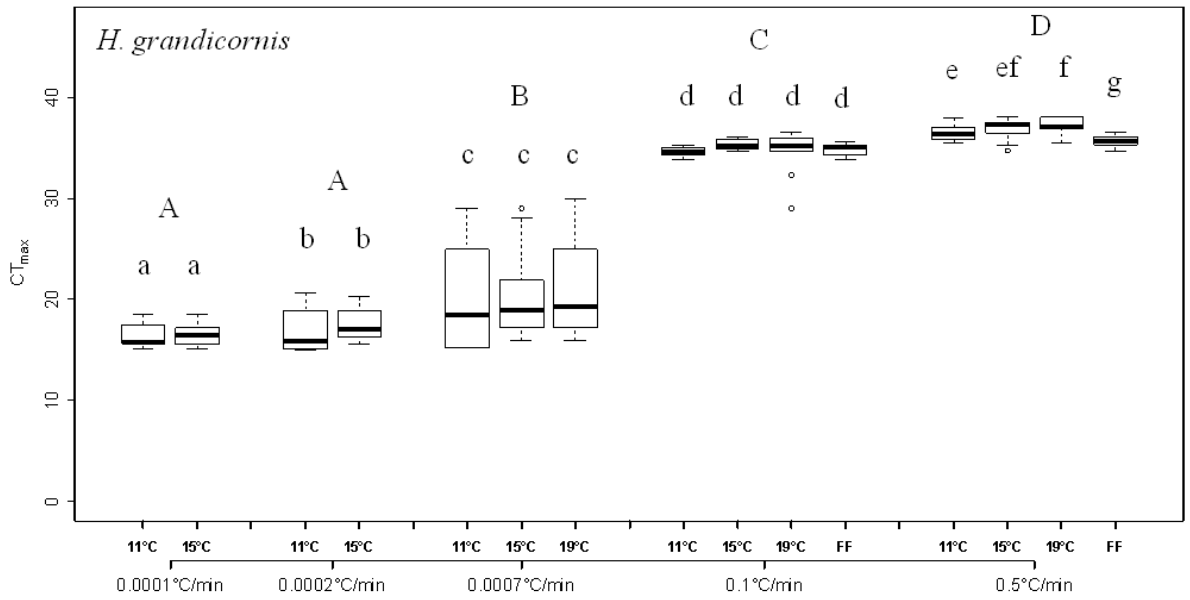

Fig. 3. Effect of rate of temperature change and acclimation on the CT_{max} (median ± 25th and 75th percentiles) of the South African species, *Hyale grandicornis*. FF denotes field fresh individuals. Numbers indicate sample sizes within each treatment. Differing upper and lower case letters indicate significant differences among rates of change and among acclimations within each rate of change, respectively (significance set at $p < 0.05$; see methods p. 42).

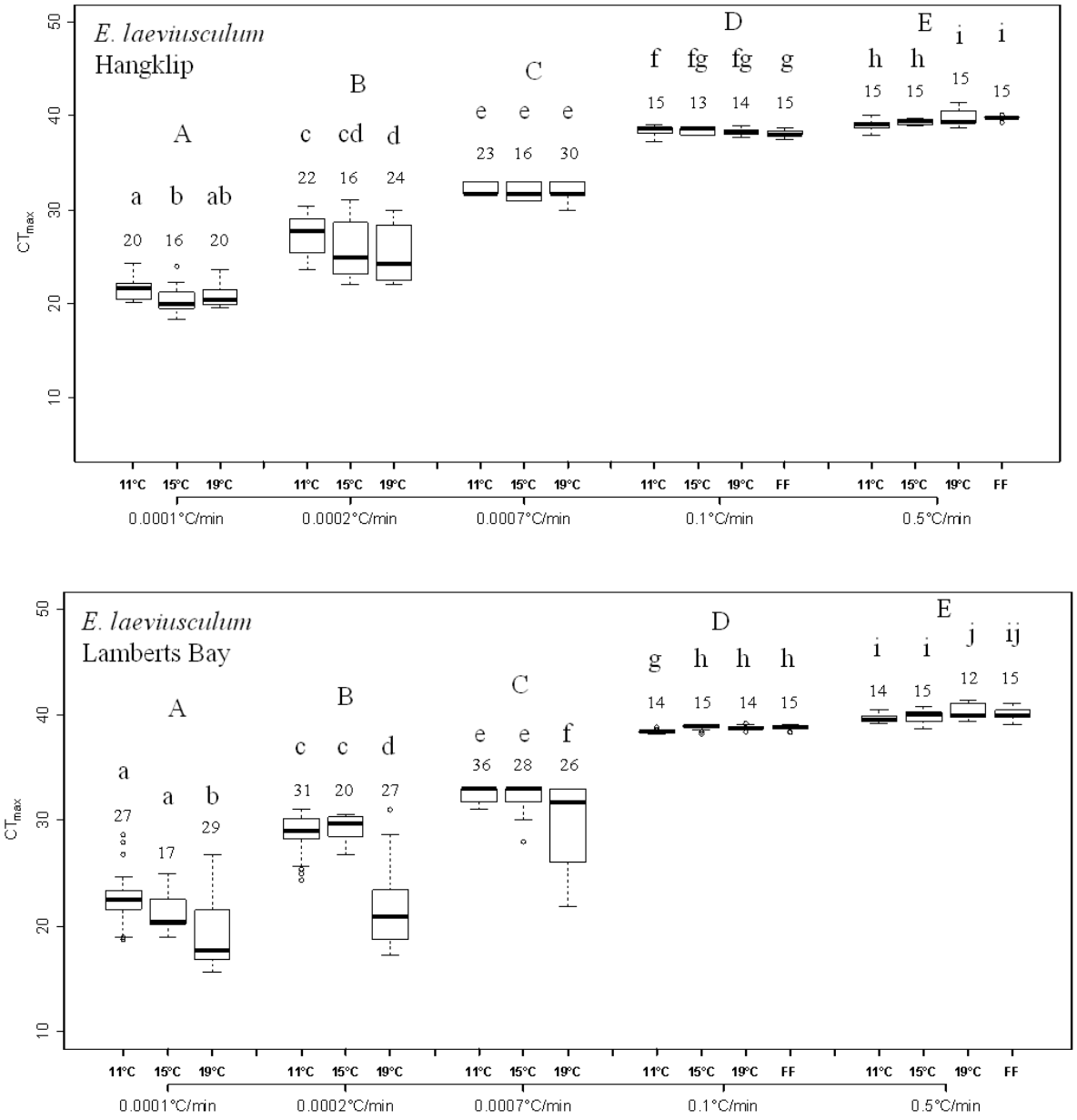

Fig. 4. Effect of rate of temperature change and acclimation on the CT_{max} (median ± 25th and 75th percentiles) of populations of the South African species, *Exosphaeroma laeviusculum*. FF denotes field fresh individuals. Numbers indicate sample sizes within each treatment. Differing upper and lower case letters indicate significant differences among rates of change and among acclimations within each rate of change, respectively (significance set at $p < 0.05$; see methods p. 42).

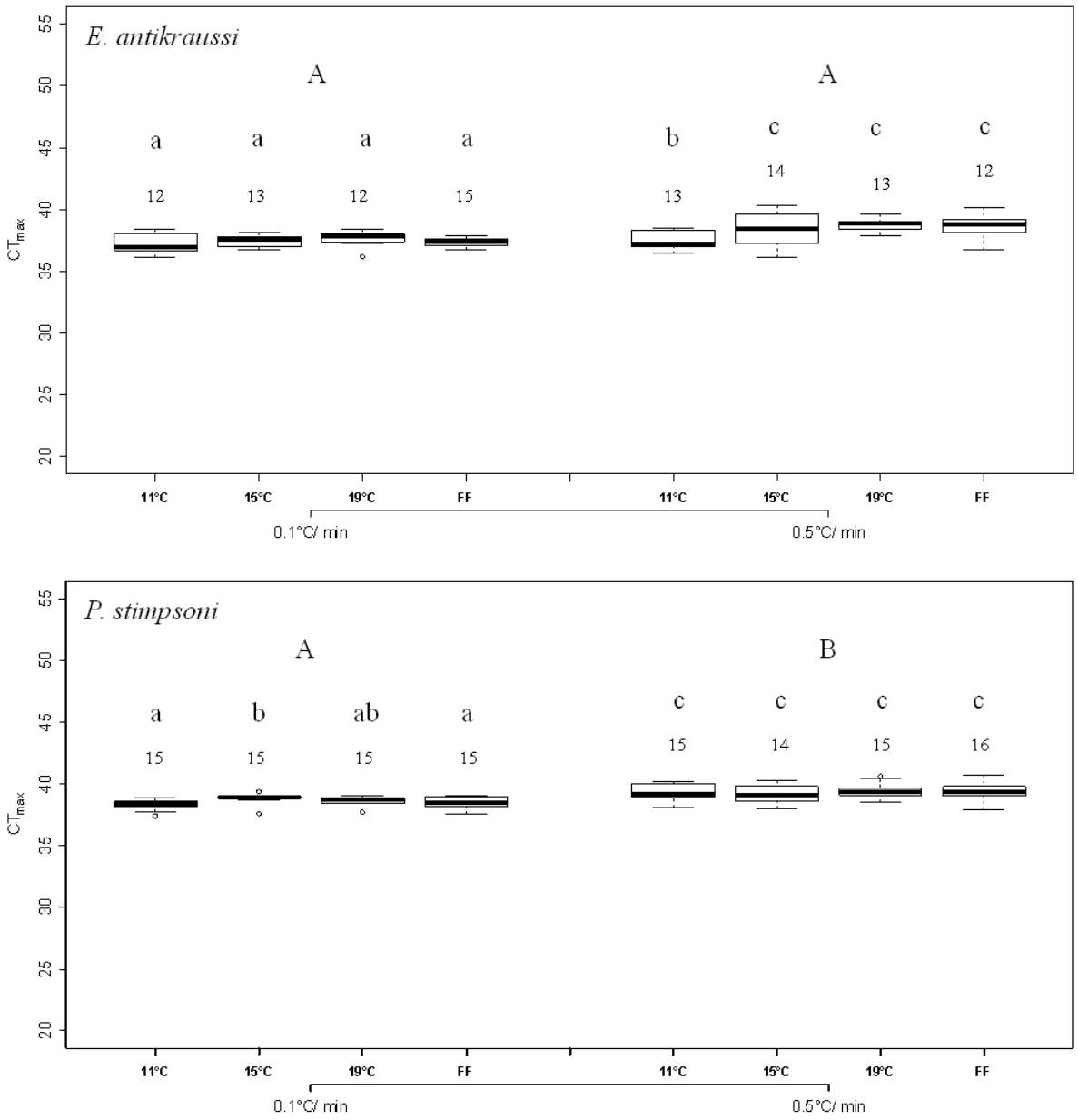

Fig. 5. Effect of rate of temperature change and acclimation on the CT_{max} (median ± 25th and 75th percentiles) of the South African species, *Exosphaeroma antikraussi* and *Parisocladus stimpsoni*. FF denotes field fresh individuals. Numbers indicate sample sizes within each treatment. Differing upper and lower case letters indicate significant differences among rates of change and among acclimations within each rate of change, respectively (significance set at $p < 0.05$; see methods p. 42).

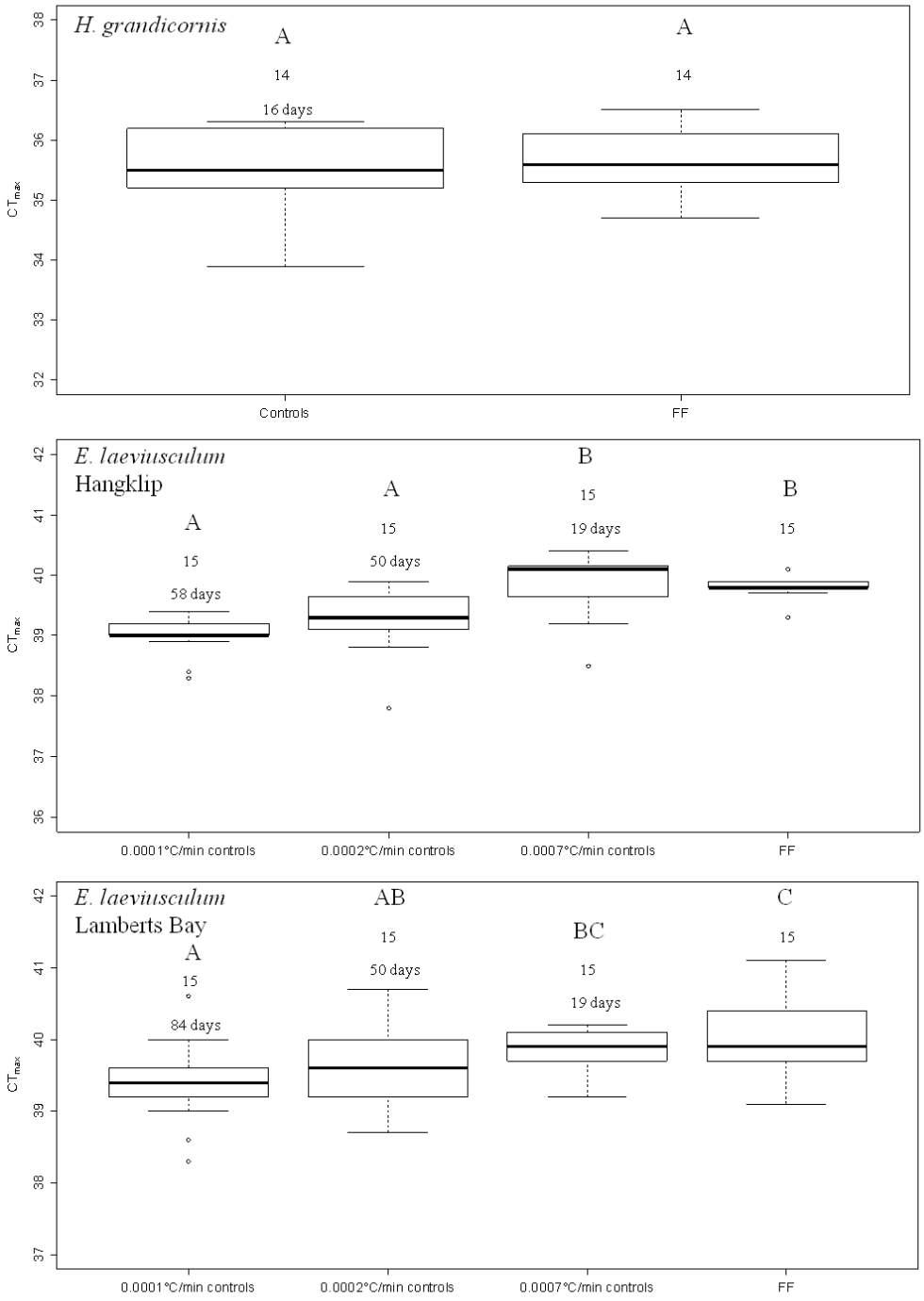

Fig. 6. The CT_{max} (median ± 25th and 75th percentiles) of slow-rate CT_{max} controls and field fresh individuals (FF) of *H. grandicornis* and *E. laeviusculum* populations. Control individuals were kept at a constant temperature (11°C for *H. grandicornis* and 15°C for *E. laeviusculum*) throughout the slow-rate CT_{max} experimental trials. Sample sizes within each treatment, as well as captivity period are presented. Differing upper case letters indicate significant differences between the treatment groups (significance set at $p < 0.05$; see methods p. 42).

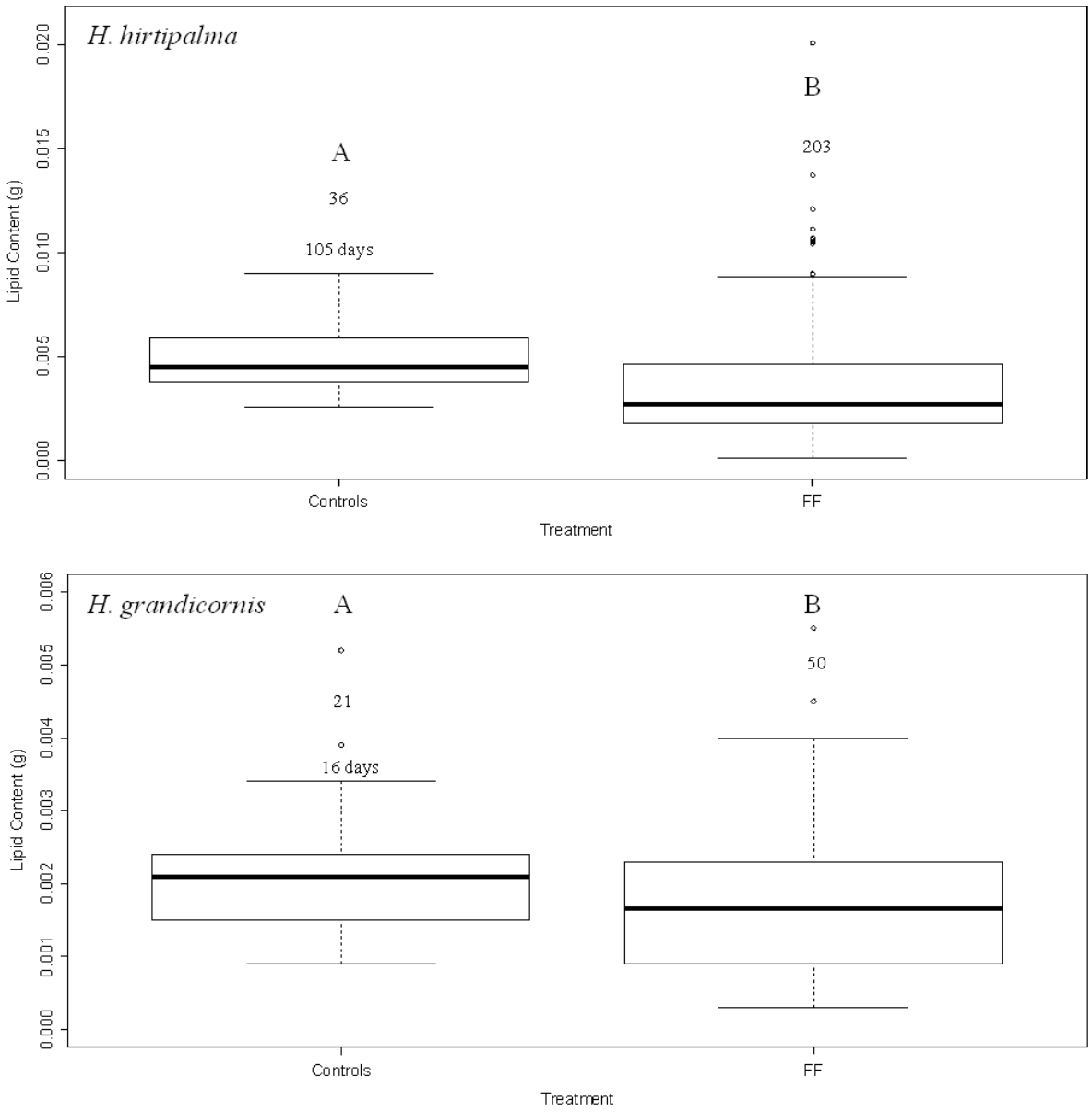

Fig. 7. The lipid content (median ± 25th and 75th percentiles) of slow-rate CT_{max} and field fresh (FF) individuals of *H. hirtipalma* and *H. grandicornis*. Control individuals were kept at a constant temperature (7°C for *H. hirtipalma* and 11°C for *H. grandicornis*) throughout the slow-rate CT_{max} experimental trials. Sample sizes within each treatment, as well as captivity period are presented. Differing upper case letters indicate significant differences between treatment groups (significance set at $p < 0.05$; see methods p. 42).

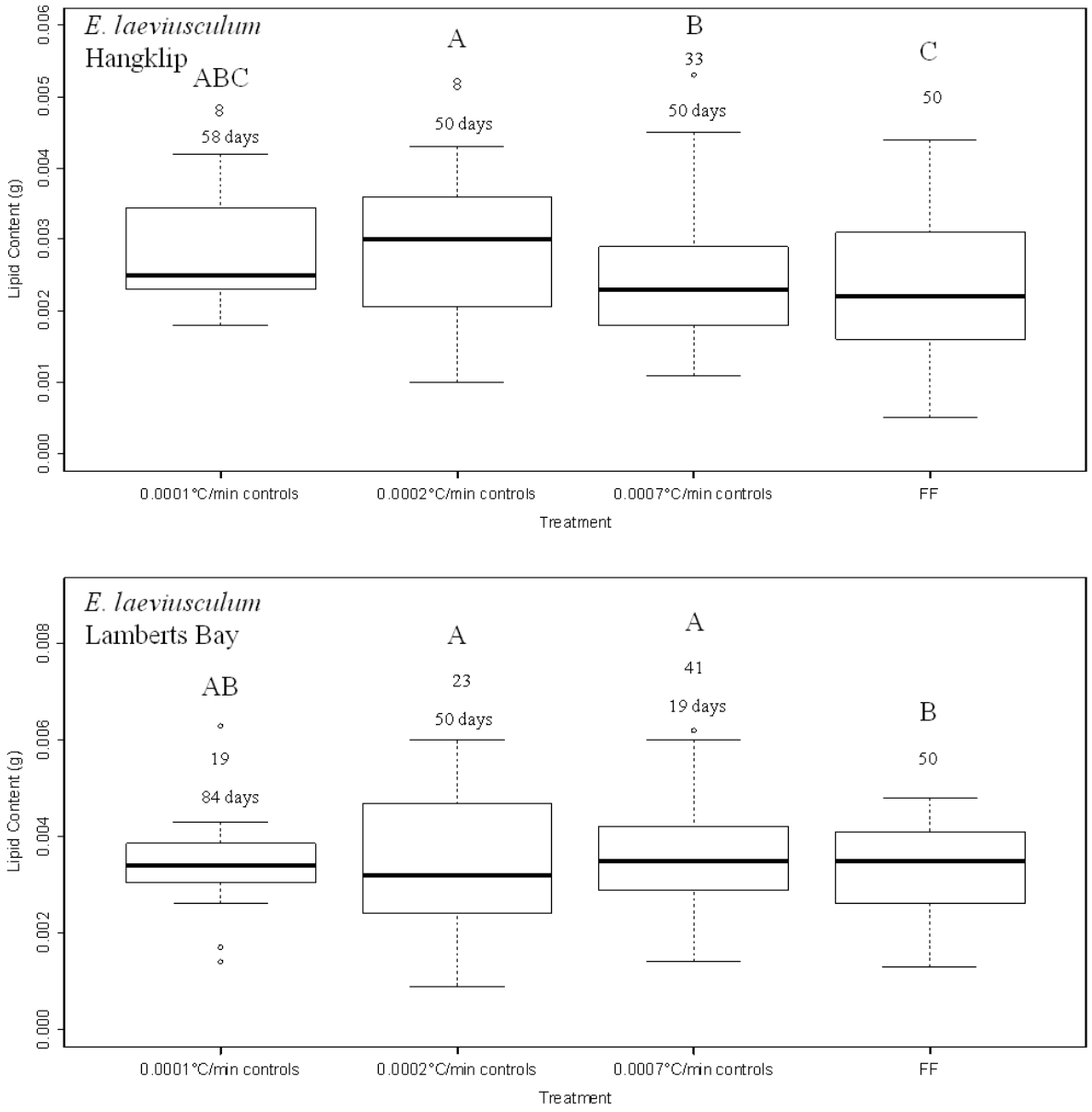

Fig. 8. The lipid content (median ± 25th and 75th percentiles) of slow-rate CT_{max} controls and field fresh (FF) individuals of Lamberts Bay and Hangklip populations of *E. laeviusculum*. Control individuals were kept at a constant temperature (15°C) throughout the slow-rate CT_{max} experimental trials. Sample sizes within each treatment, as well as captivity period are presented. Differing upper case letters indicate significant differences between treatment groups (significance set at $p < 0.05$; see methods p. 42).

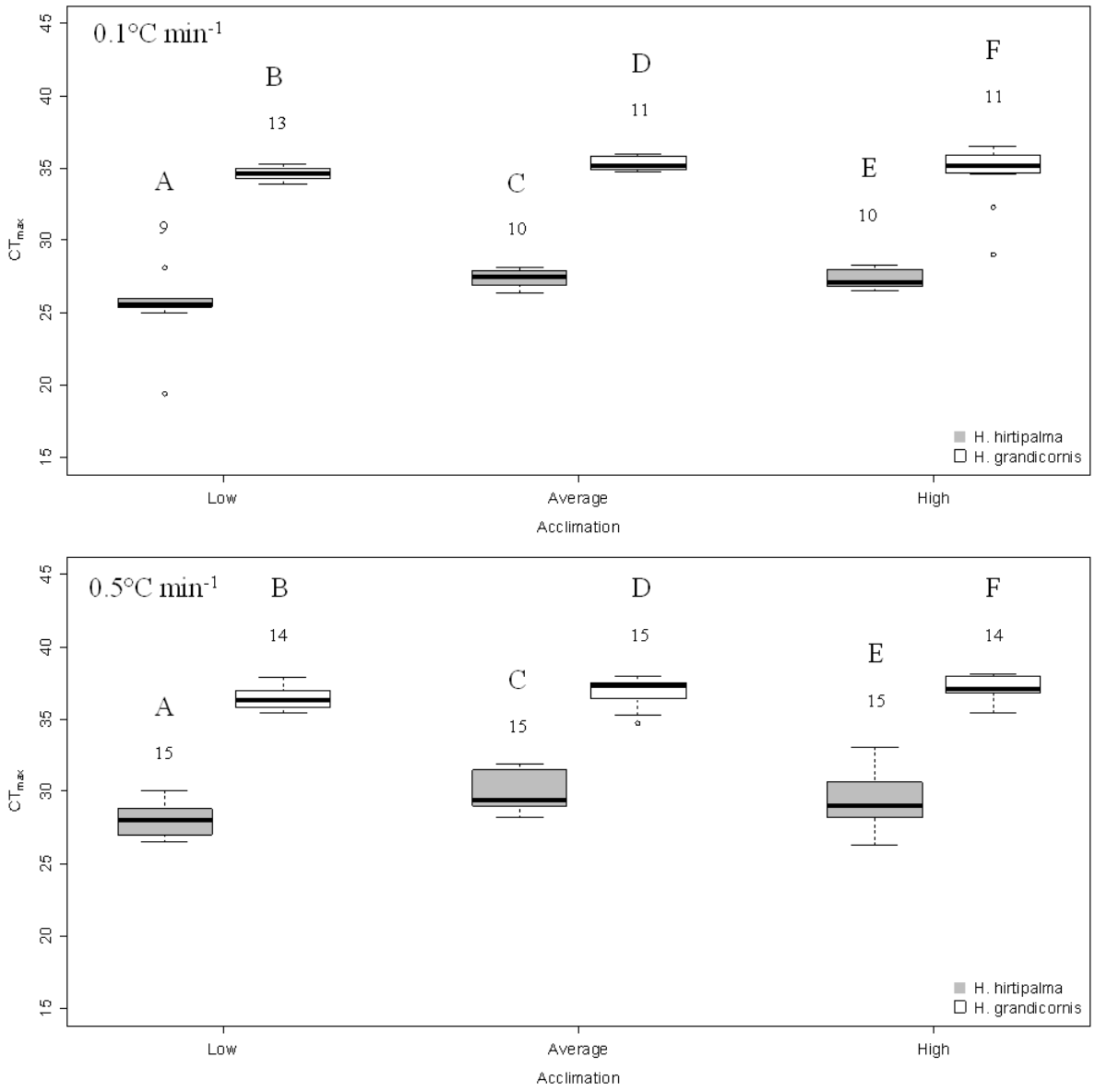

Fig. 9. The CT_{max} (median ± 25th and 75th percentiles) of *Hyale* species at fast rates of temperature change and across acclimation treatments. Numbers indicate sample sizes within each treatment. Differing upper case letters indicate significant differences between species in each acclimation treatment (significance set at $p < 0.05$; see methods p. 42).

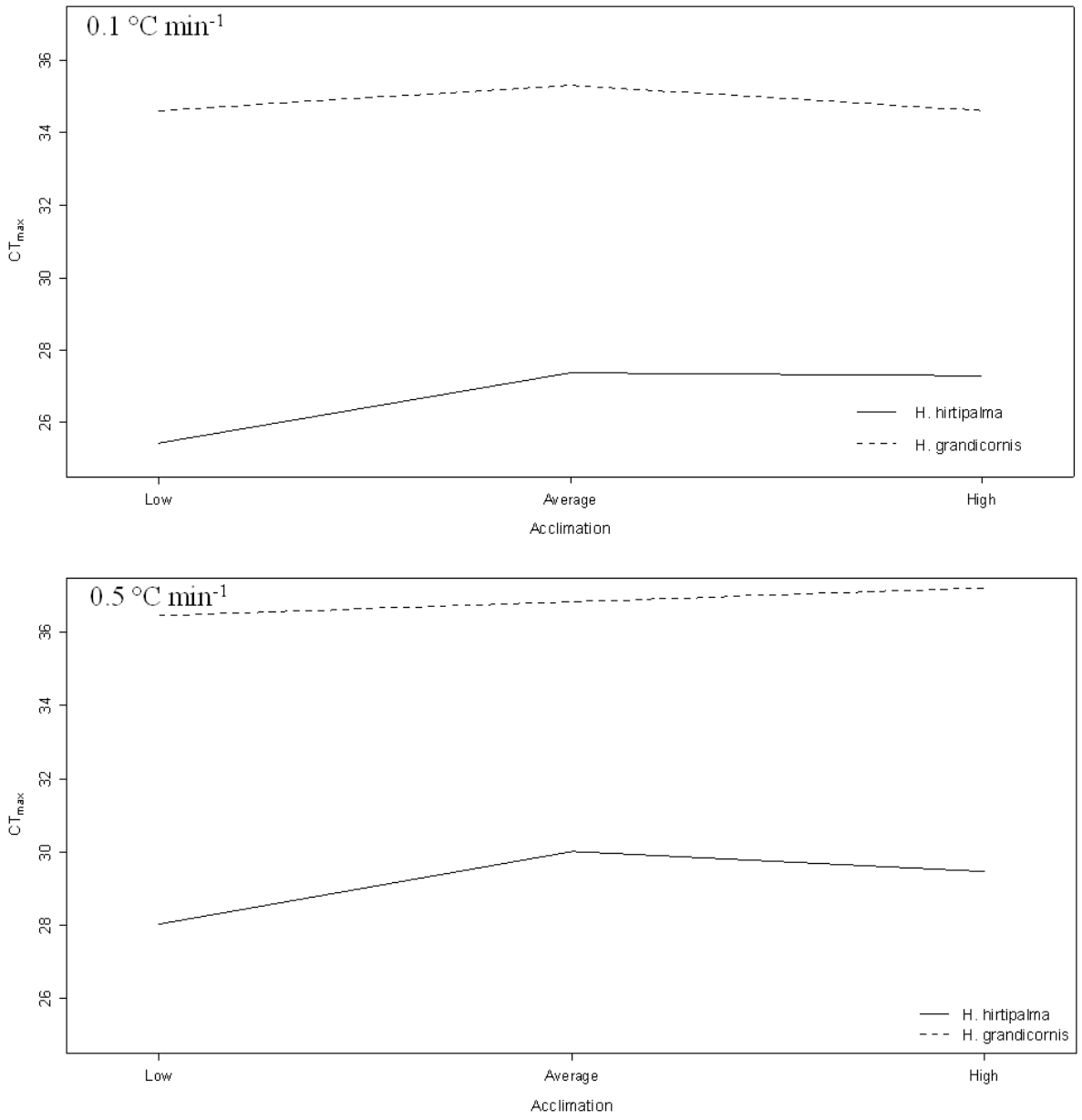

Fig. 10. The interaction between species (*H. hirtipalma* and *H. grandicornis*) and acclimation treatments at 0.5°C min^{-1}. No species x acclimation interaction was found at 0.1°C min^{-1}.

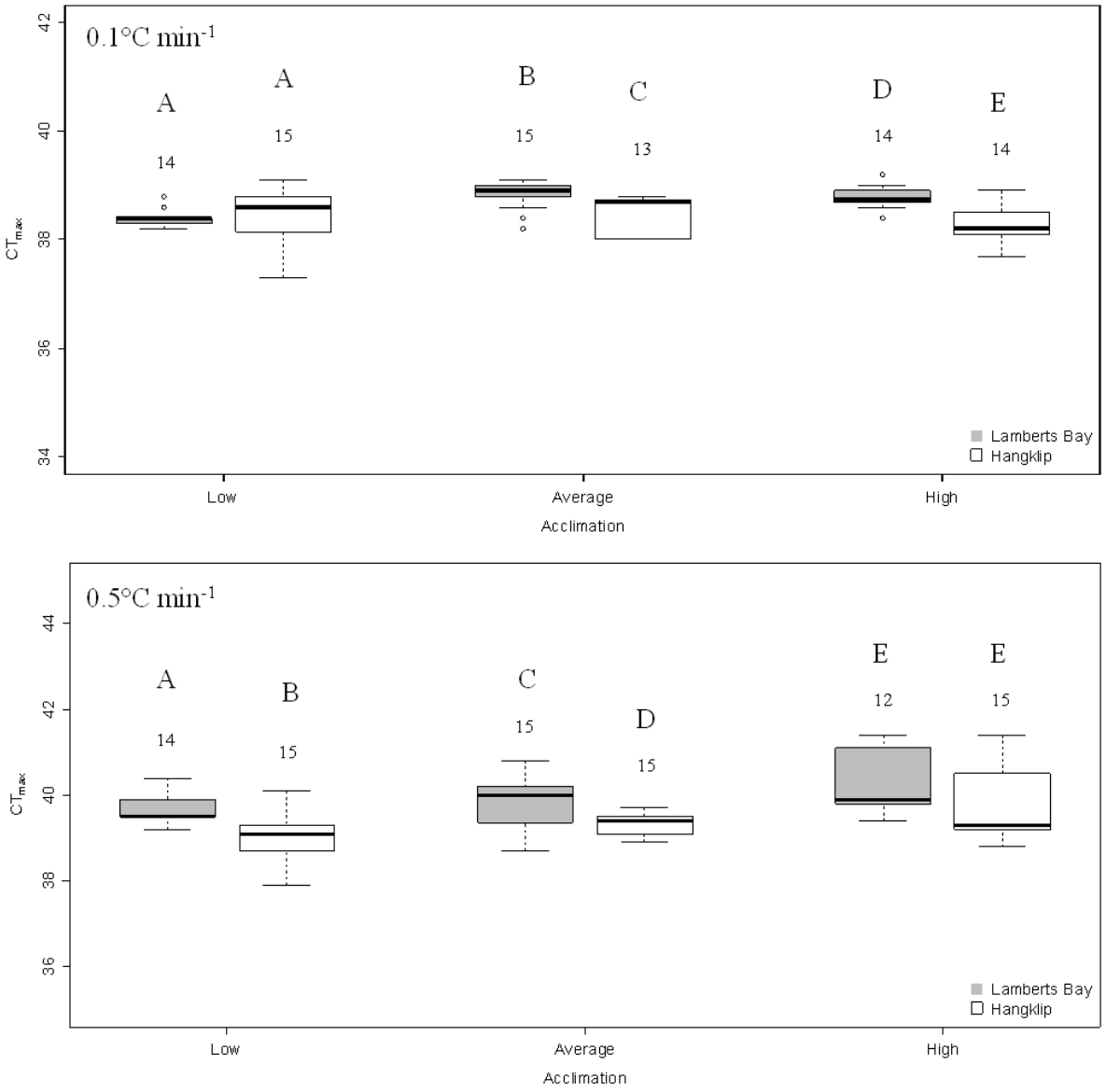

Fig. 11. The CT_{max} (median ± 25th and 75th percentiles) of *E. laeviusculum* populations at fast rates of temperature change and across acclimation treatments. Numbers indicate sample sizes within each treatment. Differing upper case letters indicate significant differences between populations in each acclimation treatment (significance set at $p < 0.05$; see methods p. 42).

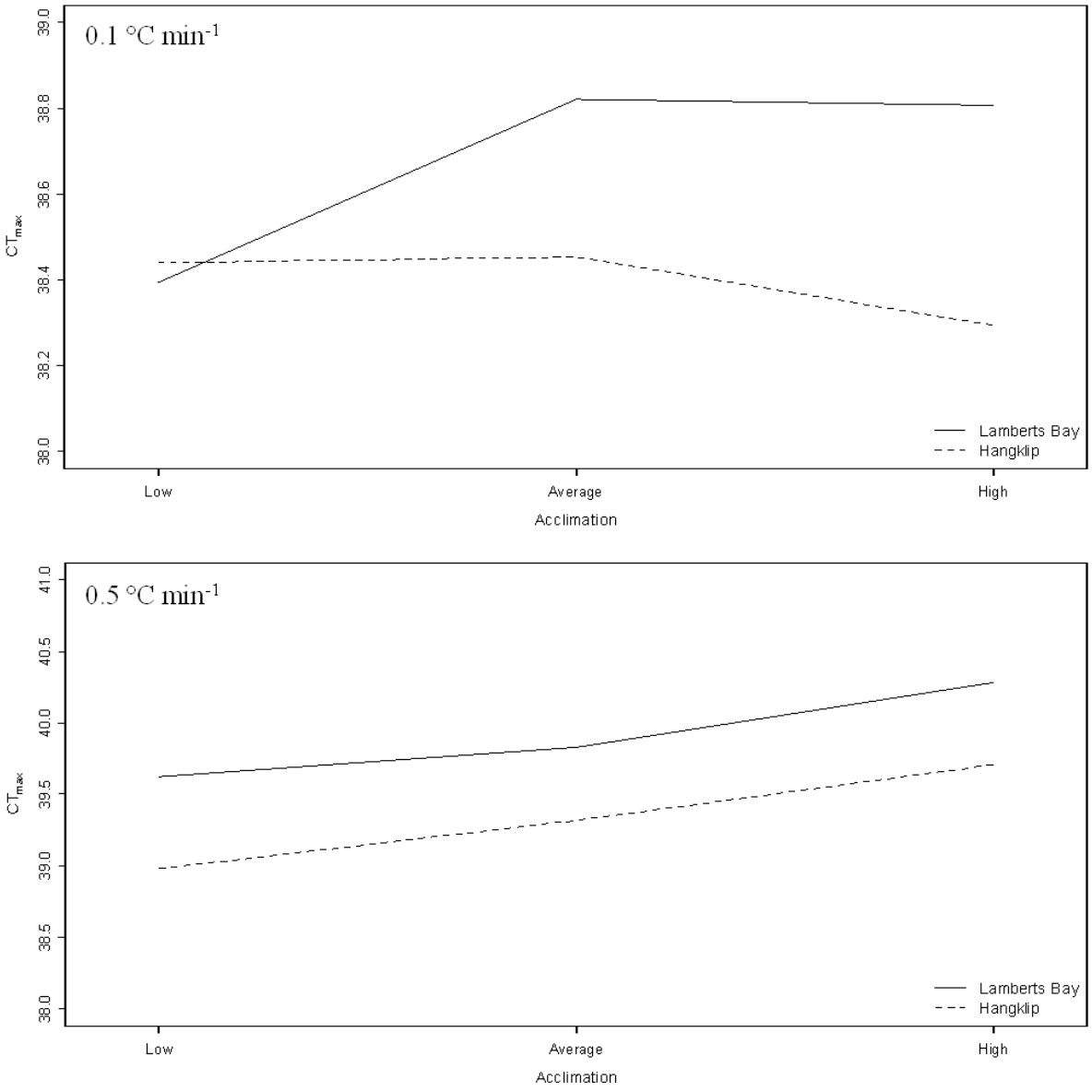

Fig. 12. The interaction between *E. laeviusculum* populations (Hangklip and Lamberts Bay) and acclimation at 0.1°C min^{-1}. No population x acclimation interaction was found at 0.5°C min^{-1}.

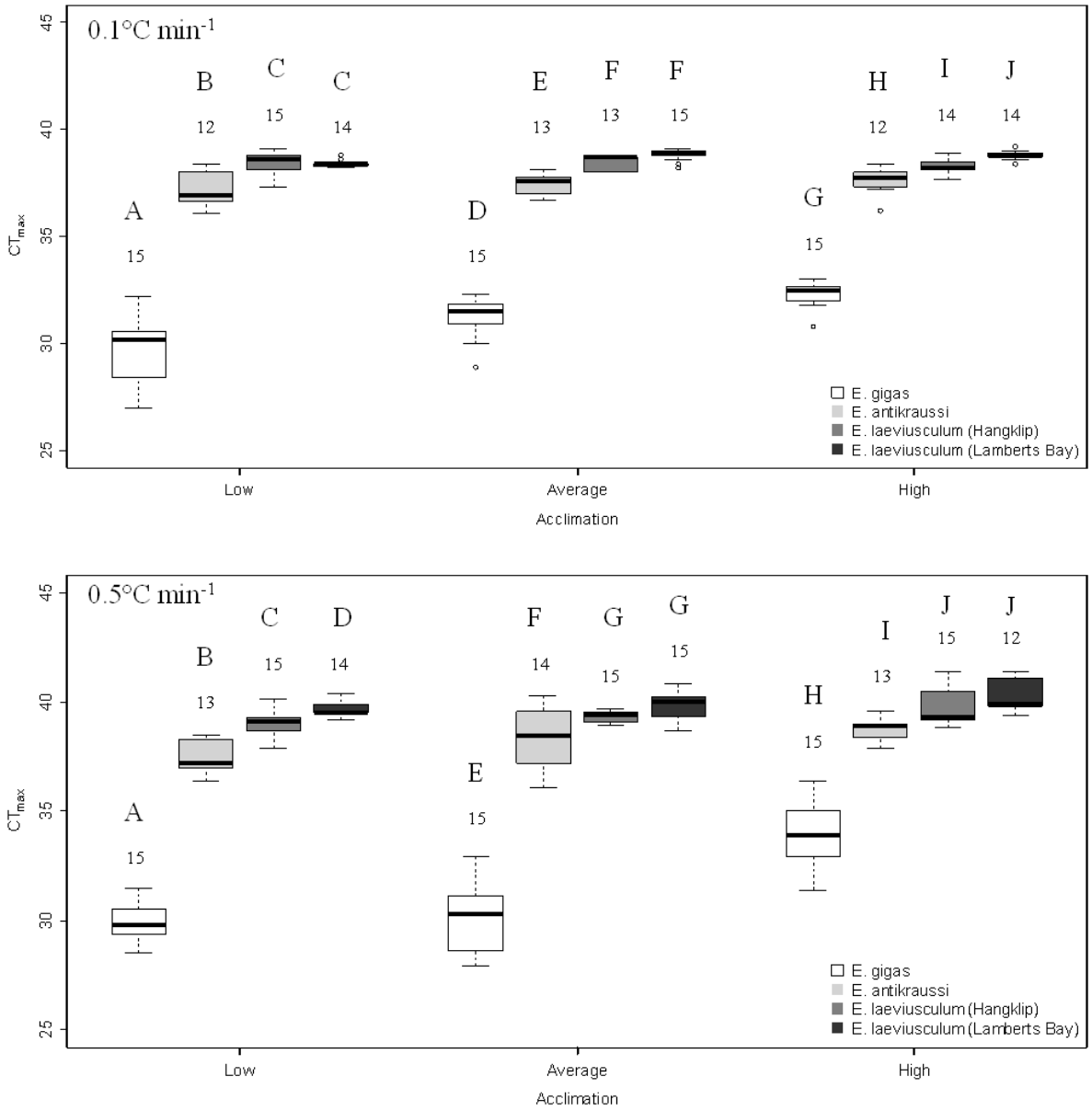

Fig. 13. The CT_{max} (median ± 25th and 75th percentiles) of *Exosphaeroma* species and populations at fast rates of temperature change and across acclimation treatments. Numbers indicate sample sizes within each treatment. Differing upper case letters indicate significant differences between species and populations in each acclimation treatment (significance set at $p < 0.05$; see methods p. 42).

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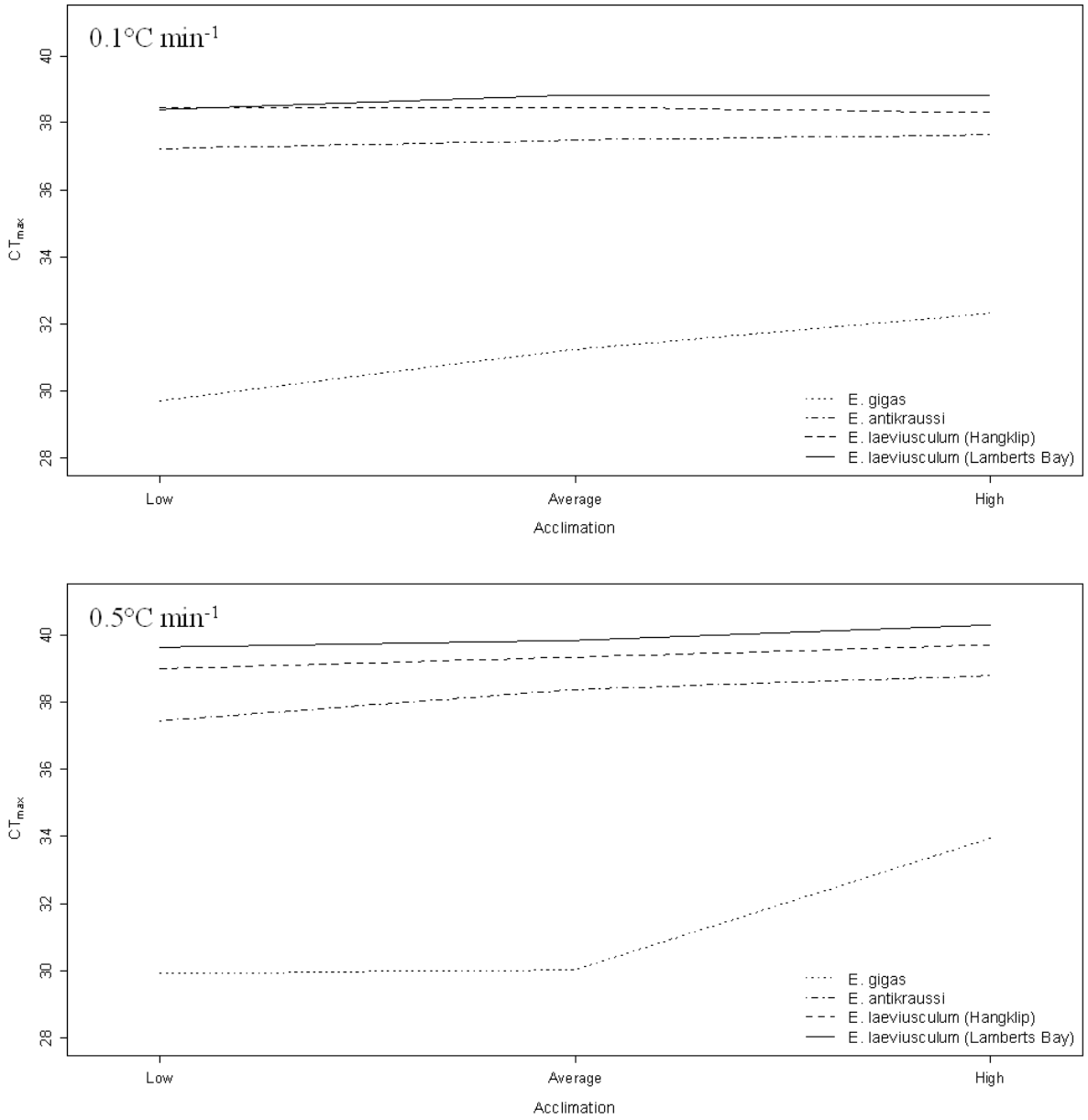

Fig. 14. The interaction between species/population (*E. gigas*, *E. antikraussi*, *E. laeviusculum* (Hangklip) and *E. laeviusculum* (Lamberts Bay)) and acclimation treatments at fast rates of temperature change.

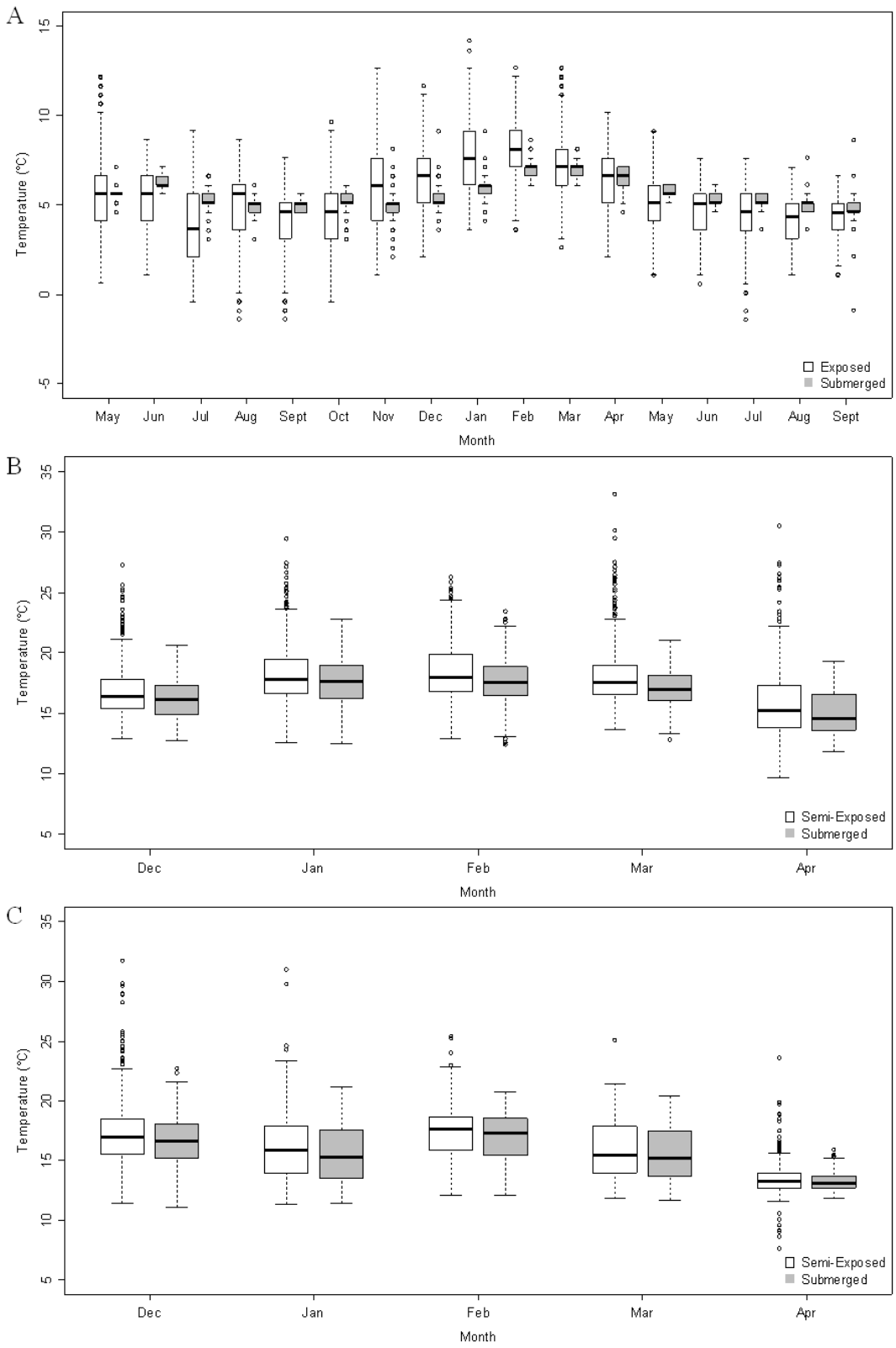

Fig. 15. Microsite data for Marion Island (A), Hangklip (B) and Lamberts Bay (C). Data were collected for Marion Island from May 2009 – September 2010, and Hangklip and Lamberts Bay from December 2010 – April 2011.

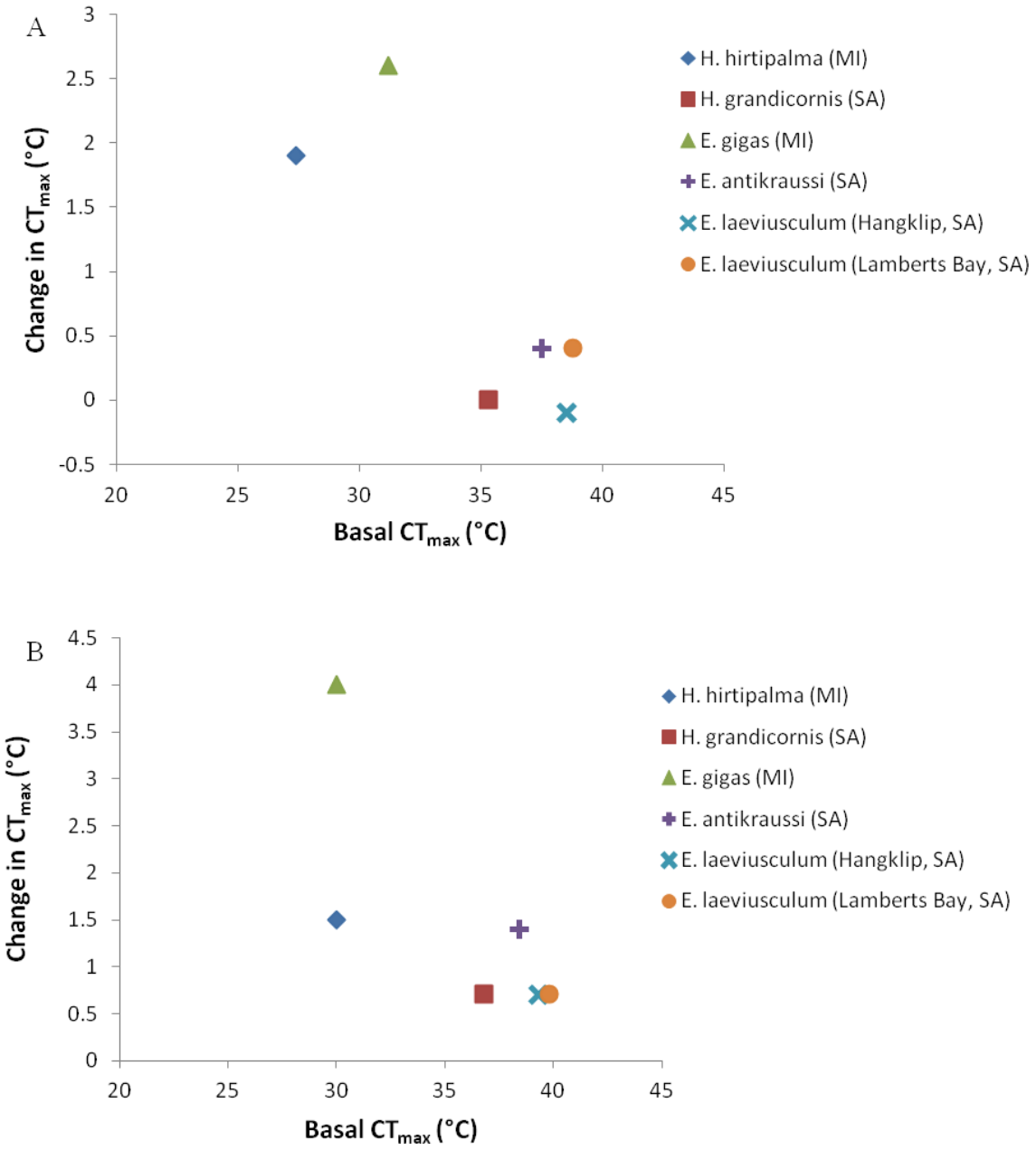

Fig. 16. The difference between mean CT_{max} when acclimated to low (3°C and 11°C for Marion Island and South African species, respectively) and high (11°C and 19°C for Marion Island and South African species, respectively) temperatures. MI and SA denote species from Marion Island and South Africa, respectively. Figure A and B show responses at 0.1 and 0.5°C min^{-1}, respectively.

Tables

Table 1. Experimental design used in this study and depicting the acclimation temperature and rate of temperature change combinations tested on each species or population. FF denotes field fresh individuals. All rates include: 0.0001, 0.0002, 0.0007, 0.1 and 0.5°C min^{-1}.

Species	Collection site and GPS location	Tidal zone inhabited	Microsite shore position	Acclimation temperature (°C)	Rate of change
Exosphaeroma gigas	Marion Island 46º53'09"S; 37º52'37"E	Infra-tidal	Submerged	3	All rates
				7	All rates
				11	All rates
				FF	0.1°C min^{-1} 0.5°C min^{-1}
Hyale hirtipalma	Marion Island 46º53'09"S; 37º52'37"E	Inter-tidal	Exposed and submerged	3	All rates
				7	All rates
				11	All rates
				FF	0.1°C min^{-1} 0.5°C min^{-1}
Exosphaeroma laeviusculum	Lamberts Bay 32º10'12"S; 18º18'42"E	Inter- to infra-tidal	Semi-exposed and submerged	11	All rates
				15	All rates
				19	All rates
				FF	0.1°C min^{-1} 0.5°C min^{-1}
Exosphaeroma laeviusculum	Hangklip 34º22'50"S; 18º49'51"E	Inter- to infra-tidal	Semi-exposed and submerged	11	All rates
				15	All rates
				19	All rates
				FF	0.1°C min^{-1} 0.5°C min^{-1}
Hyale grandicornis	Muizenberg 34º06'32"S; 18º28'08"E	Inter-tidal	Semi-exposed	11	All rates
				15	All rates
				19	0.0007°C min^{-1} 0.1°C min^{-1} 0.5°C min^{-1}
				FF	0.1°C min^{-1} 0.5°C min^{-1}
Exosphaeroma antikraussi	Hangklip 34º22'50"S; 18º49'51"E	Inter-tidal	Semi-exposed	11	0.1°C min^{-1} 0.5°C min^{-1}
				15	0.1°C min^{-1} 0.5°C min^{-1}
				19	0.1°C min^{-1} 0.5°C min^{-1}
				FF	0.1°C min^{-1} 0.5°C min^{-1}
Parisocladus stimpsoni	Hangklip 34º22'50"S; 18º49'51"E	Inter-tidal	Semi-exposed	11	0.1°C min^{-1} 0.5°C min^{-1}
				15	0.1°C min^{-1} 0.5°C min^{-1}
				19	0.1°C min^{-1} 0.5°C min^{-1}
				FF	0.1°C min^{-1} 0.5°C min^{-1}

Table 2. Mean CT_{max} (± SD) of field fresh organisms and those acclimated to mean sea water temperature (7°C for Marion Island and 15°C for South African sites) at fast rates of temperature change (0.1° and 0.5°C min^{-1}).

Species	Collection site	Field fresh (0.1°C min^{-1})	7/15°C (0.1°C min^{-1})	Field fresh (0.5°C min^{-1})	7/15°C (0.5°C min^{-1})
E. gigas	Marion Island	31.9 ± 0.3	31.2 ± 0.9	30.9 ± 1.1	30.0 ± 1.6
H. hirtipalma	Marion Island	28.4 ± 1.2	27.4 ± 0.6	28.6 ± 1.2	30.0 ± 1.3
H. grandicornis	Muizenberg	34.8 ± 0.5	35.3 ± 0.5	35.7 ± 0.5	36.8 ± 1.0
E. laeviusculum	Hangklip	38.1 ± 0.3	38.5 ± 0.3	39.8 ± 0.2	39.3 ± 0.3
	Lamberts Bay	38.8 ± 0.2	38.8 ± 0.3	40.0 ± 0.5	39.8 ± 0.6
E. antikraussi	Hangklip	37.4 ± 0.4	37.5 ± 0.5	38.4 ± 1.2	38.6 ± 0.9
P. stimpsoni	Hangklip	38.4 ± 0.5	38.8 ± 0.4	39.4 ± 0.7	39.2 ± 0.7

Table 3. Results of general linear models testing the effects of rate of change, acclimation, sex, mass and interactions on CT_{max} (see methods p. 42). Only the significant parameters from the minimum adequate model are presented.

Species	Collection site	d.f.	F	*P*	Estimate ± s.e.
E. gigas	Marion Island				
	Rate	4	283.520	< 0.001	
	Rate x Acclimation	9	13.754	< 0.001	
H. hirtipalma	Marion Island				
	Rate	4	233.326	< 0.001	
	Mass	1	15.938	< 0.001	-0.67 ± 0.18
	Rate x Acclimation	9	6.399	< 0.001	
	Rate x Sex	4	2.675	0.032	
H. grandicornis	Muizenberg				
	Rate	4	639.510	< 0.001	
	Mass	1	19.633	< 0.001	-1.23 ± 0.28
E. laeviusculum	Hangklip				
	Rate	4	456.381	< 0.001	
	Acclimation	3	3.522	0.031	
	Rate x Acclimation	9	2.178	0.024	
	Acclimation x Sex	3	2.963	0.033	
	Lamberts Bay				
	Rate	4	225.067	< 0.001	
	Acclimation	3	19.605	< 0.001	
	Sex	1	22.829	< 0.001	
	Rate x Acclimation	9	14.172	< 0.001	
	Rate x Sex	4	5.226	< 0.001	
E. antikraussi	Hangklip				
	Acclimation	3	6.410	< 0.01	
	Sex	1	5.594	0.020	
	Mass	1	19.553	< 0.001	12.28 ± 2.78
	Rate x Acclimation	3	3.726	0.014	
	Acclimation x Sex	2	7.120	< 0.01	
P. stimpsoni	Hangklip				
	Rate	1	56.794	< 0.001	

Table 4. Results of general linear models of the effects of treatment (slow-rate CT_{max} controls and field fresh), sex, mass and interactions on the CT_{max} of *H. grandicornis* and *E. laeviusculum* populations. Only the significant parameters from the minimum adequate model are presented.

Species	Collection site	d.f.	F	*P*	Estimate ± s.e.
H. grandicornis	Muizenberg				
	Mass	1	5.847	0.023	-0.75 ± 0.31
E. laeviusculum	Hangklip				
	Treatment	3	14.572	< 0.001	
	0.0001°C min^{-1} controls				39.03 ± 0.12
	0.0002°C min^{-1} controls				39.27 ± 0.12
	0.0007°C min^{-1} controls				39.86 ± 0.12
	Field Fresh				39.81 ± 0.12
	Lamberts Bay				
	Treatment	3	5.049	< 0.01	
	0.0001°C min^{-1} controls				38.93 ± 0.26
	0.0002°C min^{-1} controls				39.02 ± 0.26
	0.0007°C min^{-1} controls				39.37 ± 0.24
	Field Fresh				39.52 ± 0.24
	Mass	1	5.725	0.020	9.35 ± 3.91

Table 5. Results of general linear models testing the effects of treatment (slow-rate CT_{max} controls and field fresh), dry mass and interactions on the lipid content (log_{10} transformed in cases where assumptions were not met) of *Hyale* species and *E. laeviusculum* populations. Only the significant parameters from the minimum adequate model are presented.

Species	Collection site	d.f.	F	*P*	Estimate ± s.e.
H. hirtipalma	Marion Island				
	Treatment	1	27.999	< 0.001	
	Controls				-0.57 ± 0.12
	Field Fresh				-0.80 ± 0.12
	Dry mass	1	1790.080	< 0.001	1.07 ± 0.03
H. grandicornis	Muizenberg				
	Treatment	1	5.034	0.028	
	Controls				-1.18 ± 0.20
	Field Fresh				-1.05 ± 0.22
	Dry mass	1	667.626	< 0.001	1.00 ± 0.04
E. laeviusculum	Hangklip				
	Treatment	3	7.170	< 0.001	
	0.0001°C min^{-1} controls				0.0003 ± 0.0003
	0.0002°C min^{-1} controls				0.0005 ± 0.0003
	0.0007°C min^{-1} controls				0.00003 ± 0.0002
	Field Fresh				0.0007 ± 0.0001
	Dry mass	1	207.593	< 0.001	0.17 ± 0.01
	Lamberts Bay				
	Treatment	3	4.772	< 0.01	
	0.0001°C min^{-1} controls				-3.30 ± 0.43
	0.0002°C min^{-1} controls				-2.28 ± 0.230
	0.0007°C min^{-1} controls				-2.51 ± 0.270
	Field Fresh				-3.48 ± 0.22
	Dry mass	1	32.259	< 0.001	0.61 ± 0.11
	Treatment x Dry mass	3	5.777	< 0.001	

Table 6. Results of general linear models of the effects of species/population, acclimation, sex, mass and interactions on the CT_{max} of *Hyale* and *Exosphaeroma* species, and *E. laeviusculum* populations at fast rates of temperature change (see methods pg. 42). Only the significant parameters from the minimum adequate model are presented.

Species and collection site	Model parameters	Rate of change 0.1°C min^{-1}			Rate of change 0.5°C min^{-1}		
		d.f.	F	*P*	d.f.	F	*P*
H. hirtipalma (Marion Island)	Species	1	523.967	< 0.001	1	288.995	< 0.001
H. grandicornis (Muizenberg)	Acclimation	2	4.161	0.0203			
	Species x Acclimation				2	3.210	0.045
E. laeviusculum (Hangklip)	Population	1	17.047	< 0.001	1	21.117	< 0.001
E. laeviusculum (Lamberts Bay)	Acclimation				2	10.215	< 0.001
	Population x Acclimation	2	5.571	< 0.01			
E. gigas (Marion Island)	Species/Population	3	41.796	< 0.001	3	148.412	< 0.001
E. laeviusculum (Hangklip)	Acclimation				2	7.566	< 0.001
E. laeviusculum (Lamberts Bay)	Species/Population x Acclimation	6	11.770	< 0.001	6	17.202	< 0.001
E. antikraussi (Hangklip)	Species/Population x Mass	3	3.104	0.0284			

Table 7. The 'warming tolerance' of *Hyale* and *Exosphaeroma* species, and *E. laeviusculum* populations at fast rates of temperature change.

Species	Collection site	Exposure	Field fresh (0.1°C min^{-1})	7/15°C (0.1°C min^{-1})	Field fresh (0.5°C min^{-1})	7/15°C (0.5°C min^{-1})
H. hirtipalma	Marion Island	Exposed	22.0	22.0	23.2	24.6
		Submerged	22.8	21.8	23.0	24.4
H. grandicornis	Muizenberg	Semi-exposed	17.3	17.8	18.2	19.3
E. gigas	Marion Island	Submerged	26.3	25.6	25.3	24.4
E. laeviusculum	Hangklip	Semi-exposed	20.6	20.9	22.3	21.8
		Submerged	21.4	21.7	23.1	22.6
	Lamberts Bay	Semi-exposed	22.9	22.9	24.1	23.9
		Submerged	23.1	23.1	24.3	24.1
E. antikraussi	Hangklip	Semi-exposed	19.9	20.0	21.1	20.8

Table 8. Microsite temperature (°C) at exposed, semi-exposed and submerged positions at collection sites.

Location	Period of temperature data collection	Microsite	Mean ± SD	Coefficient of variation	Minimum	Maximum	Thermal range	Mean rate of temperature increase (°C min^{-1})	Maximum rate of temperature increase (°C min^{-1})
Marion Island	May 2009 - September 2010	Exposed Submerged	5.4 ± 2.2 5.6 ± 0.9	0.4 0.2	- 1.4 - 0.9	14.8 9.1	15.6 10.03	0.005 0.003	0.05 0.04
Hangklip	December 2010 - April 2011	Exposed Semi-exposed Submerged	18.9 ± 2.6 17.5 ± 2.7 16.7 ± 2.1	0.1 0.2 0.1	9.9 9.7 11.8	30.2 33.2 23.4	20.3 23.5 11.6	0.01 0.01 0.006	0.06 0.2 0.06
Lamberts Bay	December 2010 - April 2011	Exposed Semi-exposed Submerged	18.8 ± 4.4 15.9 ± 2.8 15.7 ± 2.4	0.2 0.2 0.2	9.6 7.6 11.1	37.2 31.7 22.7	27.6 24.1 11.7	0.02 0.008 0.004	0.07 0.1 0.06