Acclimation effects on thermal tolerance in ameronothrid mites at sub –Antarctic Marion Island

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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Abstract

Evidence for widespread ecological impacts due to rapid climate change is on the increase, with further warming predicted over the next century. This increase in warming has lead to organisms adjusting their distribution range and where this is not possible organisms must cope with the stressful environments in a different way. One potential way to handle environmental stress is via a mechanism known as phenotypic plasticity, which allows an organism to express different phenotypes depending on the biotic or abiotic environment in a way that may alter that organism's fitness. Acclimation temperatures can potentially cause plastic (reversible and irreversible) changes in the response of individuals to stressful experimental conditions that could increase their survival. The issue of whether this acclimation will be beneficial to the organism or not remains contentious, with support offered both for and against the idea of beneficial acclimation. The aims of the investigation were to determine the thermal limits of five ameronothrid mite species (*Halozetes marinus*, *H. marionensis*, *H. belgicae*, *H. fulvus* and *Podacarus auberti*) from varying terrestrial habitats and to test whether there is beneficial acclimation on the thermal traits. Along with the beneficial acclimation hypothesis several alternative hypotheses were also tested. In addition, locomotor performance was determined as it has been suggested that locomotion performance is a reliable and practical measure of potential fitness of animals. The same hypotheses testing the effects of acclimation temperature on the thermal limits were tested on three performance traits; performance breadth, optimum speed and optimum temperature. Support for beneficial acclimation was found in the performance breadth and optimum speed traits for all species except for *Halozetes marinus*. For the optimum temperature trait the prediction for the compensation hypothesis was met and that of the beneficial acclimation hypothesis rejected, with the only exception again being that of *H. marinus*. In the case of the locomotor performance traits the prediction for the beneficial acclimation hypothesis in the performance breadth and optimum speed traits are the same for that of the compensation hypothesis, therefore the overall response of the more terrestrial mite species is one of compensation. However, support for beneficial acclimation was not found in the thermal limit traits of all five species, with the higher acclimation temperatures (specifically 15° C) resulting in negative responses in LLT in *H. marinus* and *H. belgicae*, and in ULT in *H. fulvus*. Phenotypic flexibility varied between marine and terrestrial species. The less variable marine environment showed lower flexibility than that of terrestrial species in the performance breadth trait and optimum temperature trait, but there was a lack of variation between the marine and terrestrial species in the temperature tolerance traits. These results show that the effects of acclimation on various traits, especially fitness related traits, are complex and require more attention if the consequences are to be fully explained. This study therefore provides insight into the effects of acclimation on performance traits and thermal limit traits and has implications for the evolution of plastic responses in terrestrial arthropods.

Opsomming

Bewys vir wye ekologiese impakte as gevolg van vinnige klimaatsverandering is aan die toeneem, met verdere verwarming wat voorspel word gedurende die volgende eeu. Dié toename in verwarming het alreeds veroorsaak dat organismes hulle verspreidings verander, of waar dit nie moontlik is nie, moet hulle stresvolle omgewingstoestande op ‘n ander wyse hanteer. Een moontlike manier om omgewingsstres te hanteer is deur gebruik te maak van ‘n meganisme wat bekend staan as fenotipiese plastisiteit, wat dit vir ‘n organisme moontlik maak om ander fenotipes te toon afhangende van die biotiese of abiotiese omgewing in ‘n wyse wat die organisme se fiksheid beinvloed. Temperatuur akklimasie kan moontlik plastisiteit (omkeerbaar of onomkeerbaar) veranderinge in die reaksie van individue tot stresvolle eksperimentele toestande tot gevolg hé, wat gevolglik hulle oorlewing kan verhoog. Die kwessie of hierdie akklimasie wel voordelig vir die organisme is of nie is steeds betwisbaar, met beide steun vir en teen die idee van voordelige akklimasie. Die doel van hierdie ondersoek was om vas te stel wat die termiese limiete van vyf ameronothrid myt spesies (*Halozetes marinus*, *H. marionensis*, *H. belgicae*, *H. fulvus* en *Podacarus auberti*) van verskeie terrestriële habitatte is, en om te toets of daar voordelige akklimasie in die termiese eienskappe voorkom. Tesame met die voordelige akklimasie hipotese is daar verskeie alternatiewe hipoteses ook getoets. Verder, is bewegings prestasie vasgestel omdat dit al voorgestel is dat bewegings prestasie ‘n betroubare en praktiese mate van die potensiële fiksheid van ‘n dier aantoon. Dieselfde hipotese wat die effek van akklimasie temperatuur op die termiese limiete toets, is op drie prestasie eienskappe getoets; prestasie wydte, optimale spoed en optimale temperatuur. Bewyse vir voordelige akklimasie is gevind in die prestasie wydte en optimale spoed eienskappe vir alle spesies behalwe *Halozetes marinus*. Vir die optimale temperatuur eienskap was die voorspelling vir die kompensasie hipotese korrek maar dié van die voordelige akklimasie verkeerd, met *H. marinus* die enigste uitsondering. In die geval van die bewegings prestasie eienskappe is die voorspelling vir die voordelige akklimasie hipotese in die prestasie wydte en optimale spoed eienskappe die selfde as vir die kompensasie hipotese. Daarom is die algemene reaksie vir die meer terrestriële spesies een van kompensasie. Bewyse vir voordelige akklimasie is egter nie gevind in die termiese limiet eienskappe van die vyf spesies nie, met die hoér akklimasie temperature (spesifiek 15° C) wat 'n negatiewe reaksie in LLT in *H. marinus* en *H. belgicae*, en in ULT in *H. fulvus* veroorsaak het. Fenotipiese buigsaamheid het verskil tussen mariene en terrestriële spesies. Die minder variërende mariene omgewing het 'n laer buigsaamheid in die prestasie wydte en optimale temperatuur eienskappe getoon as die van terrestriële spesies, maar daar was geen variasie tussen mariene en terrestriële spesies in die temperatuur toleransie eienskappe nie. Die resultate wat hier voorgelê word wys dat die gevolge van akklimasie op verskeie eienskappe, veral fiksheids verwante eienskappe, ingewikkeld is en vereis meer aandag om die gevolge volledig te verduidelik. Hierdie studie verskaf dus insig in die gevolge van akklimasie vir prestasie eienskappe en termiese limiet eienskappe en het gevolge vir die evolusie van plastiese reaksies in terrestriële arthropoda.

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Table of Contents

Chapter 1

General Introduction

One of the most pressing threats facing modern biotas is climate change (Danks, 1992). Over the last century there has been an increase in mean and minimum global temperatures, with further warming predicted over the next century (IPCC, 2001). This warming has translated to a northward shift in climatic isotherms by 120 km and 105 km for Europe and western North America, respectively (Parmesan et al., 1999). Similar effects are also apparent in the high-latitudes of the Southern Hemisphere, where more than a 1°C warming has occurred over the last 40 years at some localities (Bergstrom and Chown, 1999; Smith, 2002; Walther et al., 2002). Attention is also being placed on the realized and expected warming of high latitude regions due to increasing atmospheric concentrations of radiatively-active gases such as CO_2, methane and nitrous oxide (Chapin et al., 1992; Maxwell, 1992; IPCC, 2001). According to Gillet and Thompson (2003), recent climate change in the Southern Hemisphere is indeed marked by strengthening of the circumpolar westerlies in both the stratosphere and the troposphere. Through climate trend simulations using an atmospheric model run with high vertical resolution (forced solely by prescribed stratospheric ozone depletion), Gillet and Thompson (2003) provide evidence that anthropogenic emissions of ozone depleting gases have impacted climate both at stratospheric levels and at the Earth's surface.

With these changing climatic conditions have come changes in the distribution, phenology and physiology of various plant and animal species (Menzel and Fabian, 1999; Parmesan et al., 1999; Sturm et al., 2001; Fitter and Fitter, 2002; Peñuelas and Boada, 2003). These climatic changes have also brought about changes in community composition and species dominance resulting in changes in ecosystem-level processes (Kennedy, 1995; Brown et al., 1997; Chapin et al., 1992).

Climate limits the distribution range of many taxa (Spicer and Gaston, 1999; Sax, 2001), and it is predicted that species will shift their distribution upwards in latitude with increased global warming (Pitelka, 1997; Parmesan et al., 1999; Helmuth et al., 2002; Walther et al., 2002). Species density at given locations may change, with the ranges of species either shifting poleward or upwards in elevation as species move to occupy areas that conform to their temperature tolerances (Parmesan et al., 1999; Thomas and Lennon, 1999; Grabherr et al., 1994). However, species that are unable to migrate (due to limited colonization or dispersal ability) have to cope with the environment in a different way.

Some species may alter their phenology in the face of environmental stress (e.g. warming: Menzel and Fabian, 1999). Many life history traits of species are triggered by temperature-related cues with change possibly occurring in the timing of events (e.g. migration or egg laying: Walther et al., 2002; Root et al., 2003). A stress may also be countered either by long-term adaptation (within a population) or by phenotypic plasticity (within-individual level). If an adaptation occurs it can include either genotypic change for the basal response, or a change in the extent of phenotypic plasticity, or both (Agrawal, 2001). By contrast, phenotypic plasticity allows an organism to express different phenotypes depending on the biotic or abiotic environment in a way that may alter that organism's fitness (Kingsolver and Huey, 1998; Agrawal, 2001; Berteaux et al., 2004).

Climate change in the sub-Antarctic

There has been a marked change in climate in the sub-Antarctic with clear trends evident on Marion Island (Smith, 2002), the Kerguelen Archipelago (Frenot et al., 1997) and Heard Island (Budd, 2000).

The trend for Marion Island shows an increase in mean annual temperature from 1969 to 1999, with a mean warming rate of 0.04°C per year, and an annual precipitation decline with an average of 25 mm per year between 1965 and 1999 (Smith, 2002). Hours of sunshine have also shown an increase, with an average of 3.3 hours each year between

1951 and 1999 (Smith, 2002). These changes are thought to be a consequence of changing atmospheric circulation patterns (Smith and Steenkamp, 1990), and the localized movement of oceanic frontal systems (Pakhomov and Chown, 2003).

The Kerguelen Archipelago shows similar trends. Mean annual temperature has increased by 0.12°C between 1951 and 1993. Precipitation decreased dramatically in the 1960s, with current levels of precipitation being 500 mm lower than those observed in the 1950s (Frenot et al., 1997). Yearly hours of sunshine have not changed detectably between 1951 and 1993 (Frenot et al., 1997).

Mean annual air temperature on Heard Island increased by 1.8°C between 1948 and 1951, with air temperatures 1.3°C higher in 1954 than in 1948 (Budd, 2000). This rise in temperature has been accompanied by glacier retreat and readvance during the 1950s and 1960s, and steady retreat since then (Budd, 2000). The cause of this increase in temperature is thought to be due to a strong and persistent shift in atmospheric circulation (Budd, 2000).

The effects of these changes might be pronounced, as the terrestrial ecosystems of sub-Antarctic islands are relatively simple and sensitive to perturbations due to the isolation, impoverished biota and harsh environments of these islands (Smith, 2002). For the sub-Antarctic direct and indirect ecological consequences of changes in climate include changes in species distributions, abundances, physiologies, phenologies and interactions between species (see Bergstrom and Chown, 1999). As individual species respond to a changing climate there may be a disrupting effect on their interactions with other species at the same or adjacent trophic levels (Walther et al., 2002).

Responses to climate change in the sub-Antarctic

Physiological responses to temperature change among sub-Antarctic plants and animals have been well studied. On Kerguelen, Frenot et al. (1997) investigated the potential effects of recent climatic change on the colonization of deglaciated areas by *Poa keguelensis* and *P. annua* and found that current climatic changes only have a minor/indirect impact in the primary succession progress of these two species in the Ampère Valley. However, the authors suggested that there is no evidence that the observed climatic conditions benefit other native vascular plants in the rest of the Kerguelen Archipelago as the reduced precipitation (related to annual drought) may have resulted in the death of *Azorella selago* cushions on several islands in the Golfe du Morbihan. Good drought resistance, however, was shown for *Acaena magellanica* with increased distribution on Kerguelen. Frenot et al. (1997) suggest that the combination of reduced precipitation and increased temperature could have a major influence on plant communities in the sub-Antarctic, with potentially serious consequences on Kerguelen.

Scott (1990) analyzed vegetation transect data from several glacier retreat zones on Heard Island, and found two plant species, *Poa annua* (one of the most common naturalized alien plants in the sub-Antarctic region) and *Ranunculus biternatus*, to be spreading outwards from their greatest areas of concentration. Changes in the distribution of *Acaena magellanica* have also been observed since 1950. Scott (1990) suggested that with continuing climatic amelioration and glacial recession there will be an expansion in the size of the vegetated areas, as areas for potential colonization become available. Such conditions may also affect vascular plants; plants that have been confined to certain parts of the island may become more wide spread (Scott, 1990).

Many studies have been conducted on the thermal biology and desiccation tolerance of invertebrates on sub-Antarctic islands; including Marion Island (Klok and Chown, 1997;

Van der Merwe et al., 1997; Klok and Chown, 2001; Sinclair and Chown, 2003), South Georgia (Bale et al., 2001) and Signy Island (Hayward et al., 2001). Klok and Chown (1998) found that *Embryonopsis halticella* (Lepidoptera: Yponomeutidae) larvae were able to increase their critical maximum temperature with acclimation. Klok and Chown (2003) found that the critical thermal minimum (CTmin) temperature and critical thermal maximum (CTmax) temperature of six weevil species at six altitudinal sites decreased and showed no change with treatment temperature respectively. Sinclair and Chown (2003) exposed *Pringleophaga marioni* (Lepidoptera: Tineidae) larvae to short-term low pre-treatment temperatures (-5, 0, 5 and 15°C) then to two hours at -7.9°C. Pre-treatments did not significantly improve the survival ability of the larvae, indicating that *P. marioni* do not show a rapid cold hardening response. The effects of successive freezing exposures on the supercooling point (SCP) and subsequent survival of summer acclimatized larvae of *Hydromedion sparsutum* have been investigated (Bale et al., 2001). Their results indicate that in larvae in which the SCP is lowered following a sub-zero exposure, the depression of the SCP is highest in individuals that did not freeze. Larval populations also showed signs of segregation into two sub-populations with different overwintering strategies after successive frost exposures in early winter (Bale et al., 2001). Hayward et al. (2001) investigated the hygropreference of adult *Cryptopygus antarcticus* and *Alaskozetes antarcticus* from Signy Island at three temperatures (5, 10 and 20°C) along various humidity gradients (9-98% Relative humidity). Relative humidity preference for both species is influenced by temperature, with *C. antarcticus* showing preference for high humidity at 20°C and *A. antarcticus* showing no humidity preference at 20°C but a preference for the lowest humidity at 5°C. These studies indicate that critical temperatures and supercooling points can be affected by pre-treatments/acclimation temperatures, and that the influence of temperature may also play a role in influencing desiccation tolerance.

Few of these authors have speculated about the interactions between tolerance limits and climate change. However, Klok and Chown (1997), in an investigation on the thermal tolerance limits of *Pringleophaga marioni* (Lepidoptera: Tineidae), suggested that a continued increase in the radiation input on Marion Island may have pronounced effects on the tolerance limits of this species as results indicated that caterpillars were not able to survive short exposures to temperatures close to their CTmax. In addition, Sinclair and Chown (2003) argued that on Marion Island, whilst the island is warming (Smith, 2002), the frequency of freeze-thaw events may be increasing due to more clear sky nights. Thus physiological responses to both upper and lower lethal temperatures are important.

Sub-Antarctic Marion Island

Marion Island (46°54'S, 37°45'E) is an isolated volcanic island in the Southern Ocean and is the larger of the two islands in the Prince Edward Island (PEI) group, situated 1770 km south east of South Africa (Fig. 1A). The PEI group is thought to be *ca.* 450 000 years old, and the two islands are separated by a shallow saddle, covered by water 45 to 260 m deep (McDougall et al., 2001; Pakhomov and Chown, 2003). Marion Island, which covers an area of approximately 290 km^2 (Fig. 1B), is built on oceanic crust about 45 Ma old and is the tip of an active oceanic intra-plate volcano which rises from sea-floor depths of > 3500 m (McDougall et al., 2001). The island is still considered an active volcano. An eruption on the west coast in September 1980 resulted in a lava flow covering about 9 ha (Smith, 1987) with the last volcanic activity being reported in 2004. The island has been exposed to three different glacial episodes in the last 300 000 years (Smith, 1987).

The annual precipitation on Marion Island is approximately 1900 mm, and is approximately equally distributed throughout the year (Smith, 2002). The island has a low mean air temperature (*c.* 5.7°C), high humidity (83%), and a high degree of cloudiness with

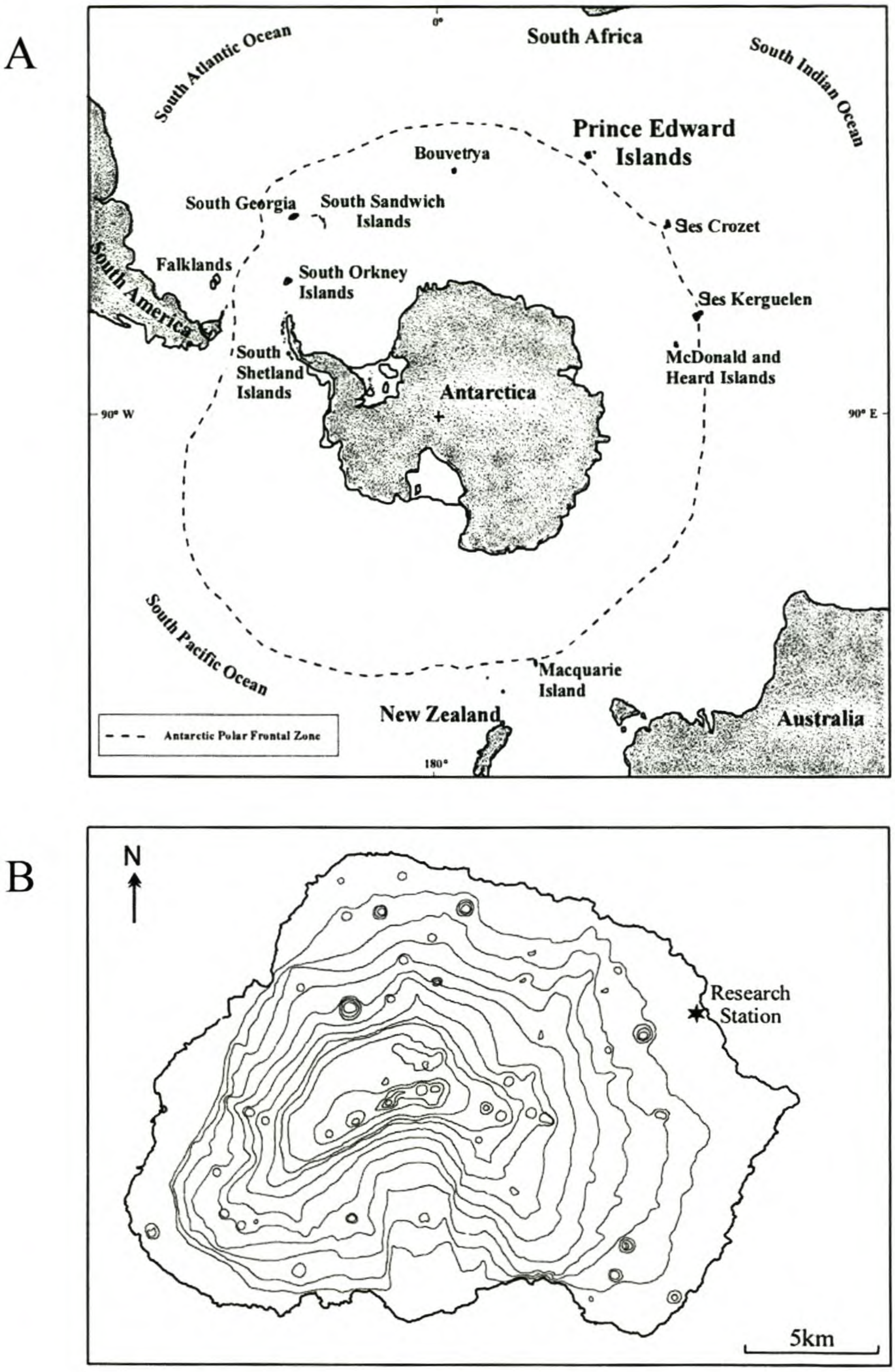

Fig. 1. A map of (A) the South Polar Region, indicating the position of the Prince Edward Islands in relation to other sub-Antarctic islands and (B) Marion Island, with the relative position of the Research Station.

a strong, predominantly westerly wind, which reaches gale force proportions through much of the year (Smith, 1987).

The vegetation on Marion Island is considered to be relatively species poor consisting of only 38 vascular species, 14 of which are introduced alien species (Smith, 1987). There are 93 species of mosses and 40 species of liver worts, which form an important component of the vegetation (V. R. Smith pers. comm.). More than 100 species of lichens have been recorded, mainly epilithic crutose forms which dominate the vegetation at high altitudes (Smith, 1987). Twenty eight bird species breed on Marion Island of which 27 are seabirds. There are no introduced bird species on the island (Hänel and Chown, 1999). The Lesser Sheathbill is the only endemic sub-species and the only true terrestrial bird inhabiting the island (Hänel and Chown, 1999). Three indigenous seal species (elephant seal, *Mirounga leonina*; Antarctic fur seal, *Arctocephalus gazella*, and the sub-Antarctic fur seal, *A. tropicalis*) breed on the island, and there is one alien rodent species (House Mouse, *Mus musculus*) (Hänel and Chown, 1999).

The arthropods include 60 mite and 14 springtail species (Barendse and Chown, 2000; Gabriel et al., 2001; Chown et al., 2002). The remaining arthropods comprise 18 indigenous and 27 introduced (12 naturalized alien species, 15 transient alien species) insect species belonging to 31 families (Hänel and Chown, 1999). In addition to the abovementioned taxa there is one indigenous species of terrestrial snail, one introduced slug species, a recently introduced terrestrial isopod (*Porcellio scaber*) (Slabber and Chown, 2002) and several introduced earthworm species (Crafford et al., 1986; Smith, 1987; Hänel and Chown, 1999). Mites and springtails dominate the arthropod fauna on Marion Island, apparently contributing to soil nutrient cycling (Chown et al., 2002). However, surprisingly little is known about the responses of these animals to the abiotic environment.

The ameronothrid mites on Marion Island occupy the littoral, marine and terrestrial habitats. Four of the species belong within the genus *Halozetes* with the remaining species, *Podacarus auberti* Grandjean, falling into a closely related genus. *H. belgicae* and *H. marinus* are widely distributed in the sub-Antarctic (this is also true for *P. auberti*) (Pugh, 1993; Marshall et al., 1999; Mercer et al., 2000), while *H. marionensis* is confined to the South Indian Ocean Province (Wallwork, 1973; Pugh, 1993). It is also thought that *H. belgicae* and *H. marinus* are evolutionarily older species (Wallwork, 1973), with *H. fulvus* considered to be recently evolved and endemic to the Prince Edward Islands (Marshall et al. unpublished data, see also Wallwork, 1973). All five species are ecologically separated in the marine and terrestrial environments (Fig. 2). *Halozetes marinus devilliersi* Engelbrecht and *H. marionensis* Engelbrecht are found in the marine littoral zone, occurring respectively in the red seaweeds in the lower shore and in the black lichen *Verrucaria* sp. which dominates the upper shore (Mercer et al., 2000). *H. marinus* also has preference for thick mats of filamentous algae on sheltered shores (Mercer et al., 2000; pers observation). *H. belgicae* Michael occurs in *Turgidosculum complicatulum* (formerly *Mastodia tesselata*), and *Caloplaca* sp. in the supra-littoral zone. *H. belgicae* and *H. marionensis* have been found to inhabit saltwater and brackish/freshwater pools in the supra-littoral zone (Marshall et al., unpublished data). *H. fulvus* and *Podacarus auberti* are found predominantly in terrestrial environments on moss (e.g. *Sanionia uncinata*) in mires and *Poa cookii* respectively (Mercer et al., 2000; Barendse et al., 2002), with *Podacarus auberti* also occuring in *T. complicatulum* in the supralittoral (Mercer et al., 2000). The five species constituting this group occupy the full range of habitats on Marion Island from sub-littoral to high elevation, and occur in distinct environments (marine littoral and terrestrial). Terrestrial habitats on Marion Island are thermally variable (Chown and Crafford, 1992), providing opportunities to compare thermal limits and activities of species occurring in the

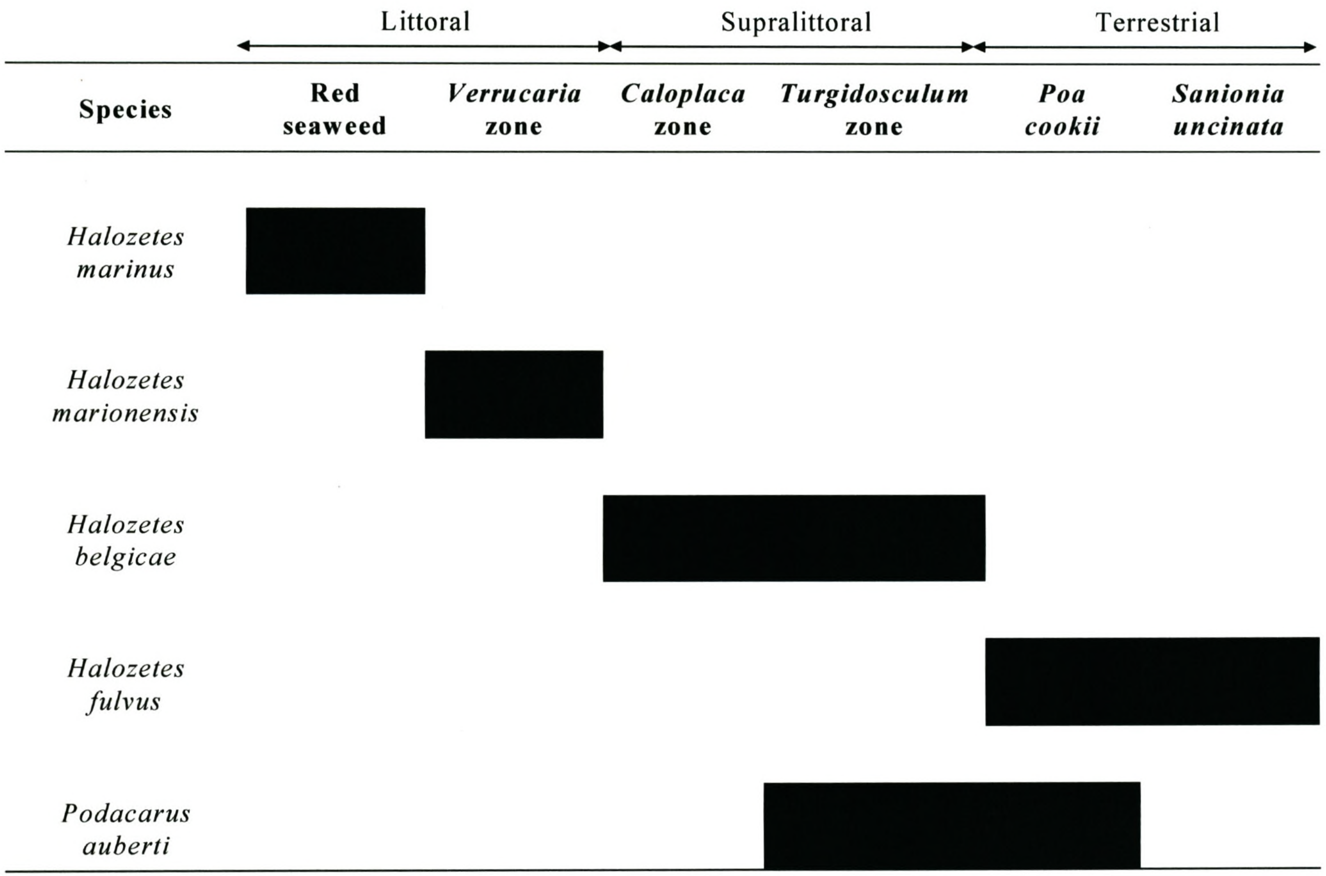

Fig. 2. Diagramatic representation of the ranges of the five species of oribatid mites along the rocky shore and terrestrial habitats.

more thermally stable marine environment with species from a thermally variable terrestrial environment, and to examine how thermal limits change when exposed to thermal stresses. Therefore in this study, investigations are focused on absolute thermal limits (upper and lower lethal temperatures), supercooling points, and the responses of the temperature dependence of locomotion.

Cold hardiness

To counter the effects of low temperatures invertebrates can respond in two ways: individuals can either have a programmed response to cold, which is a long term acclimation that can occur over a period of a few days to a few weeks (Chen et al., 1987), usually preceding winter during which physiological preparations are made (Lee et al., 1987); or individuals can show a rapid response (rapid cold hardening) which can be induced by short periods of pre-exposure (as short as two hours) to low temperatures (Chen et al., 1987).

When faced with long periods of cold exposure during winter, arthropods generally enhance survival by adopting one of two possible cold hardiness strategies: freeze-tolerance or freeze-intolerance. Some species are able to switch between the two strategies (Cannon and Block, 1988). Freeze tolerant organisms are able to survive sub-zero temperatures by tolerating the formation of extracellular (and rarely intracellular) ice within the body (Danks et al., 1994; Sinclair, 1999). Freeze-intolerance is defined as ability of the organism to survive subzero temperatures by preventing the formation of ice crystals in the body which the organisms are not able to tolerate (Danks et al., 1994; Sinclair, 1999). Species that do not survive low sub-zero temperatures have often been placed in the freeze-intolerant category. However, death by processes other than freezing does not necessarily justify placing these species in the freeze-intolerant category. Bale (1993) suggested that a distinction between freezing and chilling needs to be made and that species that do not fall into the freeze-tolerance or freeze-intolerance categories be grouped into three main categories:

- Chill-tolerant – can be divided into highly- and moderately chill-tolerant subgroups; features of these subgroups are little evidence for non-freezing mortality above the supercooling point and the ability to survive for long periods of time at low or subzero temperatures normally encountered in the winter microhabitat
- Chill-susceptible – constitutes a small group of insects that have limited cold hardiness capabilities and that can survive at low temperatures below the threshold required for normal growth and development (0°C to 5°C), but die rapidly after very brief exposures to relatively high subzero temperatures.
- Opportunistic survival – individuals in this group are killed by brief exposure to low temperature but distinguish themselves from chill-susceptible species by their inability to survive below threshold temperatures at which normal metabolism is terminated. The winter survival of these species would depend on the opportunistic exploitation of suitable warm sites.

Freeze tolerant species have limited supercooling ability, and ice nucleating agents (INA) (such as food particles; bacteria and potentially proteins, however these are not the proteins' main function) are present in the haemolymph to prevent freezing injury in closed compartments such as cells and the intestine (Zachariassen, 1985). INAs ensure that extracellular freezing is established before nucleating components in closed compartments like cells and the intestine initiate freezing in these compartments. Extracellular INAs have a high activity allowing them to induce freezing at a temperature above the nucleation temperature of intracellular and intestinal ice-nucleating components (Zachariassen, 1985). Unlike freeze-intolerant species that require the removal or inactivation of INAs during the cold season, freeze-tolerant species retain INAs (Zachariassen, 1985). Zachariassen (1985) argued that ice nucleating activity may vary seasonally because freeze-tolerant beetles from Southern California lacked ice nucleating activity when collected in summer.

In freeze-intolerant species, cold hardiness is found to be at a maximum in winter with increased cold hardiness resulting from the accumulation of cryoprotective substances (Bale, 1987; Zachariassen and Kristiansen, 2000; Sinclair and Sjursen, 2001) as well as the removal or inactivation of INAs (Zachariassen, 1985). The accumulation of cryoprotective substances helps to lower the freezing point of an animal (Bale, 1987; Zachariassen and Kristiansen, 2000; Sinclair and Sjursen, 2001). The production of antifreeze proteins (also known as thermal hysteresis proteins (THP)) assists in lowering the freezing point of the haemolymph of an individual relative to its melting point, and may also act as a stabilizing agent by preventing the growth of ice crystals when in the supercooled state (Bale, 1987; Zachariassen and Kristiansen, 2000). THPs may also protect insects against seeding of external ice through the body wall (Zachariassen, 1985). The presence of INAs poses a threat of potential ice nucleation in the intracellular or gut compartments and for insects to obtain high super cooling capacities these INAs have to be removed or inactivated (Zachariassen, 1985).

The evolution of cold-tolerance has resulted in predominantly freeze-intolerant species, and it has been suggested that freeze-intolerance is basal in the arthropods and that the freeze-tolerance strategy has frequently evolved within different taxa (Sinclair et al., 2003b).

Upper Lethal Temperature

In ectotherms, rapid exposure to high temperatures results in a form of stress known as heat shock (Chen at al., 1991). The exposure to high temperatures leads to the expression of heat shock genes and synthesis of heat shock proteins (Hsps) (Chen et al., 1990; Chen et al., 1991; Goto and Kimura, 1998), these proteins function as molecular chaperones and by interacting with stress-damaged proteins assist in the unfolding and relocalization of proteins damaged by the stresses (Krebs and Feder, 1997; Goto and Kimura, 1998). Organisms from fluctuating environments tend to exhibit the heat-shock response while organisms in more stable environments lack the ability to induce Hsps (see Goto and Kimura, 1998). In addition, the effect of acclimation on the expression of these proteins also varies amongst ectothermic organisms (Goto and Kimura, 1998). Koban et al. (1987) reported that induction temperature for Hsps at high temperatures in the channel cat fish showed no effect of acclimation whereas threshold temperature for the induction of the heat shock response in *Gillichthys mirabilis* was affected by acclimation (Dietz, 1994). Therefore changes in the temperature at which Hsps are induced due to acclimation effects could potentially affect the upper thermal tolerance of an organism.

To determine the upper thermal tolerance of an organism one of two major methods can be used, namely the static (or lethal temperature - LT) method and the dynamic method. The static method measures the time to death at constant temperatures and utilizes statistical interpolation techniques to determine values which represent the temperatures where 50% of the population survives (LT50) (Lutterschmidt and Huchison, 1997). The dynamic method involves increasing the test temperature until an end point is reached when the animal shows a characteristic visible response (e.g loss of muscle function). This method represents the concept of critical thermal maximum (CTmax) and represents functional limits to heat exposure.

Locomotion

Locomotion and its thermal sensitivity are important traits. There has been much emphasis on the measurement of the ecological relevant aspects of whole organism performance, and locomotion performance has been suggested to be a reliable and practical measure of potential fitness of animals that is functionally related to morphological variation (Semlitsch et al., 1999; Losos et al., 2002).

Locomotion studies, mostly of maximal performance capabilities, have suggested that one can predict how differences in phenotype should lead into differences in maximal capabilities, which over evolutionary time would result in selection acting on the maximal performance capabilities (Losos et al., 2002; Schulte et al., 2004). Sprint speed has been argued to be ecologically relevant in lizards and anurans, as it may affect fitness via its effect on factors such as predator avoidance, foraging success, dispersal and social dominance (Huey and Dunham, 1987; Semlitsch et al., 1999; Van Damme and Vanhooydonck, 2001). Locomotor performance also plays an important role in the daily and seasonal activities of anurans (e.g. migration of pond-breeding frogs to aquatic sites during the breeding season: Semlitsch et al., 1999).

For ectotherms with limited thermoregulatory capacity temperature often limits activity patterns, translating into differences in realized fitness (Gilchrist, 1996). Maximum swimming speed in goldfish *Carassius auratus* (Cyprinidae) is reduced by acclimation to temperatures other than 25°C at their optimal temperature (optimal temperature refers to the thermal conditions (or optimal temperature) that maximize performance) (Kingsolver and Huey, 1998). This is true also of arthropods. In *Aphidius ervi*, selection for increased walking speed at the optimal temperature resulted in a selection for decreased performance breadth (an index of the breadth of the curve) (Gilchrist, 1996).

The responses of thermal limits to acclimation

Acclimation temperatures can cause changes (potential phenotypic plasticity) in the response of individuals to stressful experimental conditions that could increase their survival (Hoffmann, 1995). As mentioned earlier, phenotypic plasticity allows an organism to express different phenotypes depending on the biotic or abiotic environment in a way that may alter that organism's fitness (Kingslover and Huey, 1998; Agrawal, 2001). The ability of an animal to respond to changing environmental conditions (daily, seasonal or due to changing climatic patterns) is important for its survival. Physiological traits reflect phenotypic plasticity in various ways (Huey and Berrigan, 1996):

- Acclimation responses - the duration of these modifications may occur over minutes to months. Such modifications are reversible within an organism's lifetime, the modifications may be active responses (to current environments) or anticipatory responses (to future environments).
- Developmental switches - with this plastic response the phenotype is fixed irreversibly by environmental conditions experienced during a critical phase of an individuals development, these switches may involve continuous (e.g. size) or discontinuous (e.g. bristle number) traits.
- Developmental pathologies - environmentally induced, pathological modifications that occur during development.
- Labile effects (also known as transient or acute effects) - are rapid modifications of phenotypic capacity or performance. These occur as a function of an organism's immediate (as opposed to cumulative) physical or physiological environment they can be represented by performance curves which can potentially be modified by acclimation or developmental switches.

- Cross-generational effects - maternally or paternally transmitted phenotypic modifications. These effects on temperature are considered potentially an important influence on phenotypic plasticity.

Many studies have examined the effects of acclimation on phenotypic plasticity (Nunney and Cheung, 1997; Woods, 1999; Thompson et al., 2001; Woods and Harrison, 2001), with acclimation being defined as "…the physiological, biochemical or anatomical responses of an individual animal that result from chronic exposure to a new, experimentally induced, environmental condition" (Woods and Harrison, 2002). A question that arises is whether the acclimation and consequence results thereof are beneficial to the organism or not?

This question is usually examined under the rubric of the Beneficial Acclimation Hypothesis (BAH), which was first defined by Leroi et al. (1994) as: "…acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment". Many studies have tested this hypothesis, providing support for it (Nunney and Cheung, 1997, Thomson et al., 2001), but, more commonly, rejected it (Leroi et al., 1994; Bennett and Lenski, 1997; Gibbs et al., 1998; Woods, 1999; Woods and Harrison, 2001).

In an investigation of the effects of temperature on body size and fecundity in female *Drosophila melanogaster,* Nunney and Cheung (1997) found that individuals reared to adulthood at 18°C versus 25°C showed a 12% increase in dry weight. Early fecundity at a given temperature was highest when the rearing temperature and test temperature were the same, indicating that the acclimation regimes were beneficial. Thomson et al. (2001) acclimated pupal and adult *Trichogramma carverae* at 33°C and 35°C respectively, and reared a control group at 25°C. Pre-treated adults and larvae showed an increase percentage survival after exposure to 40°C when compared to the control group. The authors also tested for costs involved with acclimation by determining parasitism rate. No change in parasitism rate in the field and laboratory was found indicating that no cost was incurred on this trait by acclimation. From these results acclimation was found to be beneficial. *Manduca sexta* larvae reared from the first instar on low-water (69%) or high-water (80%) artificial diets, then transferred in the early fifth instar to either the same or opposite diets, responded to short-term hydric stress by minimizing water excretion rates through increased rectal water re-absorption, and long-term hydric stress by significant reduction of evaporative water loss (Woods and Harrison, 2001). Short-term and long-term changes appeared beneficial. Animals reared on low water diets grew less rapidly, but made apparently beneficial changes to an important component of the water balance physiology. These authors reasoned that different physiological processes and levels of biological organization are affected in differing ways by environmental change. They suggested that the Beneficial Acclimation Hypothesis will not be supported whenever chronic environmental exposure negatively affects physiological processes in aggregate, regardless of the potential adaptive or maladaptive value expressed in a particular trait, because performance traits represent the aggregate outcome of mechanistic processes or traits.

Gibbs et al. (1998) found no benefits of thermal acclimation of cuticular lipid composition and water loss rates among adult *Drosophila mojavensis*: mean hydrocarbon chain length increased after acclimation but water loss rates were unaffected, suggesting that apparent adaptive changes in cuticular lipids do not result in reduced rates of water loss, as has been previously speculated. Gibbs et al. (1998) suggested that the observed phenotypic response of cuticular lipids to the environment may represent an epiphenomenon of the effects of temperature on other processes, and may not be adaptive. Variations in temperature may affect biosynthetic enzymes (Gibbs et al., 1998), resulting in differences in surface lipid composition that have no regulatory function. Leroi et al. (1994) observed in *Escherichia coli* that acclimation to 32°C relative to 41.5°C enhanced competitive fitness at 32°C, but prior acclimation to 41.5°C relative to 32°C reduced competitive fitness at 41.5°C. Acclimation is thus not always beneficial. However, prior acclimation to 41.5°C enhanced survival at 50°C, a lethal temperature for this species, and this may have been as a result of stress proteins been induced by acclimation at 41.5°C. The expression of stress proteins may impose a physiological burden when the proteins are not needed to prevent lethal damage, hence the reduced competitive fitness at 41.5°C but increased survival at 50°C (Leroi et al., 1994).

Since the proposal of the BAH, several competing hypotheses have been put forward about acclimation effects. The Colder is Better (CIB) hypothesis (Huey et al., 1999), predicts that organisms raised at cooler temperatures have a higher significant fitness across all temperatures than organisms that are raised at higher or intermediate temperatures. Hotter is Better (HIB) is the reciprocal of the CIB, i.e. organisms raised at higher temperatures will have an overall higher relative fitness across all temperatures than do organisms raised at cooler or intermediate temperatures (Wilson and Franklin, 2002). The third competing hypothesis is the Deleterious Acclimation Hypothesis (DAH) which suggests that chronic acclimation will always have a deleterious effect owing to its negative effect on physical processes in aggregate (see Loeschcke and Hoffmann, 2002).

Thus, a major goal of the present study, given the importance of phenotypic plasticity as a partial response to climate change is to investigate the BAH and its alternatives for supercooling points (SCP), lower lethal temperatures (LLT), upper lethal temperatures (ULT) and locomotion speed. Of course, if acclimation is to be considered to be beneficial it must be of adaptive value. Because adaptations are traits on character states that evolve as a consequence of natural selection (Coddington, 1988), three conditions must be satisfied (Bech et al., 1999): (a) variation in the characteristic should be related to the variation in fitness; (b) between-individual variation should be consistent, therefore variation between individuals should be greater than the variation within an individual i.e. repeatability of the variation should be high and significant; and (c) the trait must be heritable.

Variability is important to identify because if differences in a trait are found mostly among individuals, rather than within individuals, then natural selection could act on that trait, provided the among individual differences in the trait influence survival (Fournier and Thomas, 1999). Repeatability is a useful tool for measuring variability within individuals and can be defined as "the proportion of the total variance in multiple measurements of a trait that is due to differences among individuals" (Dohm, 2002). Low repeatability in a trait (i.e. if the variation is found to be within individuals) could be as a result of noise which is inherent in biological systems (Fournier and Thomas, 1999). Thus a major goal of the work here was to examine repeatability in the traits examined, where this could be done, and the temperature dependence of locomotor performance.

The repeatability of various performance and physiological traits is well studied among vertebrates. Daily field metabolic rate in female Meadow voles (*Microtus pennsylvanicus*) (Berteaux et al., 1996) have low repeatabilities (r=0.26), which was ascribed to the fact that the animals were probably not energy stressed (voles were in a non-reproductive state) and were therefore flexible in the management of their daily energy budget. In Kittiwakes the repeatability of daily field metabolic rate is high (Fyhn et al., 2001). In Merriam's kangaroo rats (*Dipodomys merriami*) Hayes et al. (1998) found repeatability to vary from 0.13 to 0.52 for evaporative water loss and 0.41 to 0.84 for oxygen consumption. Repeatability values have also been calculated for non-physiological traits, these range from survival skills in fish larvae (Fuiman and Cowan, 2003) to haematocrit levels in birds

(Potti et al., 1999) and locomotor performance in reptiles and amphibians (Huey and Dunham, 1987; Semlitsch et al., 1999). Fuiman and Cowan (2003) found varying repeatability values. Some individuals showed consistently low repeatability which the authors suggested could indicate historical selection on performance and repeatability of escape speeds and distances. Fuiman and Cowan (2003) suggested that the low repeatability was due to limited heritability of behavioural traits, a trade-off of behavioural skills with another selected trait or extreme variability in the nature of mortality in coastal environments. Potti et al. (1999) examined the repeatability of haematocrit in pied flycathers (*Ficedula hypoleuca*) and found high repeatability values (r = 0.69 to 0.80). Potti et al. (1999) propose that the low, but repeatable, haematocrit levels may be as a result of low breeding effort. Huey and Dunham (1987) found high temporal and geographical repeatabilities for average speed of lizards (*Sceloporus merriami*; Iguanidae) from two populations. Maximum sprint speed was also found to be repeatable between years, suggesting that sprint speed is a repeatable trait. However the authors reasoned that because the repeatability of locomotor performance was lower than that of many morphological measurements, the detection of selection would require large samples, strong selection, or both. Short-term repeatability of jumping performance in newly metamorphosed leopard frogs (*Rana spp*; Ranidae) was moderately high when reared at high density, possibly increasing the predictability of performance (Semlitsch et al., 1999).

Not many studies have investigated repeatability of physiological or performance traits in invertebrates. Schaus and Sakaluk (2002) focused on the repeatability of sperm number in three cricket species (r=0.36 to 0.79 across species). The lowest repeatability, in *Gryllus sigillatus*, may be due to a consequence of the males enticing females with a nuptial food gift which results in females relinquishing some control over the insemination process (Schaus and Sakaluk 2002). This allows the male to vary the number of sperm allocated to the ampula relative to males of non-gift-giving species. The authors then go on to suggest that the results of this study leave open the possibility that variation in sperm number is based to some extent on heritable genetic variation. Repeatability of metabolic rate and gas exchange characteristics have been investigated in *Perisphaeria* sp. cockroaches (Marais and Chown, 2003), the tsetse fly (*Glossina pallidipes*) (Terblanche et al., 2004a) and the whip-spider (*Damon annulatipes*) (Terblanche et al., 2004b). In a *Perisphaeria* cockroach (Marais and Chown, 2003) found four distinct gas exchange patterns, with metabolic rate (r=0.51) and the bulk of gas exchange characteristics (r=0.08-0.91. median=0.42) showing high repeatability. Marais and Chown (2003) concluded that results provide support for consistency of variation among individuals which is a prerequisite of natural selection. The results also suggest that the variation in these traits among species and populations might be adaptive. In *G. pallidipes* repeatability generally increased with age in males and females with feeding appearing to have little effect on the repeatability of metabolic rate, this may be as a result of rapid temporal change in muscle morphology in young flies (Terblanche et al., 2004a). In *D. annulatipes* repeatability was high and significant (r=0.4-0.6) with no affect on repeatability after 2 weeks of laboratory acclimation. Terblanche et al. (2004b) concluded that for this species laboratory acclimation affects can be disregarded. For both *G. pallidipes* and *D. annulatipes* the significant and high repeatabilities indicate that one of the prerequisites for natural selection has been met and therefore adaptive metabolic rate variation is not unlikely (Terblanche et al., 2004a; Terblanche et al., 2004b)

Aims of this thesis

Given the above background, the aims of this study are threefold:

i. to examine the thermal limits, including upper lethal (ULT) and lower lethal temperatures (LLT) and supercooling points, and how these limits, especially the last two, respond to acclimation effects in five ameronothrid species;

ii. to investigate phenotypic plasticity in reaction norms to test the Beneficial Acclimation Hypothesis and three alternative hypotheses, namely the Hotter is Better (HIB), Colder is Better (CIB) and Deleterious Acclimation (DAH) hypotheses;

iii. to assess repeatability in the locomotor performance curves and therefore to determine whether these curves could be subject to selection.

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Chapter 2

Locomotor performance of five oribatid mite species: testing the beneficial acclimation hypothesis and its alternatives

Introduction

Physiologists have long held the view that phenotypic change by an individual in advance of or in response to a changing environment is beneficial. Indeed, the physiological literature is replete with arguments to this effect, including those regarding seasonal and shorter term responses of individuals to changing temperatures, water availability, and resource conditions (reviews in Prosser, 1986; Chown and Nicolson, 2004). Nonetheless, over the past several years, this beneficial acclimation hypothesis (BAH) (Leroi et al., 1994) has received vigorous criticism from several perspectives. These include the failure of many studies to include, *a priori*, the wide range of alternative hypotheses for the responses of individuals to a changing environment (Huey and Berrigan, 1996; Huey et al., 1999), and the generally poor empirical support for the BAH in those tests that have examined the hypothesis explicitly (Leroi et al., 1994; Bennett and Lenski, 1997; Woods, 1999; Woods and Harrison, 2001; Sibly et al., 1997; Gibert et al., 2001; Gilchrist and Huey, 2001). Thus, much of the recent literature suggests that despite the seemingly obvious benefits of acclimation (or acclimatization), there is little support for this idea. Rather, many of the alternative hypotheses, such as optimal developmental temperatures (see Huey and Berrigan, 1996), now appear more plausible.

Given the wide range of scenarios under which adaptive phenotypic plasticity is likely to evolve (reviews in Scheiner, 1993; Agrawal, 2001; Frankino and Raff, 2004), the lack of support for the beneficial acclimation hypothesis is counter-intuitive, at least from a physiological perspective. Indeed, several authors are now of the view that many of the explicit tests of the BAH that have been undertaken (see references above) are problematic for a number of reasons. Echoing earlier distinctions between labile and fixed traits (Scheiner, 1993), Wilson and Franklin (2002a) argue that the majority of tests of the BAH from a thermal acclimation perspective are neither direct nor complete because rather than being tests of what comparative physiologists regard as acclimation (or acclimatization) (e.g. Spicer and Gaston, 1999, 32-38; Willmer et al., 1999, 9-12), they are elegant analyses of the adaptive significance of developmental plasticity. According to Wilson and Franklin (2002a) acclimation responses are reversible, facultative changes to the phenotype, whilst developmental plasticity concerns those phenotypic alterations made as a consequence of different developmental environments. Woods and Harrison (2002) offer a different perspective, arguing that most explicit tests of the BAH compare animals in optimal environments with those under stressful conditions. Long-term exposure to the latter is likely to impair organismal performance in all other environments, so compromising tests of the BAH. In addition, Woods and Harrison (2002) suggest that tests of the BAH should focus on fitness consequences of individual traits, not on the entire suite of characters that constitute fitness, and that failure to do so has again meant that the relationship between fitness and acclimation has been obscured.

Both Woods and Harrison (2002) and Wilson and Franklin (2002a) include in their critiques several suggestions for ways in which tests of the BAH might be improved, especially with regard to examinations of this idea for labile traits. In essence, they concur that the rigorous, strong inference approach adopted in analyses of developmental plasticity (e.g. Huey et al., 1999) should be applied to tests of the BAH for labile traits, that the range of environments investigated should perhaps not include those that impose considerable stress (but see also Hoffmann, 1995; Loeschcke and Hoffmann, 2002), and that a focus on individual traits is required. Whilst acknowledging that aspects of these explicit critiques of previous, development-based tests of the BAH are open for discussion, it is clear that the recommendations they make for future tests of the BAH in labile traits could go a substantial way to resolving the surprising mismatch between what comparative physiologists consider to be self-evident (beneficial acclimation), and what tests of the idea have indicated to date.

Here, these recommendations are implemented for assessing the plasticity of the temperature sensitivity of locomotor performance in five, closely-related species of oribatid mite that occur over a range of environments from strictly marine to terrestrial at sub-Antarctic Marion Island. Locomotor performance was chosen as a trait because it is a convenient measure of physiological performance in oribatid mites (Marshall and Chown, 1995; Marshall et al., 1995), and is directly linked to fitness in a wide variety of organisms (Huey and Stevenson, 1979; Gilchrist, 1996; Gibert et al., 2001; but see also Angilletta et al., 2002 for exceptions), including mites. Mites were chosen because they are so small (Mercer et al., 2001) that they are unlikely to be able to regulate their internal thermal environment in the face of changing external conditions (Stevenson, 1985), so making them ideal for examining environmental sensitivity in physiological traits (Gilchrist, 1996). Moreover, the phylogenetic propinquity, but different habitat preferences of the particular species investigated here (Barendse et al., 2002) (Fig. 1), suggest that the extent of plasticity is likely to differ between them. Specifically, the very narrow range of sea temperatures, compared with a broader range of temperatures on rocky substrates, and an intermediate range in vegetated areas at Marion Island (Schulze, 1971; Chown and Crafford, 1992), lead to the expectation that the marine species should show much less flexibility than their epilithic and vegetation-bound counterparts.

In particular, acclimation effects on the responses of three aspects of thermal performance curves were examined: optimum speed (≈ maximum speed, u_{max}), temperature of the optimum speed (optimum temperature, T_{opt}), and performance breadth (T_{br}) (see Huey and Stevenson, 1979; Gilchrist, 1996). Although there have been several predictions made previously with regard to acclimation hypotheses both for these traits and for their covariation, we revisited these *a priori* predictions because in some instances it is clear that several quite similar predictions can be made for different combinations of the hypotheses. For example, Gilchrist (1996) suggests that the "hotter is better" hypothesis predicts a positive relationship between optimum temperature and maximum velocity (see also Carrière and Boivin, 1997). However, as we demonstrate below, a positive relationship between these variables, over a range of acclimation temperatures, can arise in two different ways. Thus, our predictions for each of the three traits and their interactions are as follows.

In the case of optimum speed, the BAH and temperature compensation (COMP) (the maintenance of rate in the face of temperature changes – see Clarke, 2003) hypotheses make equivalent predictions of no change in speed with short-term acclimation. That is acclimation at each temperature should result in optimum speed at that temperature. "Colder is better" (CIB) predicts an inverse relationship between speed and acclimation temperature, "hotter is better" (HIB) a positive relationship, and the deleterious acclimation hypothesis (DAH) (see Loeschcke and Hoffmann, 2002; Wilson and Franklin, 2002b; Woods and Harrison, 2002) predicts a substantial decline in performance at all temperatures relative to animals that have not been held in the laboratory for the acclimation period (Fig. 2a). The same predictions can be made for performance breadth. By contrast, for temperature of optimal speed (T_{opt}) the predictions are quite different. Here, the BAH predicts a positive relationship between T_{opt} and acclimation temperature (and therefore incorporates both the HIB and CIB), COMP predicts no change with acclimation, the DAH predicts a substantial difference between field fresh and acclimated individuals, and possibly also an inverse relationship between T_{opt} and acclimation temperature. The latter could also be considered "reverse acclimation" (RAH), similar to the reverse compensation that has occasionally been documented in investigations of acclimation effects on metabolic rate (see Huey and Berrigan, 1996 for review) (Fig. 2b).

When covariation of the above traits is examined, the predictions for each of the hypotheses are more complex, and require not only that covariation of the traits is examined, but also that the same is done for the relative positions of each acclimation trial along the curve (Fig. 3a - c). Moreover, in the case of the relationship between maximum speed and performance breadth, the assumption has to be made that the influence of a tradeoff between maximum speed and performance breadth is likely to be minimal (for discussions of this tradeoff see Huey and Hertz, 1984; Gilchrist, 1996; Angilletta et al., 2002).

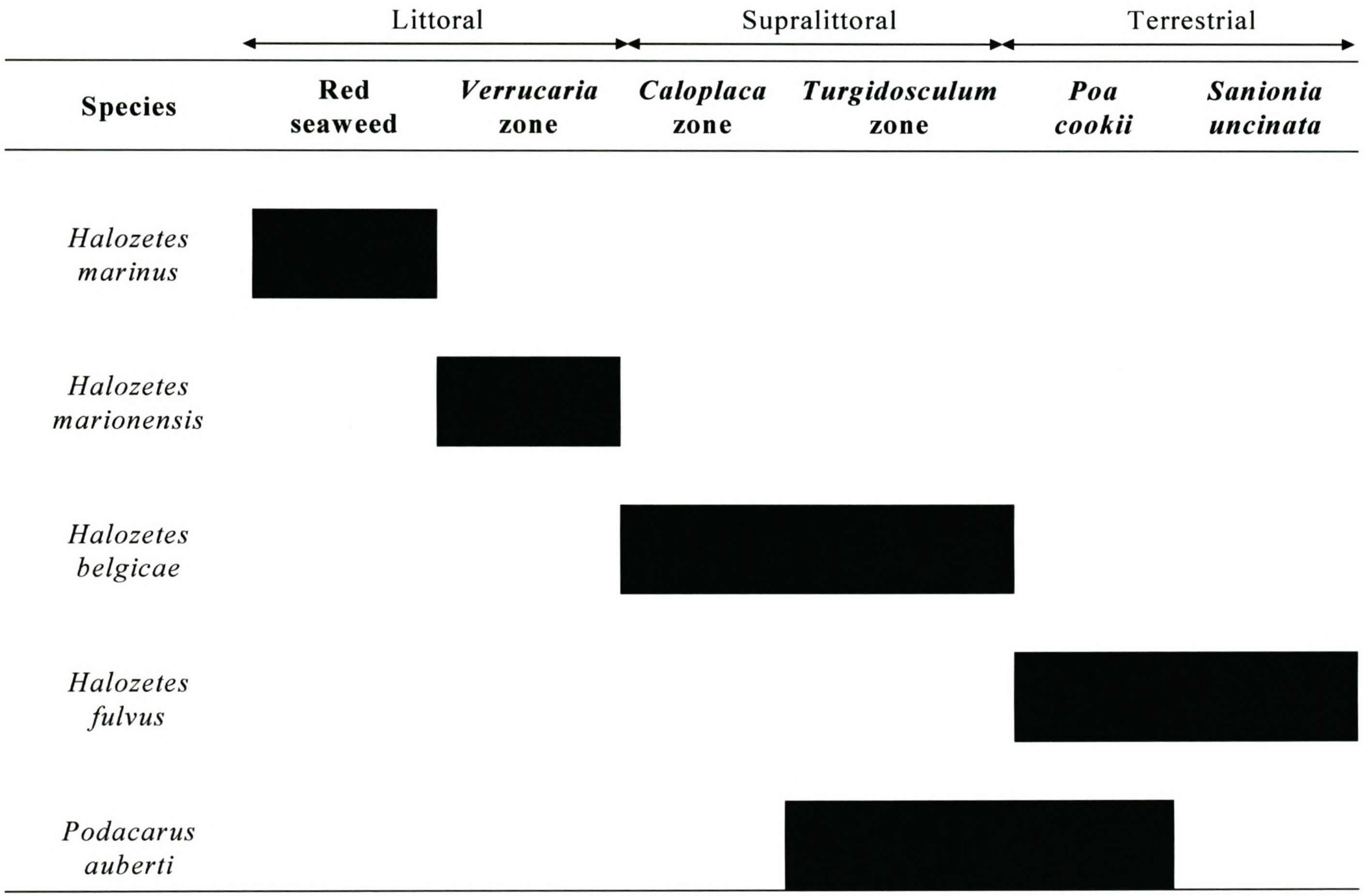


Fig. 1. Diagramatic representation of the ranges of the five species of oribatid mites along the rocky shore and terrestrial habitats.

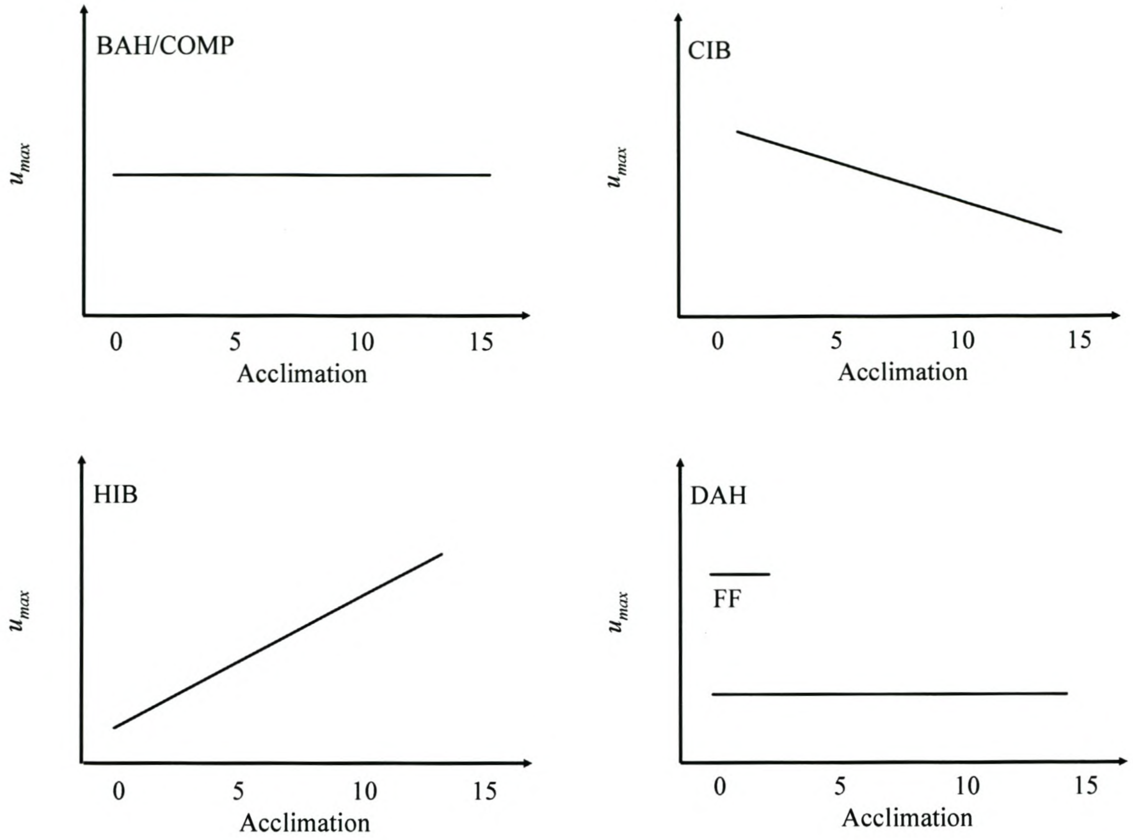


Fig. 2a. Predictions made for variation in optimum speed (u_{max}) with acclimation temperature by the Beneficial Acclimation Hypothesis (BAH) which in this case ≈ the Compensation Hypothesis (COMP); Colder is Better (CIB); Hotter is Better (HIB) and (D) Deleterious Acclimation (DAH) Hypotheses. The FF symbol represents field fresh animals that have not been exposed to acclimation. The same predictions hold for performance breadth (T_{br}).

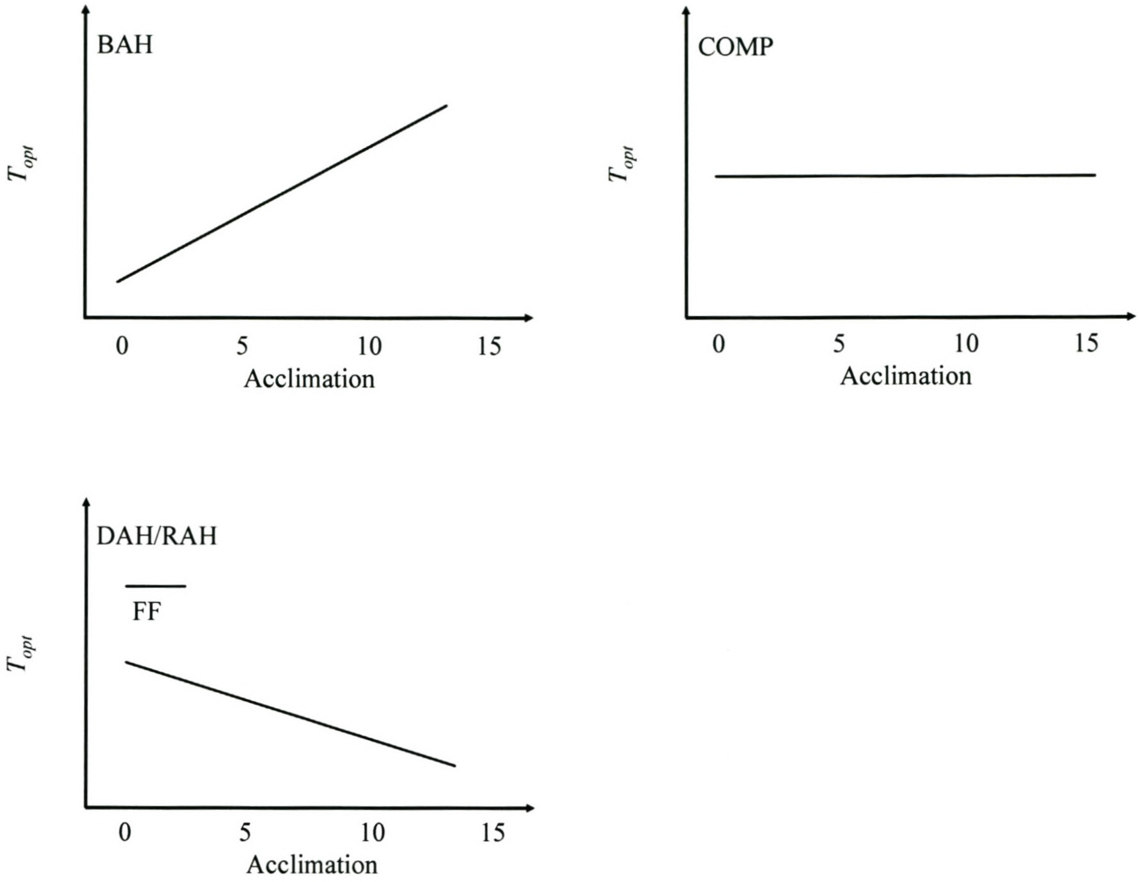


Fig. 2b. Predictions made for variation in the optimum temperature (T_{opt}) with acclimation temperature by the Beneficial Acclimation Hypothesis (BAH), which in this case includes "Colder is Better" and "Hotter is Better", and the Compensation (COMP); Deleterious Acclimation (DAH) and Reverse Acclimation (RAH) Hypotheses.

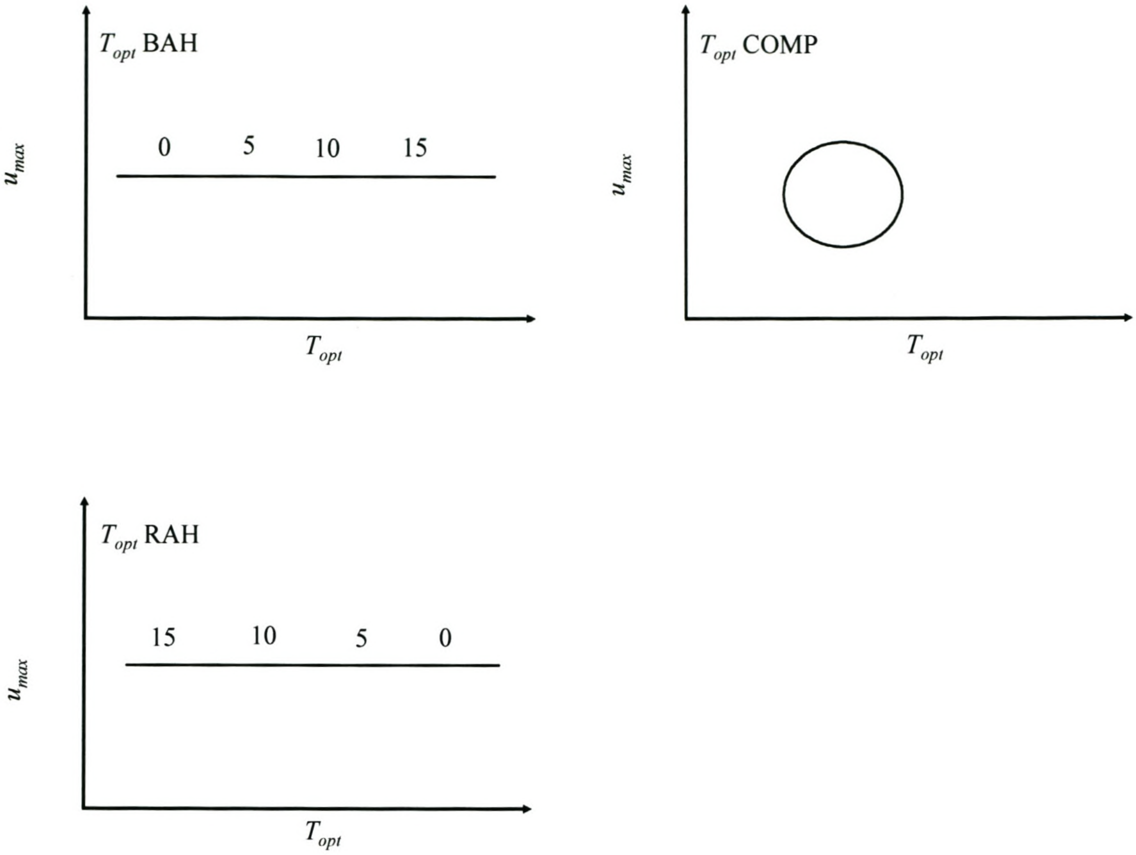


Fig. 3a. Predictions of the covariation of optimum speed (u_{max}) and optimum temperature (T_{opt}) when optimum speed shows beneficial acclimation, and the optimum temperature shows (A) beneficial acclimation, (B) compensation (COMP), or (C) reverse acclimation. The circle in the second panel represents a tight clustering of values for all of the acclimation temperatures.

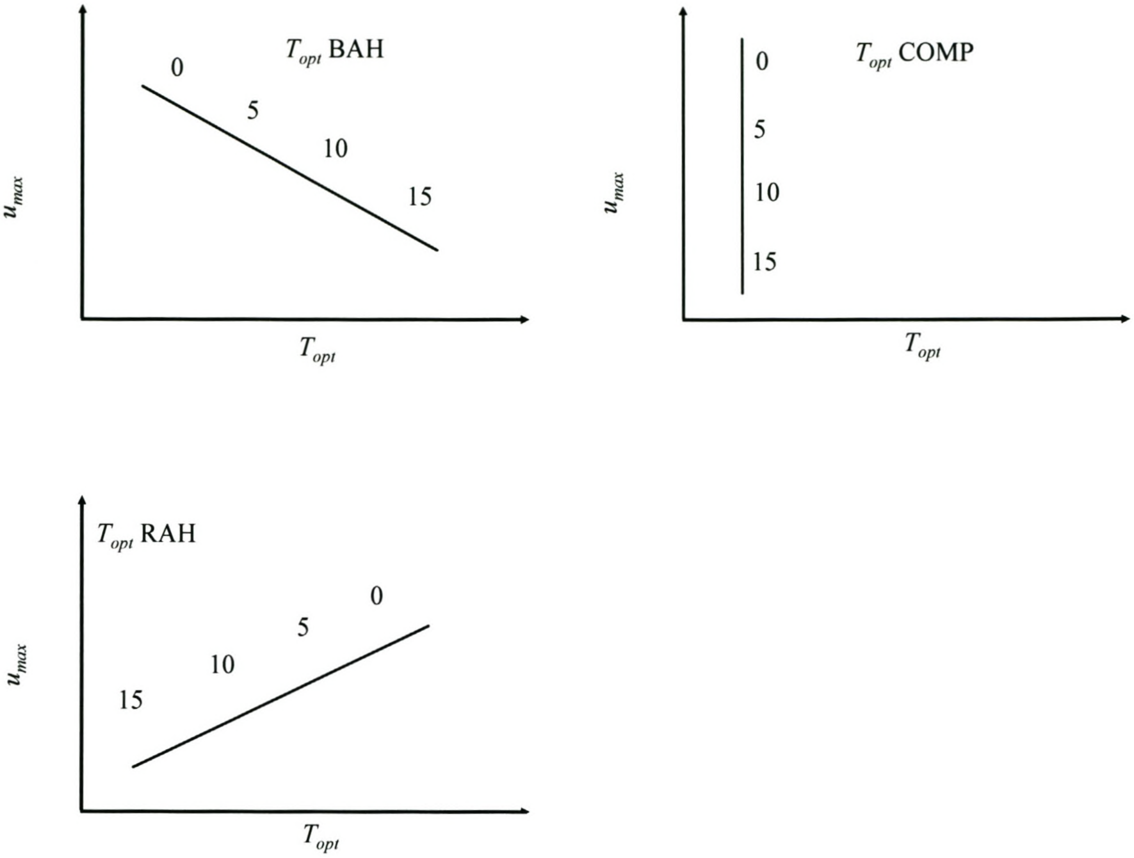


Fig. 3b. Predictions of the covariation of optimum speed (u_{max}) and optimum temperature (T_{opt}) when "colder is better" for optimum speed and the optimum temperature shows (A) beneficial acclimation (BAH), (B) compensation (COMP), or (C) reverse acclimation (RAH).

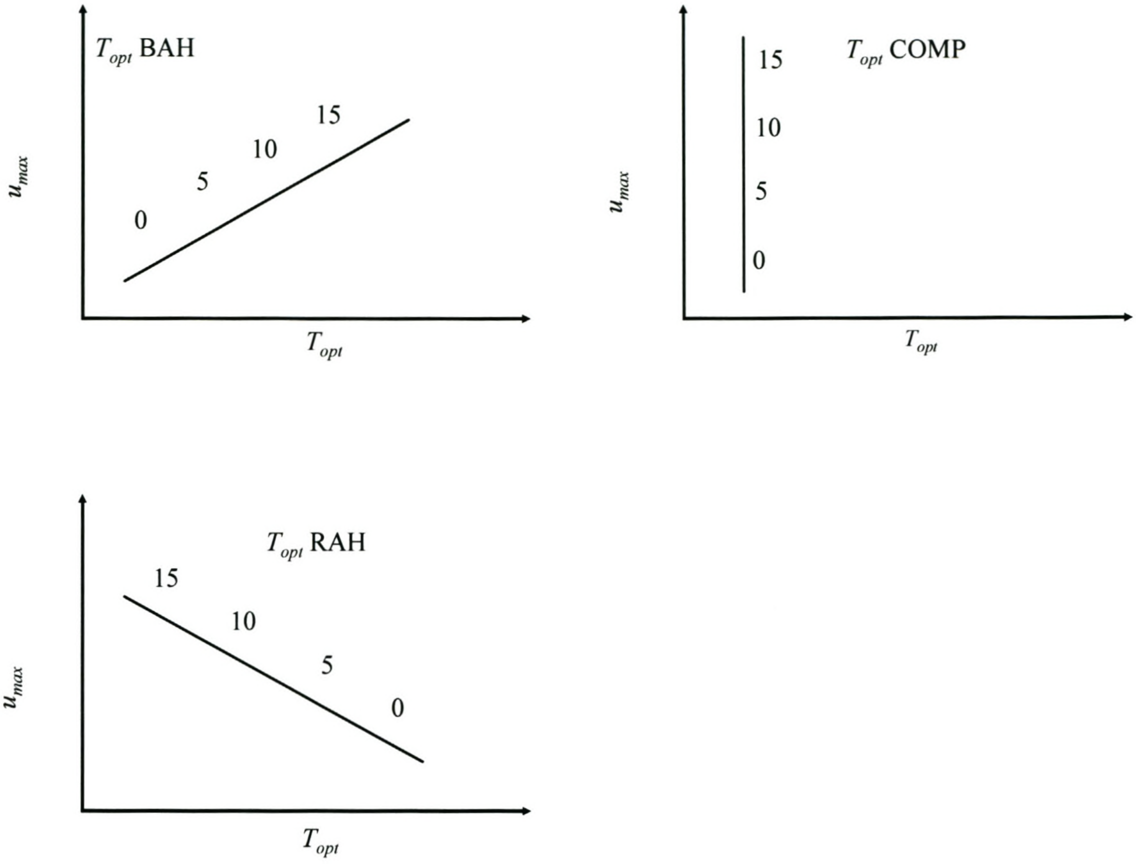


Fig. 3c. Predictions of the covariation of optimum speed (u_{max}) and optimum temperature (T_{opt}) when "hotter is better" for optimum speed, and the optimum temperature shows (A) beneficial acclimation, (B) compensation (COMP), or (C) reverse acclimation (RAH).

Materials and methods

Study site and animals

Specimens were collected from April 2002 – April 2003 on the East coast of sub-Antarctic Marion Island (46°54'S, 37°45'E), a small (290 km^2), isolated volcanic island in the Southern Ocean, situated 1770 km south east of South Africa. The island has a low mean annual air temperature (*c.* 5.7°C), high humidity (83%), and an annual precipitation of approximately 1900 mm (which is distributed evenly throughout the year) (Smith, 2002). The island is also characterized by a high degree of cloudiness and strong, predominantly westerly winds, which reach gale force proportions throughout much of the year (Schulze, 1971).

Five ameronothrid mite species were studied. *Halozetes marinus* and *H. marionensis* are found in the littoral zones along the rocky shores, *H. belgicae* is found in rocky supra-littoral zones, whilst *H. fulvus* and *Podacarus auberti* are terrestrial and occupy a wide range of habitats (Fig. 1) (Mercer et al., 2000; Barendse et al., 2002). For all species (except *Halozetes marionensis*, which was collected *in situ* from the rocky substrate into moistened, Plaster-of-Paris-lined vials) the vegetation on which the mite species occurred was collected and brought back to the laboratory and animals were extracted by hand. Animals were randomly assigned to five groups of 20 - 40 individuals each. The performance of individuals in one group was assessed immediately (labelled "field-fresh"). Each one of the remaining groups was placed in a jar containing a moistened Plaster-of-Paris substrate, provided with food and water, and assigned to one of four acclimation temperatures (0°, 5°, 10°, 15°C, ± 1°C in each case), obtained using temperature-regulated climate chambers, and held there for seven days. Day length was set to 14:10 (L:D) for the 10°C and 15°C acclimation treatments, and to 9:15 (L:D) in the 0°C and 5°C acclimation treatments. Because the mites are small, the tubes within which they were held occupied little space and therefore shelf effects in the climate chambers are unlikely to have had any influence on the temperature at which the individuals were held. Moreover, the same areas in the climate chambers were used for each species.

Locomotion trials

Individual mites were observed on a temperature-controlled stage using a stereo-microscope (X16 magnification). To induce walking, mites were exposed to a cold light source, and the frequency of leg movements of the front right leg was counted for each mite over a range of temperatures (Marshall et al., 1995). The number of leg strokes was counted over a 20s period, but only after 20s had elapsed once the mite had started walking. Counts were abandoned if mites faltered. A sample size of 20 animals was used per acclimation temperature.

The locomotion data were used to construct performance curves (Gilchrist, 1996) (see Fig. 4), of which the key traits are: T_{br} – index of the breadth of the curve, or performance breadth; T_{opt} – the thermal conditions (or optimal temperature) that maximize performance, or the optimum temperature; and u_{max} – the maximum rate of performance, or optimum speed (Gilchrist, 1996). T_{opt} and u_{max} were obtained from the experimental data and were used to calculate T_{br} using Gilchrist (1996)'s formula:

$$\mathrm{T_{br}} = \sqrt{\sum\left[\frac{u_i(T_i - T_{opt})}{u_{\max}}\right]^2} \qquad \text{(Eq. 1)}$$

where T_{opt} is the temperature at which an individual had the most number of leg strokes/20 seconds; u_{max} is the number of leg strokes/20 seconds at T_{opt}; u_i is number of leg strokes/20 seconds at temperature T_i. These traits are in many ways similar to those that have been developed for reaction norms (see David et al., 1997). However, unlike some authors, we do not consider the acute effects of temperature on performance to be similar to the effects of acclimation, but rather prefer to draw a distinction between the two (see also Huey and Berrigan, 1996). Thus, in this case we are interested in the effects of acclimation on the key traits that can be derived from the performance curve. Thus we prefer not to term this performance curve a reaction norm (as Angilletta et al., 2003 do).

Data analysis

To determine whether the data were representative of the capabilities of each animal, the number of leg strokes per 20 s was counted three times for each assessment temperature to provide a short-term assessment of the reliability of the counts. On the basis of these repeated counts, three performance curves were constructed for each individual at each acclimation temperature, and repeatability of each of the key traits derived from the performance curve was assessed using the intraclass correlation coefficient (Falconer and Mackay, 1996, Lessells and Boag, 1987).

To test the predictions of each of the acclimation hypotheses (see Fig. 2) the effects of the acclimation treatments on each of the traits (T_{opt} and T_{br} and u_{max}) in each of the species was assessed by plotting the trait mean (± S.E.) against each acclimation temperature (including the field fresh value), and by formally assessing the effects of the acclimation temperatures on the traits using a generalized linear model (GLZ) based on individual data points. For optimum temperature and performance breadth a GLZ assuming a normal distribution with a log link function was used, whilst optimum speed (which represents count data) was analysed using a GLZ assuming a Poisson distribution with a log link function. Covariation of the traits was examined using bi-plots (see Fig. 3) for each combination thereof, and the significance of this covariation was assessed using Pearson's product-moment correlation coefficients.

Because the relationship between optimum speed and performance breadth assumes that there is only a limited trade-off between these traits, we assessed their phenotypic covariation using a reduced major axis regression of the long-transformed values of these traits for each individual, for each species, at each of the acclimation temperatures, using RMA (v. 1.14b: Reduced Major Axis Regression, Andrew J. Bohonak, San Diego State University), and 10 000 bootstraps (with replacement) to provide confidence intervals for the slope.

Finally, to test the prediction that the species from the less variable marine environment would show a reduced phenotypic flexibility relative to the species from the more variable terrestrial environment, the largest difference in each of the traits across the four acclimation temperatures for each of the species was compared. In this case a mean and variance for this acclimation-associated difference in a given trait was produced by randomly drawing values for each trait for acclimation temperature from the data available for each individual at each temperature, and calculating the largest absolute difference between them. This was repeated 10 000 times (without replacement) to obtain the mean and variance for each species and these were compared using generalized linear models with distributions and link functions as above.

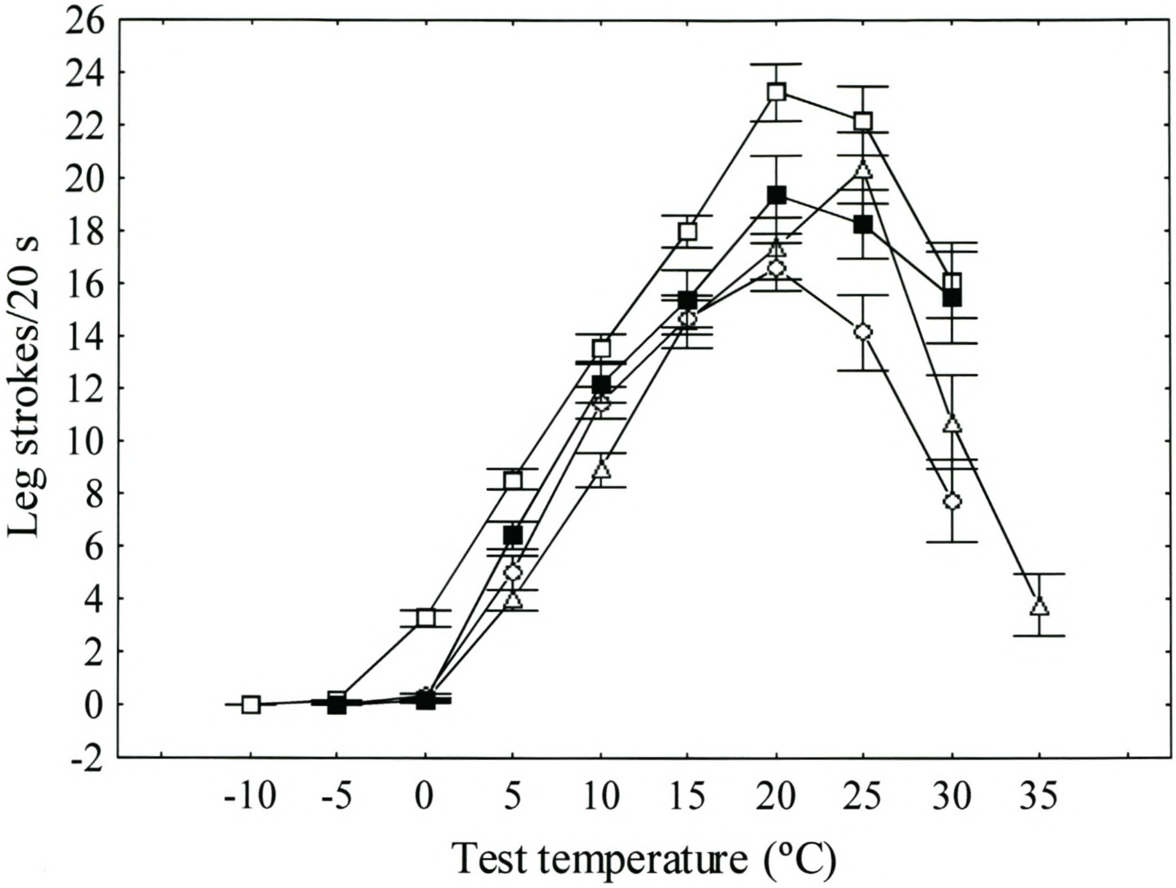


Fig. 4 Performance curves for four experimental acclimation groups (0°C (□), 5°C (Δ), 10°C (○) and 15°C (■) acclimation treatments) for *Halozetes belgicae* tested across a range of temperatures.

Results

Repeatability was generally higher for u_{max} than for T_{br} and T_{opt} in all of the species across all of the acclimation temperatures, and low repeatability values were occasionally found for T_{br} and T_{opt} especially in those individuals acclimated to 0 °C and 5 °C (Table 1). Nonetheless, it is clear that there was generally consistent performance within individuals and that the counts provided a reasonably accurate estimate of that performance. The statistical analyses, as described above, were therefore based on the mean values of the three counts for each individual at each assessment temperature.

In the case of both optimum speed (u_{max}), and performance breadth (T_{br}), there were generally no consistent differences associated with acclimation temperature for any of the species (Table 2). Moreover, the values of the traits in the acclimation groups did not differ either significantly or substantially from the values obtained from the field-fresh group of individuals (Figs 5 & 6, Table 2). Rather, there was remarkable similarity in trait values across the acclimation temperatures, with the mean difference in u_{max} and in T_{br} between acclimation temperatures across all species amounting to 4.4 strokes/20s and 3.9 units, respectively. The only possible exceptions were T_{br} in *P. auberti* (Fig. 6) where there was a small, but non-significant tendency for breadth to decline with increasing temperature, and u_{max} in *H. marinus* which also tended to decline with temperature (but only at 15°C) (Fig. 5). Optimum temperature showed very much the same pattern of little variation associated with acclimation temperature, and no substantial or consistent differences from field-fresh values. The sole exception was *H. marinus*, where T_{opt} declined significantly at the highest acclimation temperature (Fig. 7).

The plots and correlation coefficients revealed little in the way of covariation of T_{opt} and u_{max} (Fig. 8; Table 3). Rather, the values for each of the acclimation temperatures tended to cluster together, with the cluster including values for field-fresh individuals. This conclusion is substantiated by the fact that even with rescaling of the axes of the plots, there is little consistent progression of difference between the acclimation temperatures as the *a priori* predictions (Fig. 3) suggest there should be. *H. marinus* was the exception, and here there was a strong positive relationship between T_{opt} and u_{max}, with the 15°C treatment having the lowest and the 0°C treatment the highest values. Largely the same situation was found for covariation between T_{opt} and T_{br} (Fig. 9, Table 3), again with *H. marinus* being the notable exception. As with the previous variables, in this species there is a strong positive relationship between T_{opt} and T_{br}, with the 15°C treatment having the lowest and the 0°C treatment the highest values. In the case of the covariation of T_{br} and u_{max}, variation between the acclimation temperatures, at least for T_{br}, tended to be larger, but again, with the exception of *H. marinus* there were no consistent patterns of variation. In the latter species there was a positive relationship between T_{br} and u_{max}, with the 15°C treatment having the lowest and the 0°C treatment the highest values (Fig. 10, Table 3).

At the individual level, of the 20 relationships between T_{br} and u_{max} examined (four acclimation temperatures for each of the five species), three were significantly negative, and one was significantly positive (Table 4, Appendix 1). Thus, there was typically no relationship between T_{br} and u_{max} which is indicative of no tradeoff such that increased performance breadth is associated with a decline in optimum speed (and vice versa).

Interspecific differences in the extent of plasticity of the traits were significant (Table 5). Over the 15°C range of acclimation temperatures, T_{opt} differed by 4.3-7.3°C, T_{br} by 5.3 to 7.9 units, and u_{max} by 3.7 to 11.8 leg strokes/20s. For T_{opt} and T_{br} plasticity was always less in the marine species compared to the terrestrial species with the opposite trend found for u_{max} where plasticity in this trait was higher in the marine species when compared to that of the terrestrial species.

Table 1 Results of analysis of variance to calculate the repeatability (*r*) of performance breadth (T_{br}), optimum temperature (T_{opt}) and optimum speed (u_{max}) of five ameronothrid mite species for field fresh (FF) values and at four acclimation temperatures. Overlap in the 95% confidence intervals (LCL and UCL) indicate that repeatability was consistent across the species and acclimation temperatures.

	Source of variation	DF	MS	*F* - ratio	*P*	Repeatability (r)	UCL (0.95)	LCL (0.95)
u_{max}								
H. marinus								
FF	Among	19	10.38	11.12	< 0.0001	0.77	0.88	0.63
	Within	40	0.93					
0°C	Among	16	0.01	15.34	< 0.0001	0.830	0.92	0.70s
	Within	34	0.0008					
5°C	Among	17	0.05	24.74	< 0.0001	0.89	0.94	0.80
	Within	36	0.002					
10°C	Among	17	20.87	13.75	< 0.0001	0.81	0.90	0.67
	Within	36	1.52					
15°C	Among	18	0.31	9.52	< 0.0001	0.74	0.86	0.57
	Within	38	0.03					
H. marionensis								
FF	Among	19	15.98	13.90	< 0.0001	0.81	0.90	0.68
	Within	40	1.15					
0°C	Among	19	31.84	13.18	< 0.0001	0.80	0.90	0.67
	Within	40	2.42					

Table 1 cont.

	Source of variation	DF	MS	*F* - ratio	*P*	Repeatability (r)	UCL (0.95)	LCL (0.95)
5°C	Among	19	0.25	10.59	< 0.0001	0.76	0.87	0.61
	Within	40	0.02					
10°C	Among	18	13.59	8.71	< 0.0001	0.72	0.85	0.55
	Within	38	1.56					
15°C	Among	18	21.12	9.41	< 0.0001	0.74	0.86	0.57
	Within	38	2.25					
H. belgicae								
FF	Among	17	28.62	9.48	< 0.0001	0.74	0.86	0.57
	Within	36	3.02					
0°C	Among	18	74.53	30.34	< 0.0001	0.91	0.95	0.83
	Within	38	2.46					
5°C	Among	17	96.74	22.91	< 0.0001	0.88	0.94	0.79
	Within	36	4.22					
10°C	Among	19	0.03	23.06	< 0.0001	0.88	0.94	0.79
	Within	40	0.001					
15°C	Among	19	0.07	40.83	< 0.0001	0.93	0.96	0.88
	Within	40	0.002					
H. fulvus								
FF	Among	18	0.008	11.4	< 0.0001	0.77	0.88	0.63
	Within	38	0.0007					
0°C	Among	19	9.39	4.11	< 0.0001	0.51	0.71	0.29
	Within	40	2.28					

Table 1 cont.

	Source of variation	DF	MS	*F* - ratio	*P*	Repeatability (r)	UCL (0.95)	LCL (0.95)
5°C	Among	17	0.13	4.13	< 0.0001	0.51	0.72	0.28
	Within	36	0.03					
10°C	Among	18	6.18	3.71	< 0.001	0.47	0.69	0.24
	Within	38	1.67					
15°C	Among	19	0.21	4.69	< 0.0001	0.55	0.74	0.34
	Within	40	0.04					
P. auberti								
FF	Among	19	14.22	5.47	< 0.0001	0.60	0.77	0.39
	Within	40	2.60					
0°C	Among	19	47.42	29.33	< 0.0001	0.90	0.95	0.83
	Within	40	1.62					
5°C	Among	19	22.81	9.50	< 0.0001	0.74	0.86	0.58
	Within	40	2.40					
10°C	Among	18	7.02	1.375	0.20	0.11	0.37	0.00
	Within	38	5.11					
15°C	Among	19	22.40	8.90	< 0.0001	0.73	0.85	0.56
	Within	40	2.52					
T_{opt}								
H. marinus								
FF	Among	19	0.003	1.57	0.11	0.16	0.42	0.00
	Within	40	0.002					

Table 1 cont.

	Source of variation	DF	MS	*F* - ratio	*P*	Repeatability (r)	UCL (0.95)	LCL (0.95)
0°C	Among	16	0.0003	0.9	0.60	0.00	0.23	0.00
	Within	34	0.0004					
5°C	Among	17	0.004	3.52	< 0.001	0.46	0.68	0.22
	Within	36	0.001					
10°C	Among	17	0.001	2.4	< 0.05	0.32	0.58	0.08
	Within	36	0.0004					
15°C	Among	18	0.007	2.11	< 0.05	0.27	0.53	0.04
	Within	38	0.003					
H. marionensis								
FF	Among	19	0.01	4.85	< 0.0001	0.56	0.75	0.35
	Within	40	0.002					
0°C	Among	19	0.005	2.62	< 0.01	0.35	0.59	0.12
	Within	40	0.002					
5°C	Among	19	0.004	3.91	< 0.001	0.49	0.70	0.27
	Within	40	0.001					
10°C	Among	18	0.005	3.32	< 0.001	0.44	0.66	0.20
	Within	38	0.002					
15°C	Among	18	0.006	2.93	< 0.01	0.39	0.63	0.16
	Within	38	0.002					
H. belgicae								
FF	Among	17	0.004	2.62	< 0.01	0.35	0.60	0.11
	Within	36	0.002					

Table 1 cont.

	Source of variation	DF	MS	*F* - ratio	*P*	Repeatability (r)	UCL (0.95)	LCL (0.95)
0°C	Among	18	0.005	5.0	< 0.0001	0.57	0.76	0.36
	Within	38	0.001					
5°C	Among	17	0.002	1.70	0.09	0.19	0.46	0.00
	Within	36	0.001					
10°C	Among	19	0.02	13.81	< 0.0001	0.81	0.90	0.68
	Within	40	0.001					
15°C	Among	19	0.01	5.09	< 0.0001	0.58	0.76	0.37
	Within	40	0.002					
H. fulvus								
FF	Among	18	0.002	6.0	< 0.0001	0.62	0.79	0.42
	Within	38	0.0004					
0°C	Among	19	0.007	9.3	< 0.0001	0.73	0.86	0.57
	Within	40	0.001					
5°C	Among	17	0.002	2.0	< 0.05	0.25	0.52	0.01
	Within	36	0.001					
10°C	Among	18	0.007	2.49	< 0.001	0.33	0.58	0.10
	Within	38	0.003					
15°C	Among	19	0.01	3.37	< 0.001	0.44	0.66	0.21
	Within	40	0.003					
P. auberti								
FF	Among	19	0.01	3.63	< 0.001	0.47	0.68	0.24
	Within	40	0.004					

Table 1 cont.

	Source of variation	DF	MS	F - ratio	P	Repeatability (r)	UCL (0.95)	LCL (0.95)
0°C	Among	19	0.01	3.30	< 0.001	0.43	0.65	0.21
	Within	40	0.004					
5°C	Among	19	0.001	0.91	0.57	0.00	0.22	0.00
	Within	40	0.001					
10°C	Among	19	0.01	6.96	< 0.0001	0.67	0.81	0.48
	Within	40	0.001					
15°C	Among	19	0.006	1.89	< 0.05	0.23	0.49	0.01
	Within	40	0.003					
T_{br}								
H. marinus								
FF	Among	19	0.03	2.05	< 0.05	0.26	0.51	0.03
	Within	40	0.01					
0°C	Among	16	0.01	4.56	< 0.0001	0.54	0.75	0.31
	Within	34	0.002					
5°C	Among	17	0.02	5.32	< 0.0001	0.59	0.77	0.37
	Within	36	0.003					
10°C	Among	17	0.01	2.65	< 0.01	0.35	0.61	0.11
	Within	36	0.003					
15°C	Among	18	0.04	6.05	< 0.0001	0.63	0.79	0.42
	Within	38	0.01					

Table 1 cont.

	Source of variation	DF	MS	*F* - ratio	*P*	Repeatability (r)	UCL (0.95)	LCL (0.95)
H. marionensis								
FF	Among	19	19.50	4.41	< 0.0001	0.53	0.73	0.32
	Within	40	4.42					
0°C	Among	19	0.02	4.44	< 0.0001	0.53	0.73	0.32
	Within	40	0.004					
5°C	Among	19	0.01	5.07	< 0.0001	0.58	0.76	0.37
	Within	40	0.003					
10°C	Among	18	8.50	4.11	< 0.001	0.51	0.71	0.28
	Within	38	2.07					
15°C	Among	18	0.01	2.53	< 0.01	0.34	0.59	0.10
	Within	38	0.003					
H. belgicae								
FF	Among	17	0.01	3.74	< 0.001	0.48	0.70	0.24
	Within	36	0.003					
0°C	Among	18	0.01	7.21	< 0.0001	0.67	0.82	0.49
	Within	38	0.002					
5°C	Among	17	0.01	2.53	< 0.01	0.34	0.59	0.10
	Within	36	0.004					
10°C	Among	19	0.05	10.72	< 0.0001	0.76	0.87	0.61
	Within	40	0.004					
15°C	Among	19	0.44	6.17	< 0.0001	0.63	0.79	0.44
	Within	40	0.07					

Table 1 cont.

	Source of variation	DF	MS	*F* - ratio	*P*	Repeatability (r)	UCL (0.95)	LCL (0.95)
H. fulvus								
FF	Among	18	0.24	3.72	< 0.001	0.48	0.69	0.25
	Within	38	0.06					
0°C	Among	19	0.02	6.45	< 0.0001	0.64	0.80	0.45
	Within	40	0.004					
5°C	Among	17	0.02	4.58	< 0.0001	0.54	0.74	0.32
	Within	36	0.004					
10°C	Among	18	0.01	1.82	0.06	0.21	0.48	0.00
	Within	38	0.01					
15°C	Among	19	0.02	4.32	< 0.0001	0.53	0.72	0.31
	Within	40	0.004					
P. auberti								
FF	Among	19	0.47	3.417	< 0.001	0.45	0.66	0.22
	Within	40	0.14					
0°C	Among	19	31.36	4.30	< 0.0001	0.52	0.72	0.31
	Within	40	7.30					
5°C	Among	19	11.79	4.23	< 0.0001	0.52	0.72	0.30
	Within	40	2.79					
10°C	Among	19	0.04	9.11	< 0.0001	0.73	0.85	0.57
	Within	40	0.004					
15°C	Among	19	0.01	1.23	0.28	0.07	0.33	0.00
	Within	40	0.005					

*Values rounded to zero (includes negative values)

Table 2 Mean (±S.E) optimum temperature (T_{opt}), mean (±S.E) performance breadth (T_{br}) and mean optimum speed (u_{max}) for the five species of mites at the different acclimation temperatures. Superscript letters indicate significant differences between acclimation temperatures on 95% confidence intervals following a generalized linear model (for T_{br} and T_{opt} a normal distribution with log link function was selected in the GLZ; for u_{max} a poisson distribution with log link function was selected).

Acclimation (°C)	T_{opt} (°C)^1	Minimum	Maximum	T_{br} (°C)^2	Minimum	Maximum	u_{max} (°C)^3	Minimum	Maximum	N
Halozetes marinus										
FF	25.00 ± 0.51^A	20.00	30.00	16.95 ± 0.54^A	11.30	18.30	14.97 ± 0.54^A	14.66	24.38	20
0	25.00 ± 0.00^A	25.00	25.00	13.64 ± 0.41^B	13.00	23.30	16.63 ± 0.63^A	10.85	16.46	17
5	24.74 ± 0.46^A	20.00	30.00	13.04 ± 0.62^B	6.67	20.00	14.05 ± 0.84^A	6.01	18.58	19
10	$24.7\ 4\pm 0.26^A$	20.00	25.00	12.98 ± 0.33^B	9.00	20.00	16.12 ± 0.70^A	9.75	15.02	19
15	21.05 ± 0.48^B	20.00	25.00	11.22 ± 0.84^B	1.30	12.00	9.26 ± 0.62^B	6.35	17.83	18

1 T_{opt} $\chi^2_{(4)} = 61.63$, $p < 0.0001$

2 T_{br} $\chi^2_{(4)} = 61.16$; $p < 0.0001$

3 u_{max} $\chi^2_{(4)} = 44.62$; $p < 0.0001$

Table 2 cont.

Acclimation (°C)	T_{opt} (°C)[1]	Minimum	Maximum	T_{br} (°C)[2]	Minimum	Maximum	u_{max} (°C)[3]	Minimum	Maximum	N
H. marionensis										
FF	24.00 ± 0.46^{A}	20.00	25.00	14.52 ± 0.54^{A}	10.33	18.67	14.65 ± 0.52^{AB}	8.64	17.95	20
0	21.00 ± 0.46^{B}	20.00	25.00	11.35 ± 0.50^{BC}	9.67	21.33	16.15 ± 0.73^{A}	7.77	16.77	20
5	21.75 ± 0.55^{B}	20.00	25.00	13.55 ± 0.59^{AB}	11.33	9.33	15.55 ± 0.49^{A}	10.59	21.47	20
10	21.84 ± 0.57^{B}	20.00	25.00	9.94 ± 0.38^{C}	8.33	16.33	12.56 ± 0.49^{B}	6.56	13.68	19
15	24.47 ± 0.65^{A}	20.00	30.00	14.98 ± 0.68^{A}	10.00	21.00	16.79 ± 0.61^{A}	11.94	24.47	19

1 T_{opt} $\chi^2_{(4)} = 33.80$, $p < 0.001$

2 T_{br} $\chi^2_{(4)} = 56.71$; $p < 0.0001$

3 u_{max} $\chi^2_{(4)} = 13.58$; $p < 0.01$

Table 2 cont.

Acclimation (°C)	T_{opt} (°C)^{1}	Minimum	Maximum	T_{br} (°C)^{2}	Minimum	Maximum	u_{max} (°C)^{3}	Minimum	Maximum	N
H. belgicae										
FF	23.33 ± 0.57^{AB}	20.00	25.00	13.45 ± 0.47^{A}	19.33	30.00	24.43 ± 0.73^{A}	9.14	15.87	18
0	21.58 ± 0.55^{AC}	20.00	25.00	12.94 ± 0.45^{A}	13.67	31.67	23.67 ± 1.2^{AB}	10.44	18.40	19
5	25.00 ± 0.40^{B}	20.00	30.00	12.19 ± 0.41^{A}	10.33	28.67	21.37 ± 1.3^{ABC}	9.30	16.81	18
10	20.75 ± 0.85^{AC}	15.00	30.00	11.71 ± 0.86^{A}	10.67	28.67	17.38 ± 0.99^{C}	5.78	21.16	20
15	20.75 ± 0.55^{C}	15.00	25.00	13.00 ± 0.48^{A}	6.67	28.33	19.85 ± 1.28^{BC}	8.12	17.36	20

1 $T_{opt}\ \chi^2_{(4)} = 38.54$, $p < 0.0001$

2 $T_{br}\ \chi^2_{(4)} = 6.18$; $p = 0.19$

3 $u_{max}\ \chi^2_{(4)} = 29.28$; $p < 0.0001$

Table 2 cont.

Acclimation (°C)	T_{opt} (°C)[1]	Minimum	Maximum	T_{br} (°C)[2]	Minimum	Maximum	u_{max} (°C)[3]	Minimum	Maximum	N
H. fulvus										
FF	28.95 ± 0.48^{A}	25.00	30.00	17.03 ± 0.49^{A}	13.67	19.67	16.84 ± 0.45^{A}	14.32	22.89	19
0	26.75 ± 0.75^{AB}	25.00	35.00	15.24 ± 0.97^{AB}	13.00	18.67	16.23 ± 0.39^{A}	11.56	29.58	20
5	25.56 ± 0.38^{B}	25.00	30.00	14.93 ± 0.71^{AB}	11.67	17.67	15.00 ± 0.37^{A}	11.04	12.47	18
10	24.47 ± 0.75^{B}	20.00	30.00	14.34 ± 0.77^{B}	12.00	17.33	14.91 ± 0.33^{A}	11.46	26.22	19
15	28.25 ± 0.83^{A}	25.00	35.00	18.56 ± 1.07^{A}	11.33	19.00	15.07 ± 0.45^{A}	12.01	32.26	20

1 T_{opt} $\chi^2_{(4)} = 32.10$, $p < 0.0001$

2 T_{br} $\chi^2_{(4)} = 18.90$; $p < 0.0001$

3 u_{max} $\chi^2_{(4)} = 3.74$; $p = 0.44$

Table 2 cont.

Acclimation (°C)	T_{opt} (°C)[1]	Minimum	Maximum	T_{br} (°C)[2]	Minimum	Maximum	u_{max} (°C)[3]	Minimum	Maximum	N
Podacarus auberti										
FF	24.00 ± 1.06^{AB}	20.00	30.00	15.16 ± 0.77^{AB}	13.33	19.67	15.97 ± 0.38^{A}	9.53	21.66	20
0	26.50 ± 1.09^{AC}	15.00	30.00	18.47 ± 0.88^{C}	10.67	25.00	17.10 ± 0.83^{A}	12.54	24.89	20
5	25.25 ± 0.25^{A}	25.00	30.00	14.52 ± 0.55^{AB}	14.33	24.00	19.10 ± 0.62^{A}	9.70	22.07	20
10	22.89 ± 0.58^{B}	20.00	25.00	12.69 ± 0.63^{B}	13.33	19.33	16.23 ± 0.41^{A}	6.73	16.99	20
15	27.75 ± 0.68^{C}	20.00	30.00	16.46 ± 0.52^{AC}	11.00	20.33	16.23 ± 0.58^{A}	12.70	21.69	20

1 T_{opt} $\chi^2_{(4)} = 23.99$, $p < 0.0001$

2 T_{br} $\chi^2_{(4)} = 42.12$; $p < 0.0001$

3 u_{max} $\chi^2_{(4)} = 7.79$; $p = 0.10$

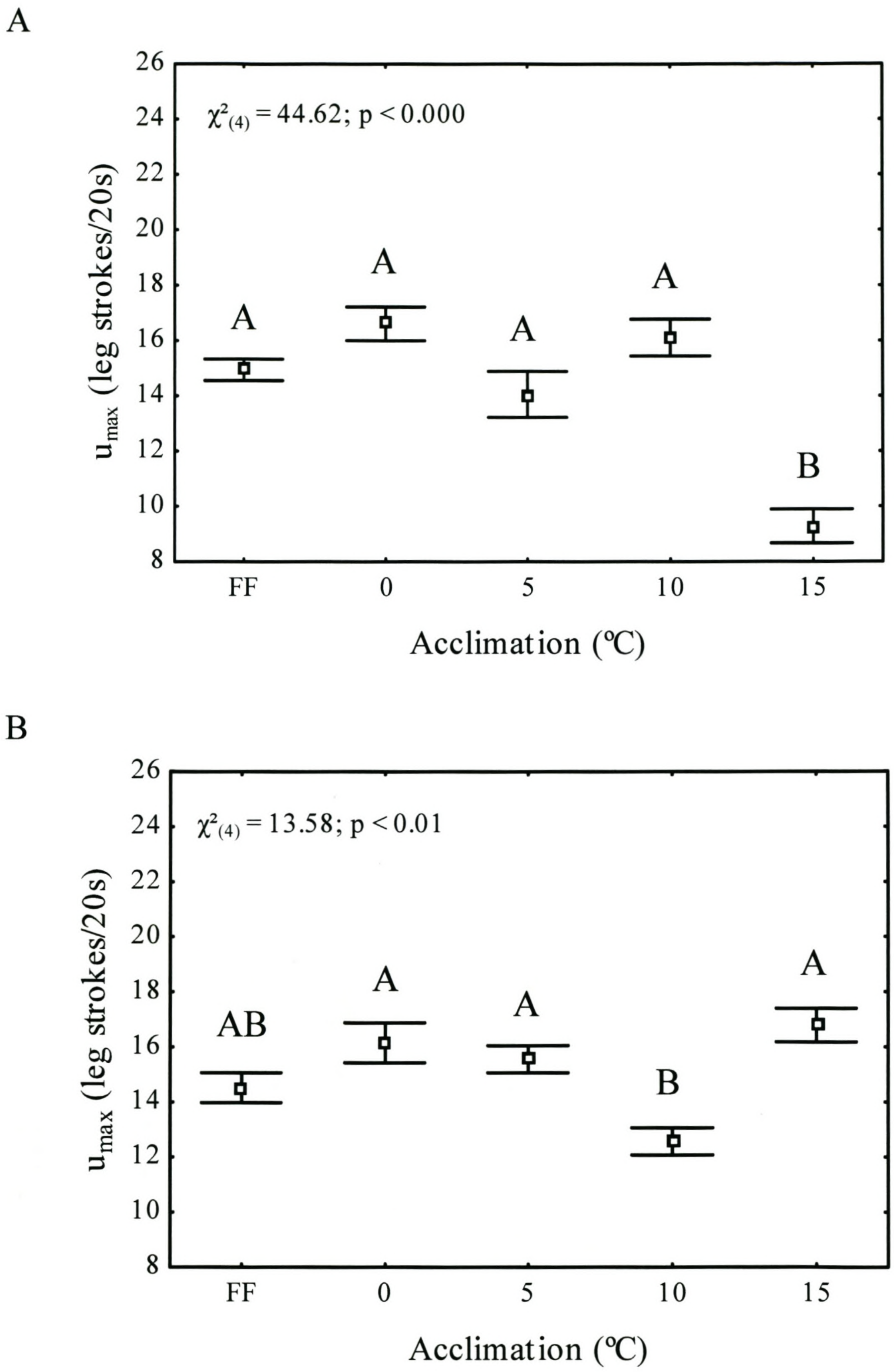
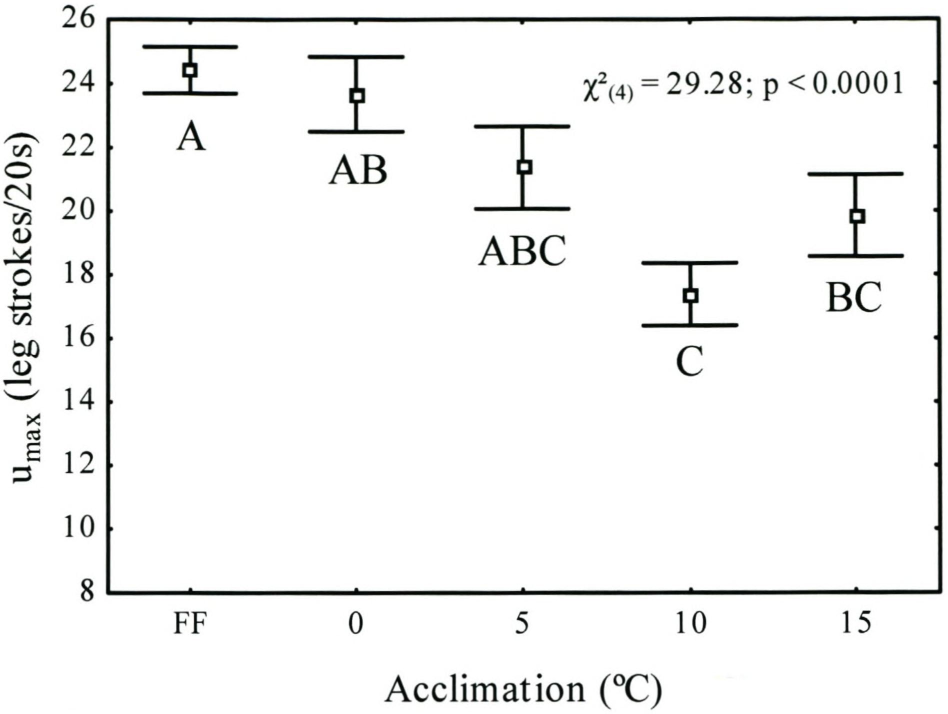

A
26
24
22
20
18
16
14
12
10
8
$\chi^2_{(4)} = 44.62; p < 0.000$
A
A
A
A
B
u_{max} (leg strokes/20s)
FF
0
5
10
15
Acclimation (°C)
B
26
24
22
20
18
16
14
12
10
8
$\chi^2_{(4)} = 13.58; p < 0.01$
AB
A
A
B
A
u_{max} (leg strokes/20s)
FF
0
5
10
15
Acclimation (°C)

Fig. 5 a – b

C


$\chi^2_{(4)} = 29.28; p < 0.0001$
u_max (leg strokes/20s)
26
24
22
20
18
16
14
12
10
8
A
AB
ABC
C
BC
FF
0
5
10
15
Acclimation (ºC)

D

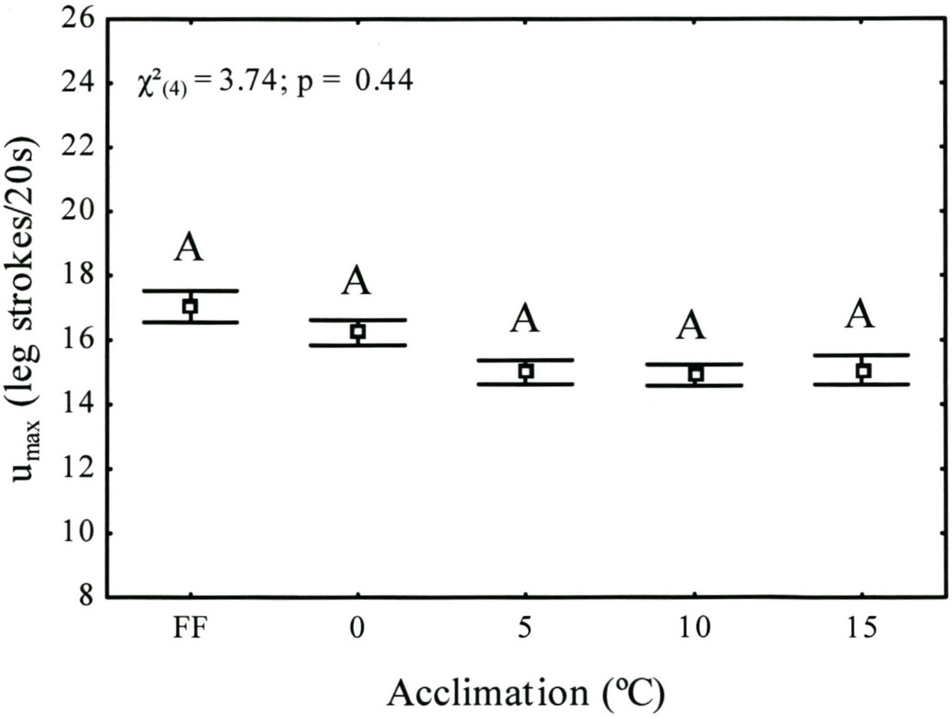

$\chi^2_{(4)} = 3.74; p = 0.44$
u_max (leg strokes/20s)
26
24
22
20
18
16
14
12
10
8
A
A
A
A
A
FF
0
5
10
15
Acclimation (ºC)

Fig. 5 c – e

E

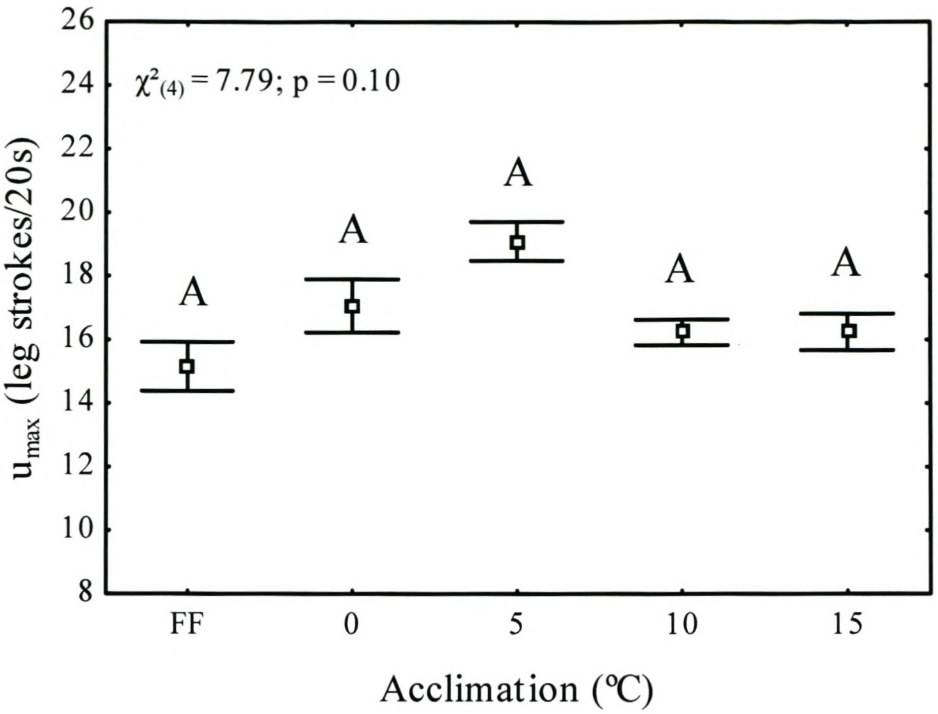


Fig. 5. Mean (± S.E.) optimum speed (u_{max}) for (A) *Halozetes marinus*, (B) *H. marionensis*, (C) *H. belgicae*, (D) *H. fulvus* and (E) *Podacarus auberti* for field fresh (FF) individuals and at four acclimation temperatures. Letters refer to significant differences based on 95% confidence intervals following a generalized linear model assuming a Poisson distribution with a log link function.

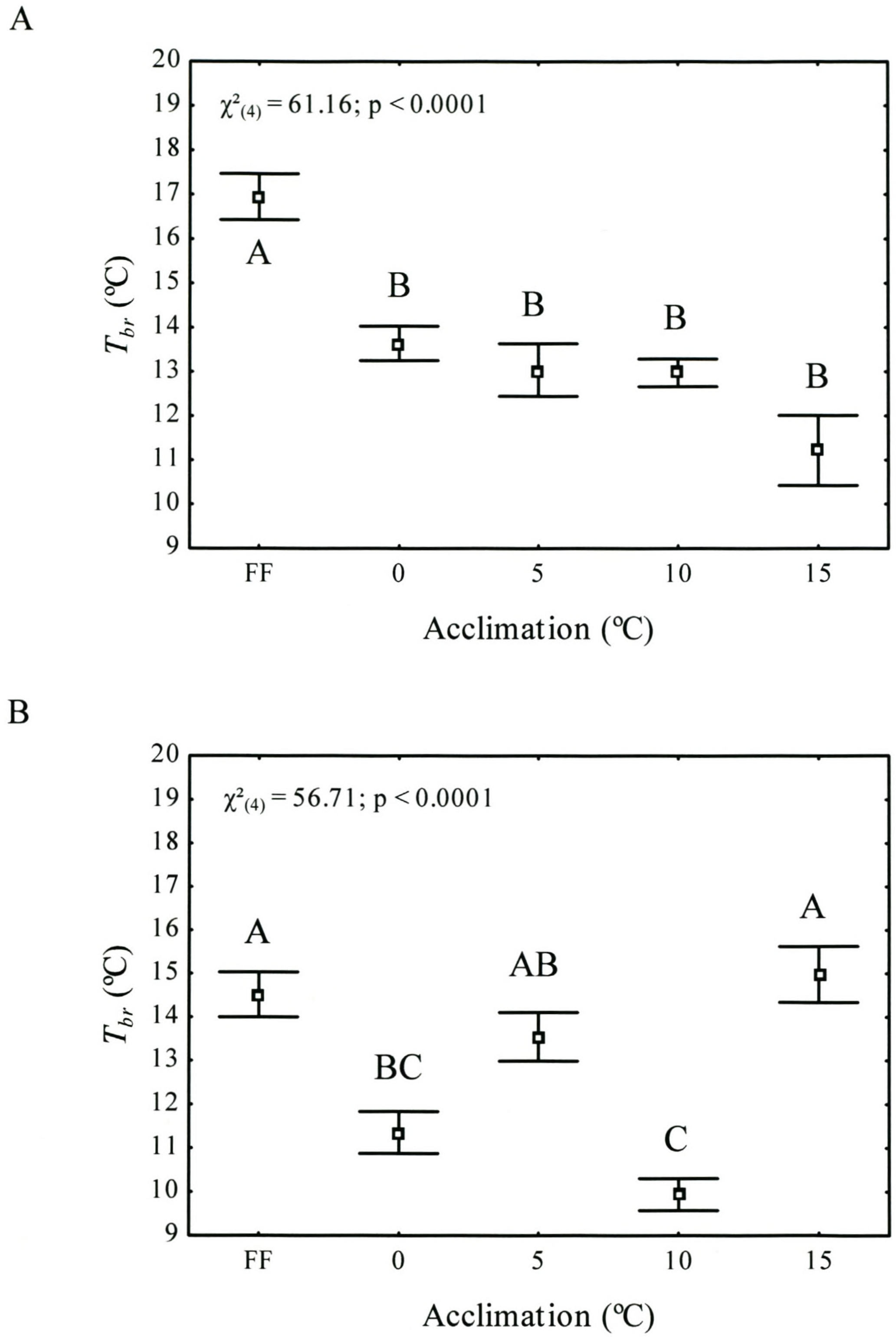
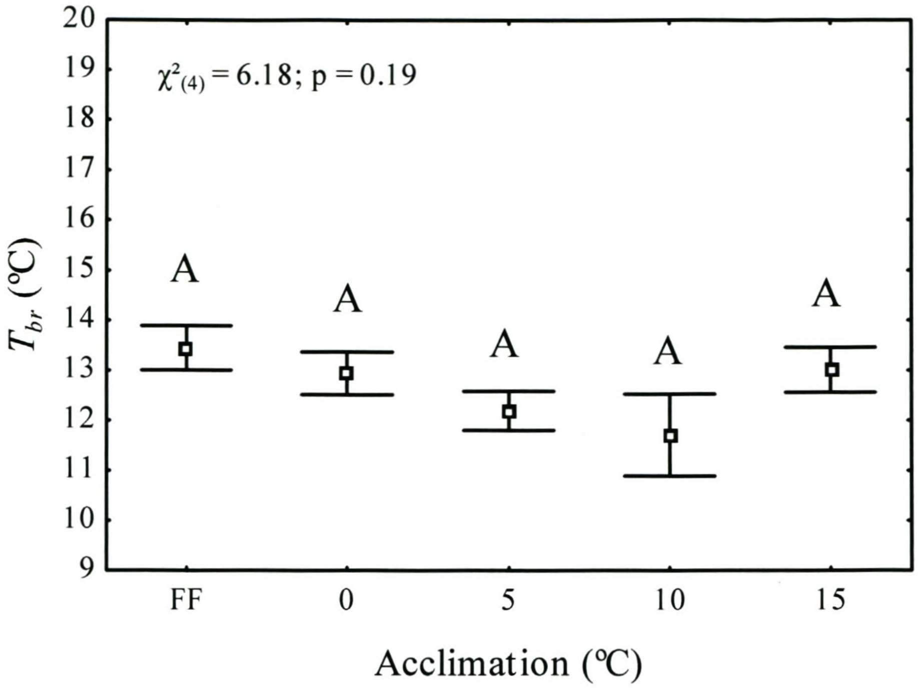

A
$\chi^2_{(4)} = 61.16; p < 0.0001$
20
19
18
17
16
15
14
13
12
11
10
9
T_{br} (ºC)
A
B
B
B
B
FF
0
5
10
15
Acclimation (ºC)
B
$\chi^2_{(4)} = 56.71; p < 0.0001$
20
19
18
17
16
15
14
13
12
11
10
9
T_{br} (ºC)
A
BC
AB
C
A
FF
0
5
10
15
Acclimation (ºC)

Fig. 6 a – b

C


$\chi^2_{(4)} = 6.18$; $p = 0.19$
20
19
18
17
16
15
14
13
12
11
10
9
T_{br} (°C)
A
A
A
A
A
FF
0
5
10
15
Acclimation (°C)

D

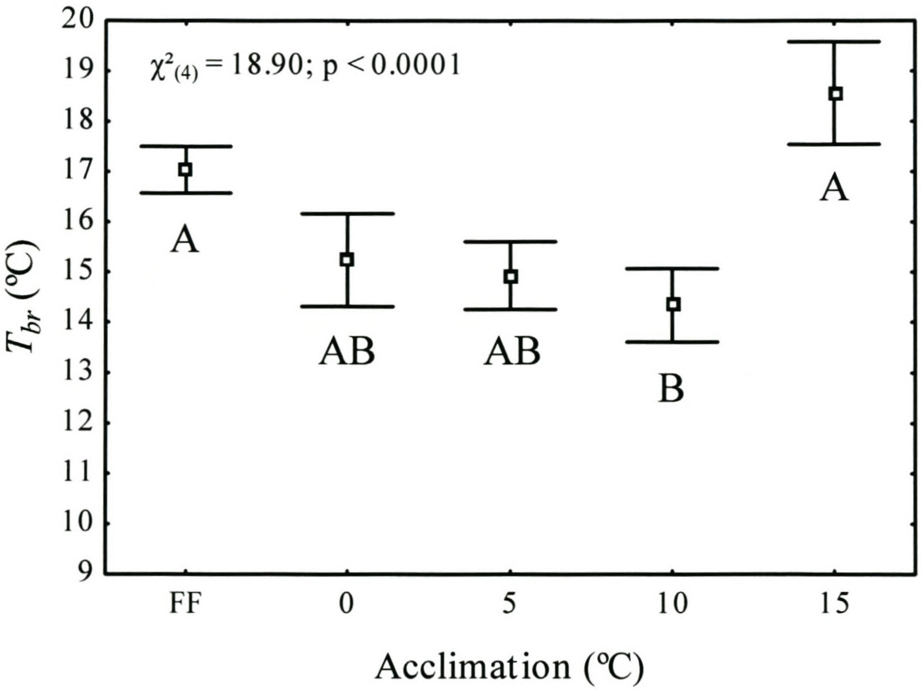

$\chi^2_{(4)} = 18.90$; $p < 0.0001$
20
19
18
17
16
15
14
13
12
11
10
9
T_{br} (°C)
A
AB
AB
B
A
FF
0
5
10
15
Acclimation (°C)

Fig. 6 c – d

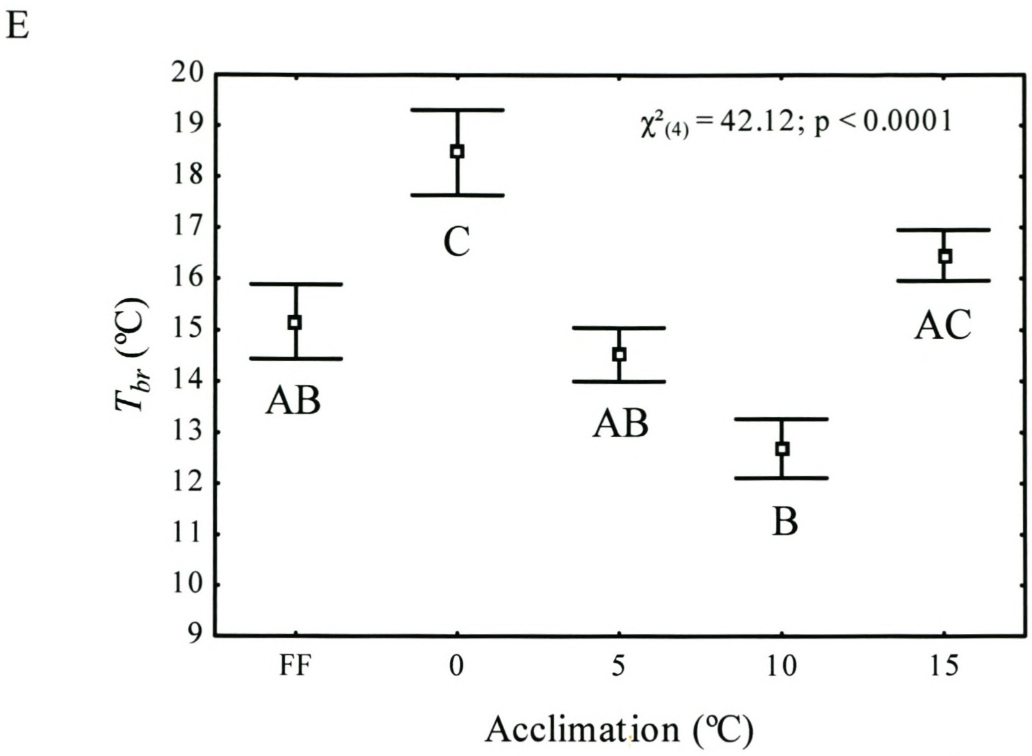


Fig. 6. Mean (± S.E.) performance breadth (T_{br}) (°C) for (A) *Halozetes marinus*, (B) *H. marionensis*, (C) *H. belgicae*, (D) *H. fulvus* and (E) *Podacarus auberti* for field fresh (FF) individuals and at four acclimation temperatures. Letters refer to significant differences based on 95% confidence intervals following a generalized linear model assuming a normal distribution with a log link function.

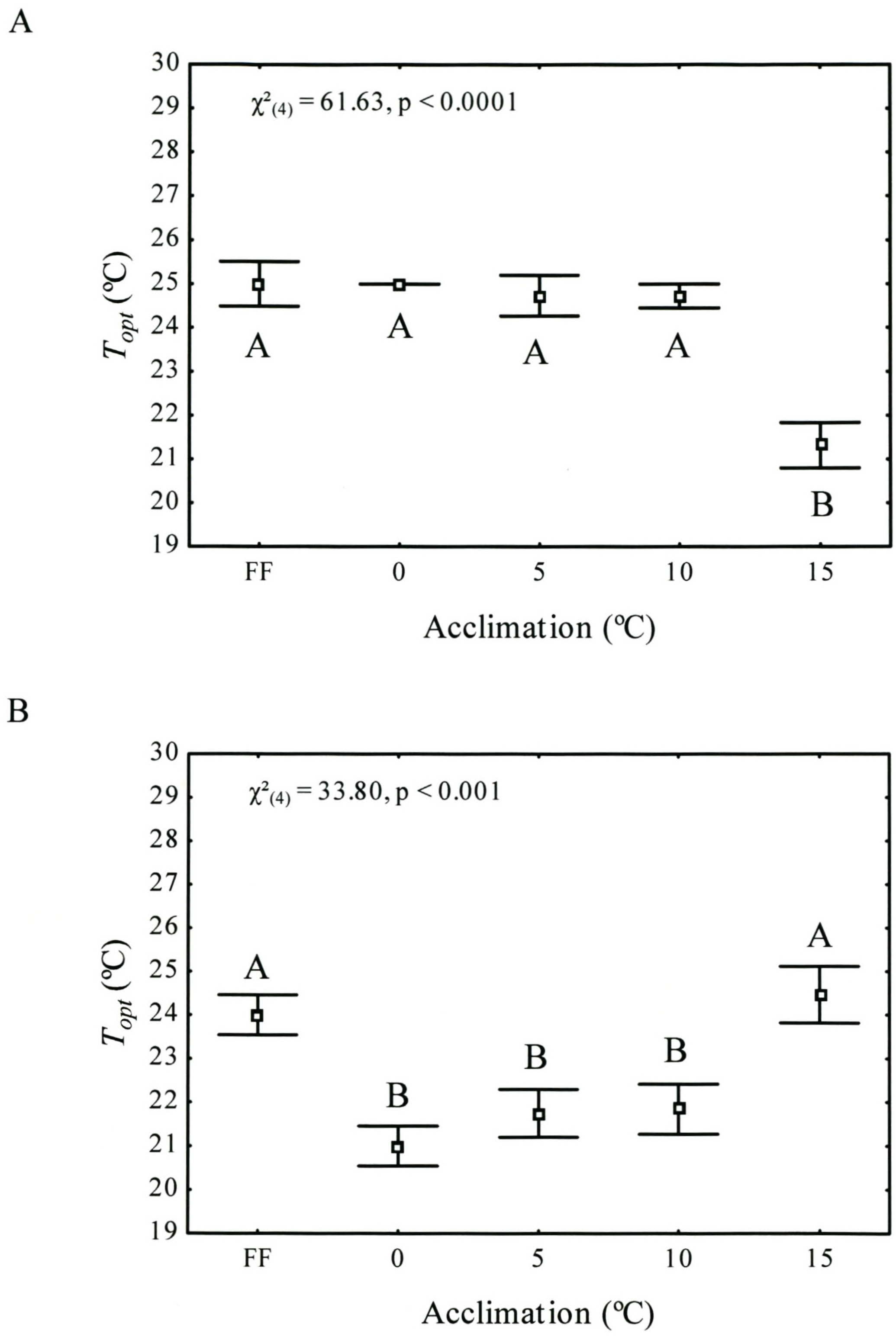
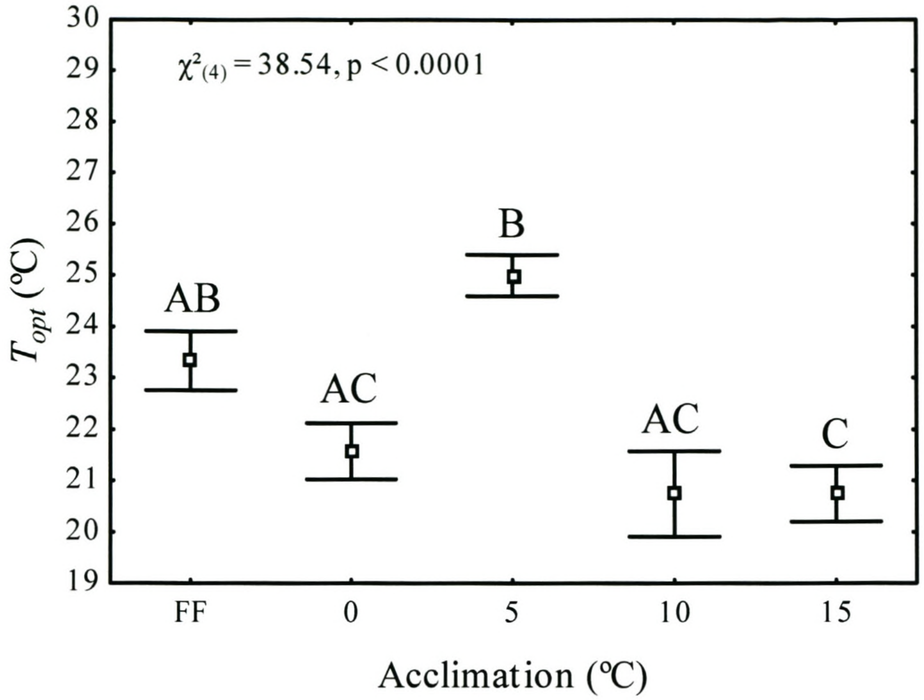

A
$\chi^2_{(4)} = 61.63, p < 0.0001$
T_{opt} (°C)
30
29
28
27
26
25
24
23
22
21
20
19
A
A
A
A
B
FF
0
5
10
15
Acclimation (°C)
B
$\chi^2_{(4)} = 33.80, p < 0.001$
T_{opt} (°C)
30
29
28
27
26
25
24
23
22
21
20
19
A
B
B
B
A
FF
0
5
10
15
Acclimation (°C)

Fig. 7 a – b

C


$\chi^2_{(4)} = 38.54$, p < 0.0001
30
29
28
27
26
25
24
23
22
21
20
19
T_{opt} (°C)
AB
AC
B
AC
C
FF
0
5
10
15
Acclimation (°C)

D

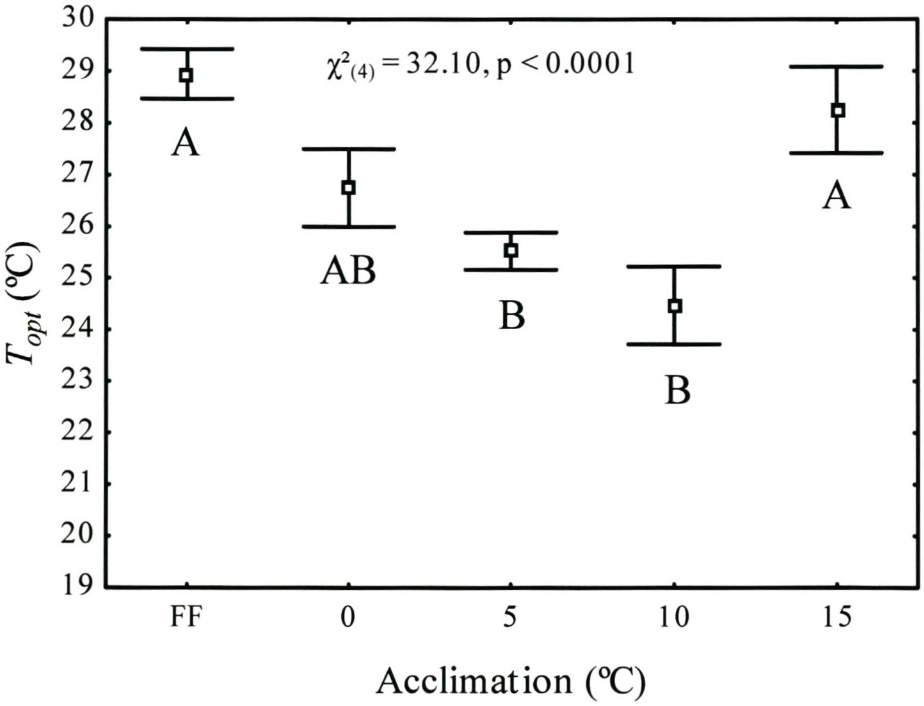

$\chi^2_{(4)} = 32.10$, p < 0.0001
30
29
28
27
26
25
24
23
22
21
20
19
T_{opt} (°C)
A
AB
B
B
A
FF
0
5
10
15
Acclimation (°C)

Fig. 7 c – d

E

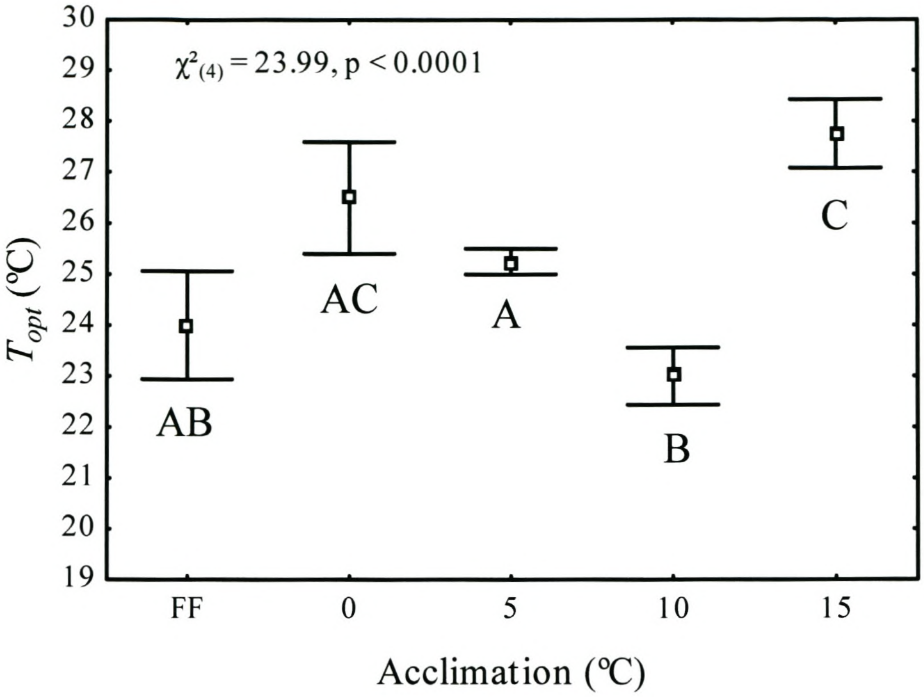


Fig. 7. Mean (± S.E.) optimum temperature (T_{opt}) for (A) *Halozetes marinus*, (B) *H. marionensis*, (C) *H. belgicae*, (D) *H. fulvus* and (E) *Podacarus auberti* for field fresh (FF) individuals and four acclimation temperatures. Letters refer to significant differences based on 95% confidence intervals following a generalized linear model using a normal distribution with a log link function.

Table 3 Results of the covariation of optimum temperature (T_{opt}) and optimum speed (u_{max}); optimum temperature T_{opt} and performance breadth (T_{br}); and performance breadth (T_{br}) and optimum speed (u_{max}) assessed using Pearson's product-moment correlation coefficients for each species.

T_{opt} and u_{max}	N	r	p
Halozetes marinus	4	0.97	< 0.05
H. marionensis	4	0.73	> 0.05
H. belgicae	4	-0.20	> 0.05
H. fulvus	4	0.91	> 0.05
Podacarus auberti	4	0.82	> 0.05
T_{opt} and T_{br}			
H. marinus	4	0.96	< 0.05
H. marionensis	4	0.38	> 0.05
H. belgicae	4	0.14	> 0.05
H. fulvus	4	0.3	> 0.05
P. auberti	4	-0.03	> 0.05
T_{br} and u_{max}			
H. marinus	4	0.96	< 0.05
H. marionensis	4	0.79	> 0.05
H. belgicae	4	0.76	> 0.05
H. fulvus	4	-0.09	> 0.05
P. auberti	4	-0.04	> 0.05

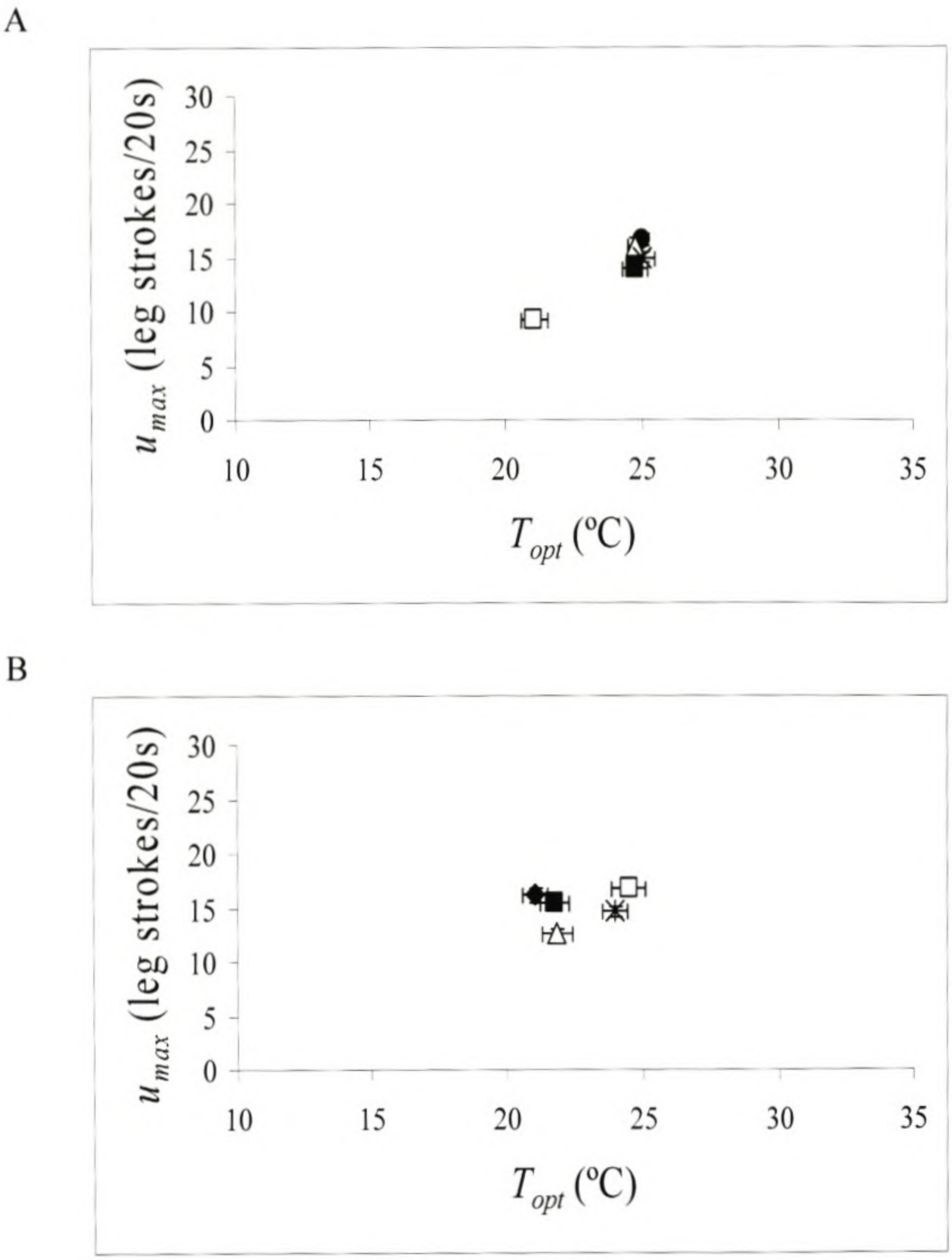
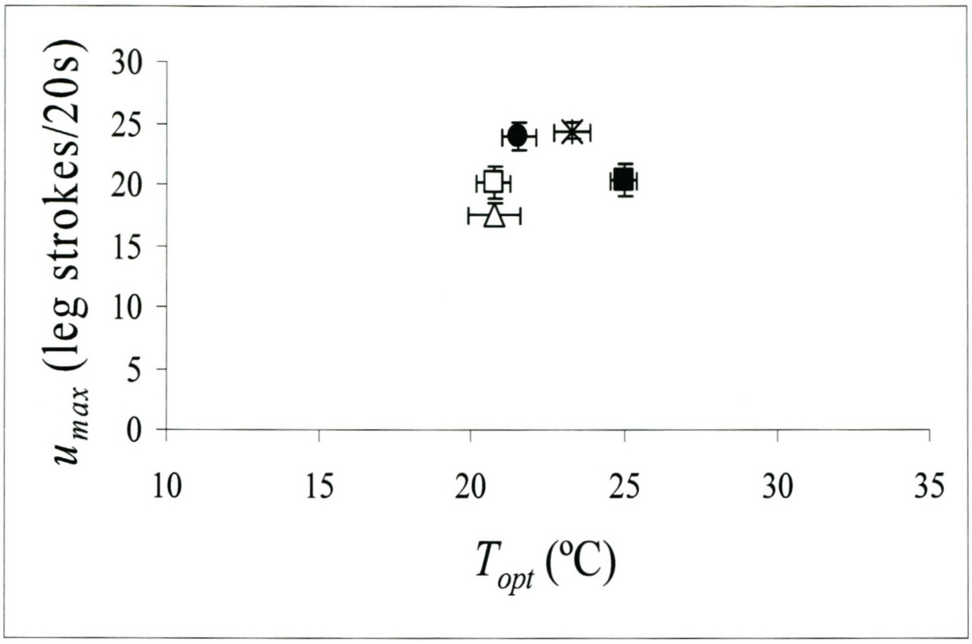
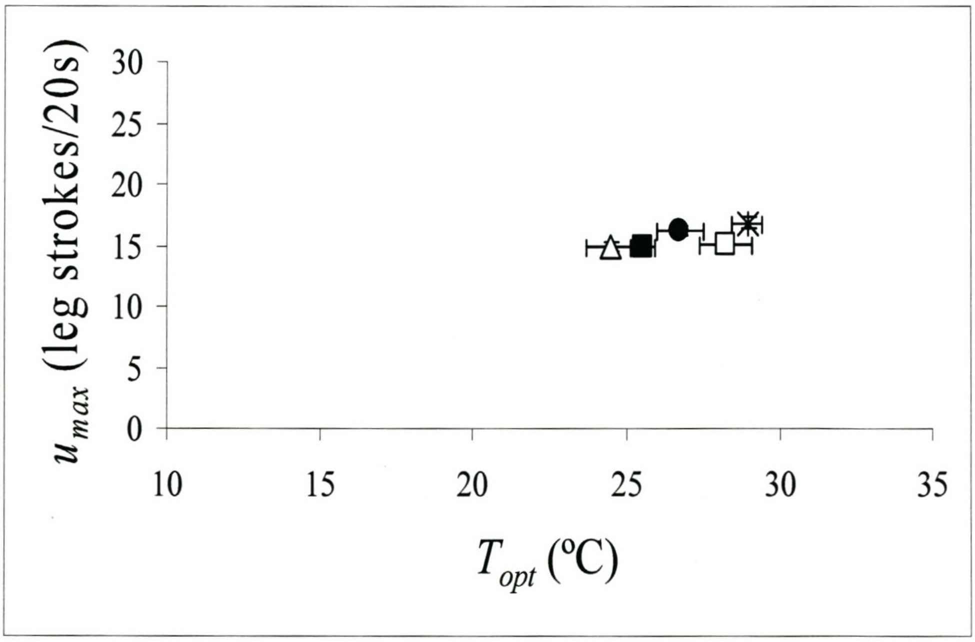

A
u_{max} (leg strokes/20s)
30
25
20
15
10
5
0
10
15
20
25
30
35
T_{opt} (ºC)
B
u_{max} (leg strokes/20s)
30
25
20
15
10
5
0
10
15
20
25
30
35
T_{opt} (ºC)

Fig. 8 a – b

C


u_{max} (leg strokes/20s)
30
25
20
15
10
5
0
10
15
20
25
30
35
T_{opt} (ºC)

D


u_{max} (leg strokes/20s)
30
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35
T_{opt} (ºC)

Fig. 8 c – d

E

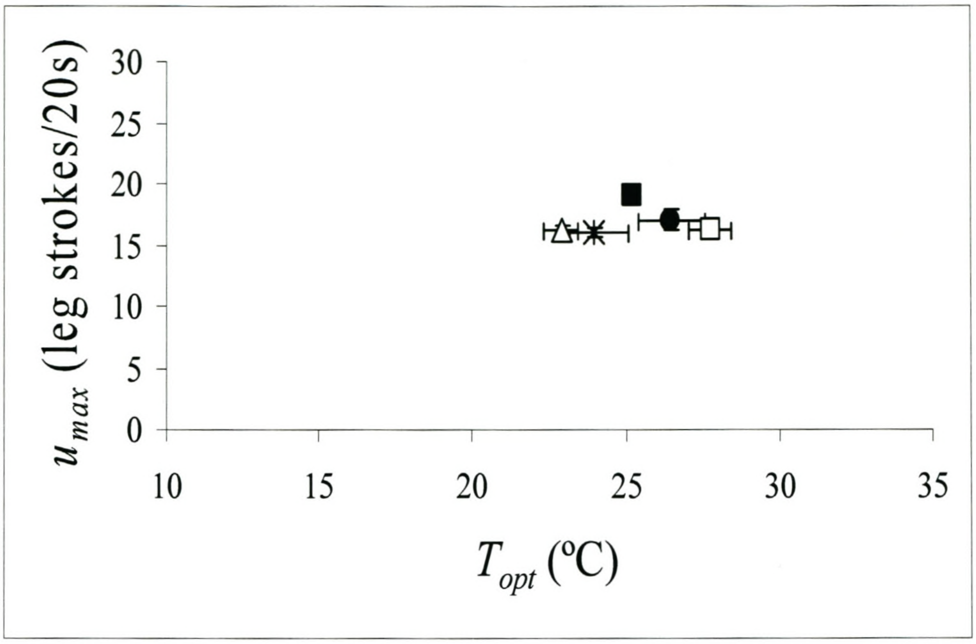
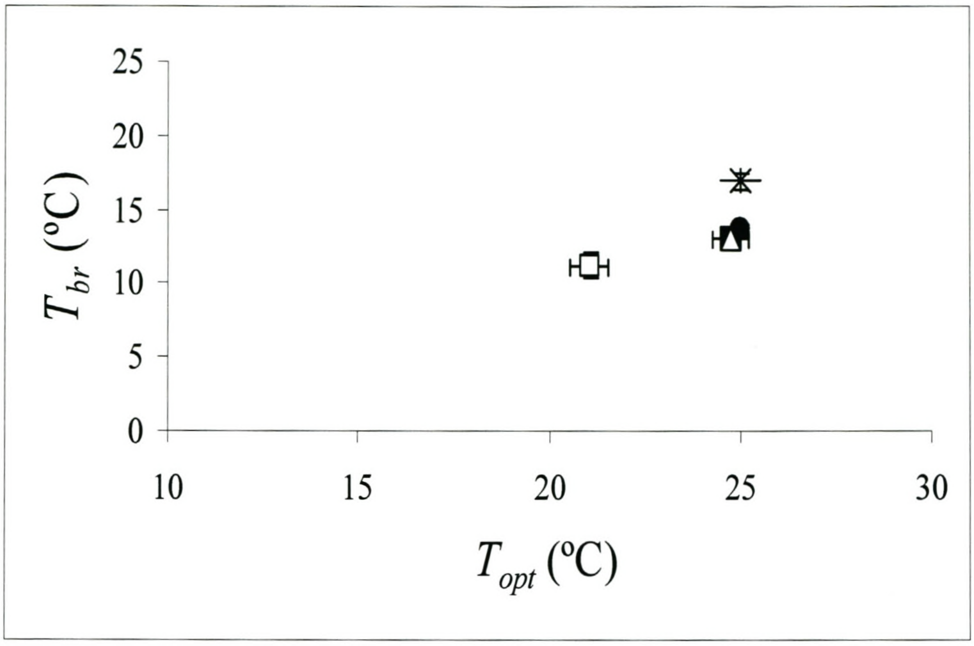


Fig. 8. Bi-plots of optimum temperatures (T_{opt}) and optimum speed (u_{max}) for (A) *Halozetes marinus*, (B) *H. marionensis*, (C) *H. belgicae*, (D) *H. fulvus* and (E) *Podacarus auberti*. Plots include four acclimation temperatures: 0°C (●), 5°C (■), 10°C (Δ), 15°C (□) and field fresh (✱) individuals.

A


25
20
15
10
5
0
T_{br} (ºC)
10
15
20
25
30
T_{opt} (ºC)

B

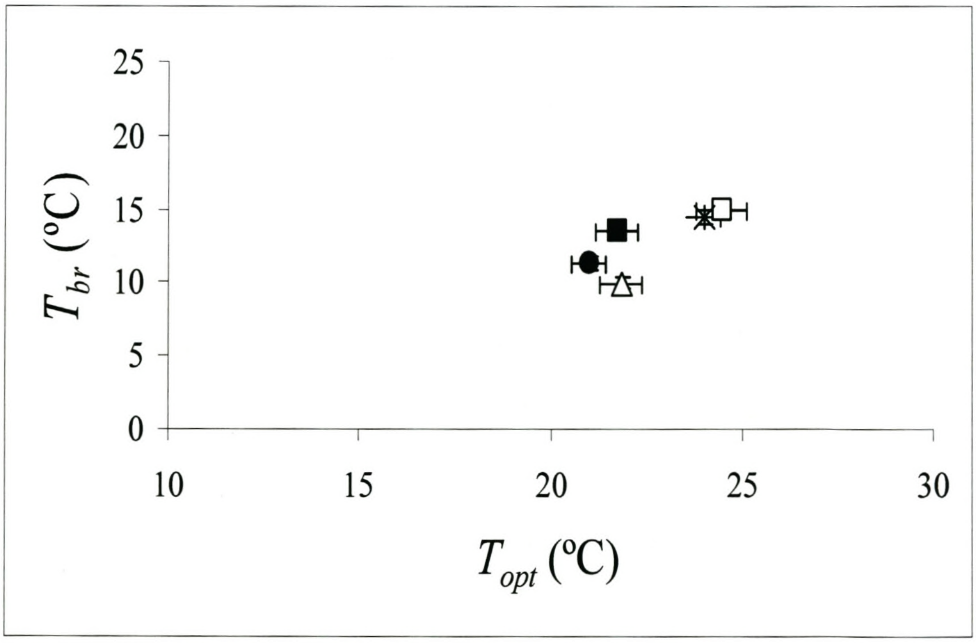
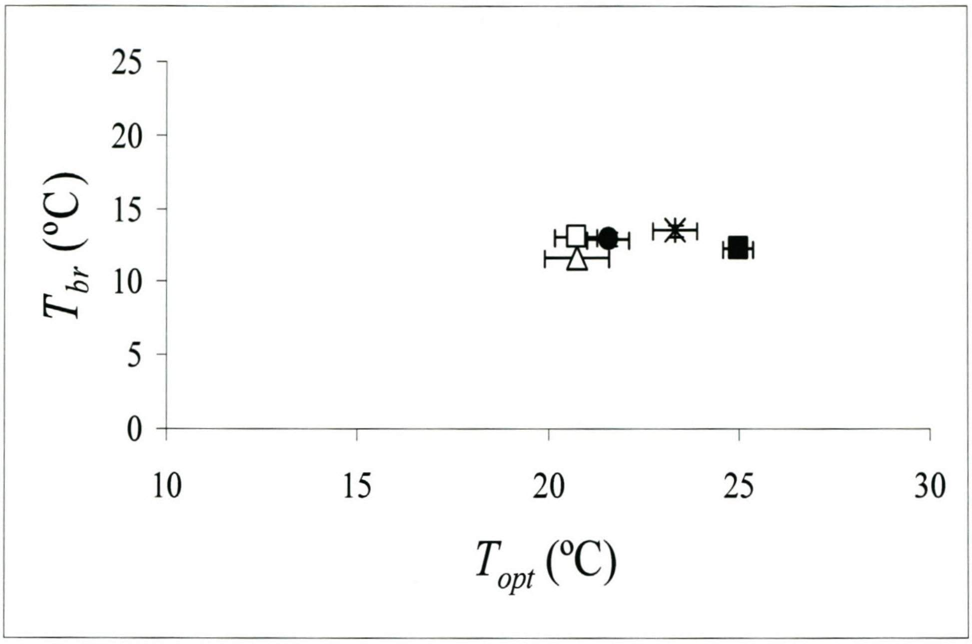
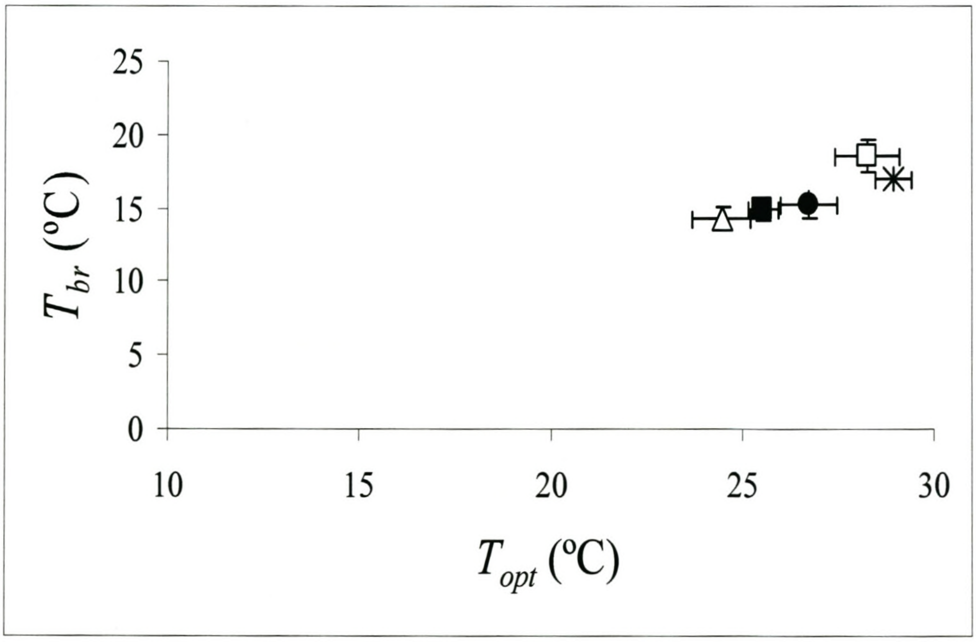

25
20
15
10
5
0
T_{br} (ºC)
10
15
20
25
30
T_{opt} (ºC)

Fig. 9 a – b

C


25
20
15
10
5
0
T_{br} (ºC)
10
15
20
25
30
T_{opt} (ºC)

D


25
20
15
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0
T_{br} (ºC)
10
15
20
25
30
T_{opt} (ºC)

Fig. 9 c – d

E

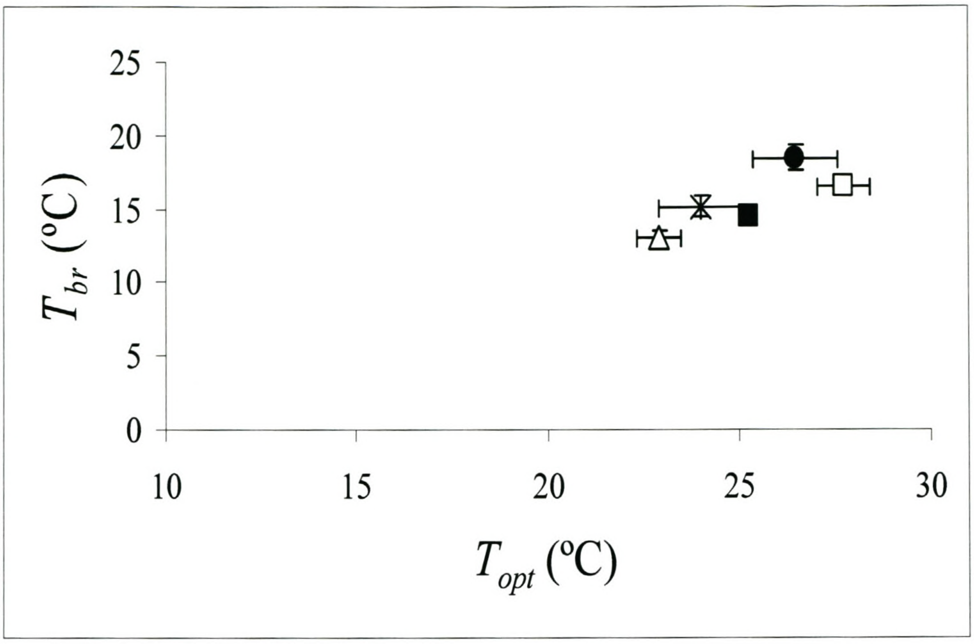
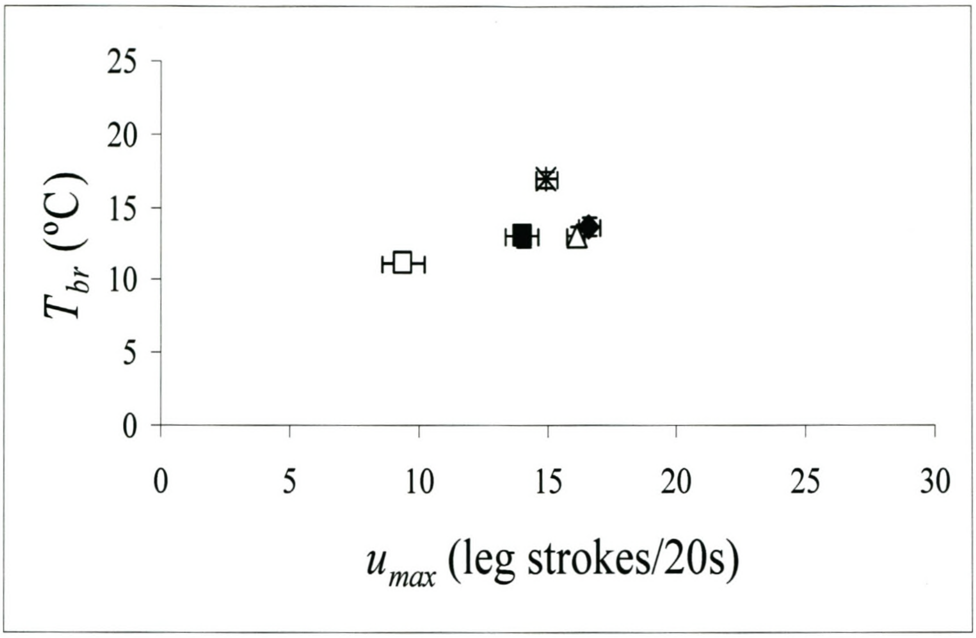


Fig. 9. Bi-plots of optimum temperature (T_{opt}) and performance breadth (T_{br}) for (A) *Halozetes marinus*, (B) *H. marionensis*, (C) *H. belgicae*, (D) *H. fulvus* and (E) *Podacarus auberti*. Plots include four acclimation temperatures: 0°C (●), 5°C (■), 10°C (Δ), 15°C (□) and field fresh (✱) individuals.

A


25
20
15
10
5
0
T_{br} (°C)
0
5
10
15
20
25
30
u_{max} (leg strokes/20s)

B

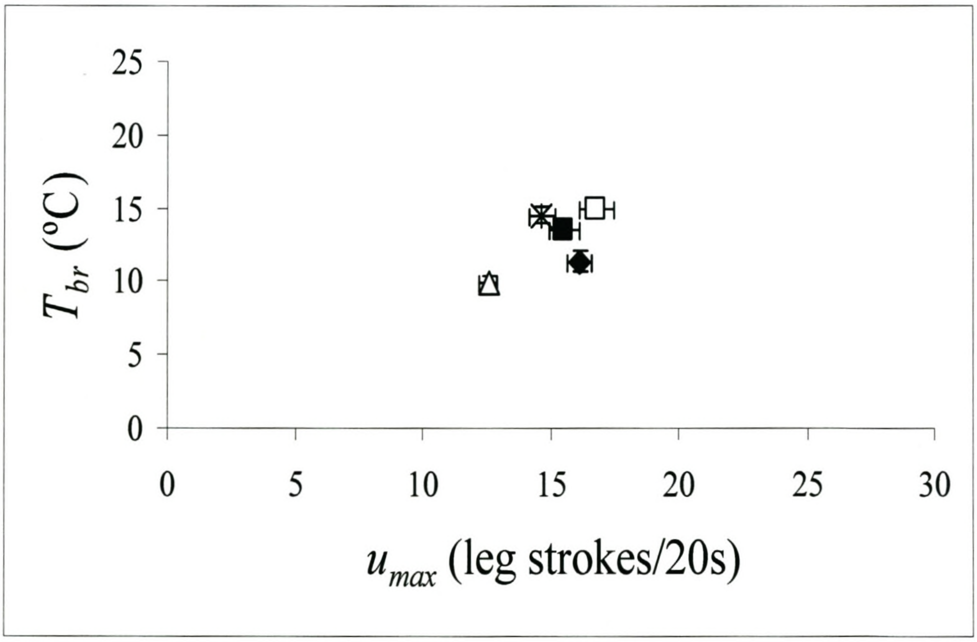
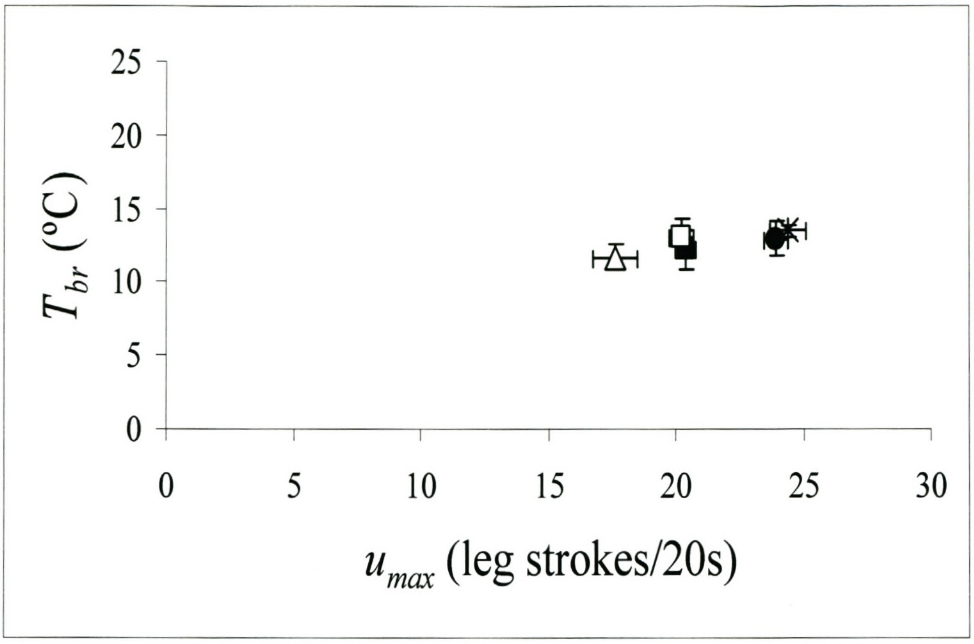

25
20
15
10
5
0
T_{br} (°C)
0
5
10
15
20
25
30
u_{max} (leg strokes/20s)

Fig. 10 a – b

C


T_{br} (°C)
25
20
15
10
5
0
0
5
10
15
20
25
30
u_{max} (leg strokes/20s)

D

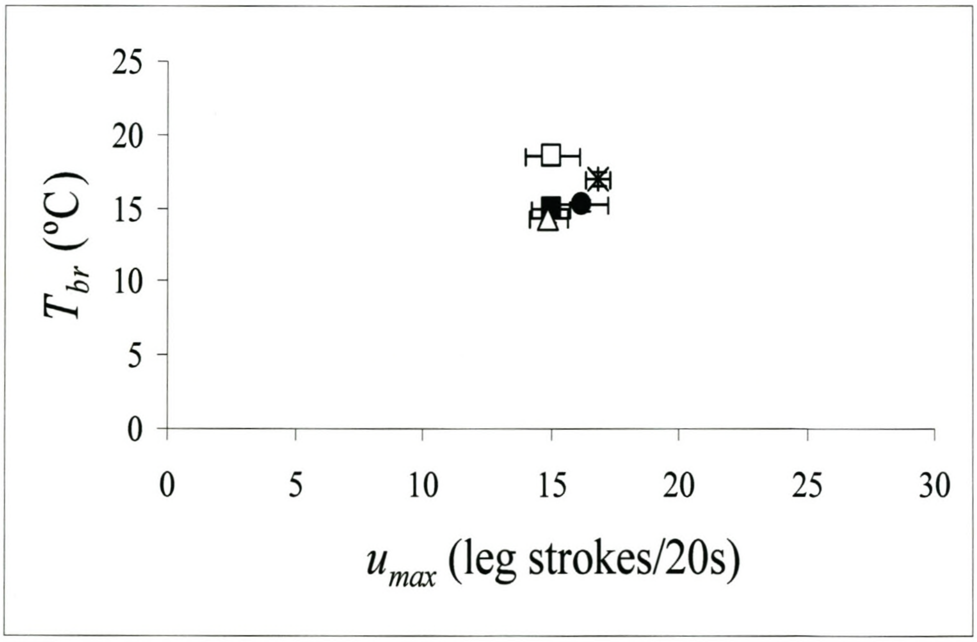

T_{br} (°C)
25
20
15
10
5
0
0
5
10
15
20
25
30
u_{max} (leg strokes/20s)

Fig. 10 c – d

E

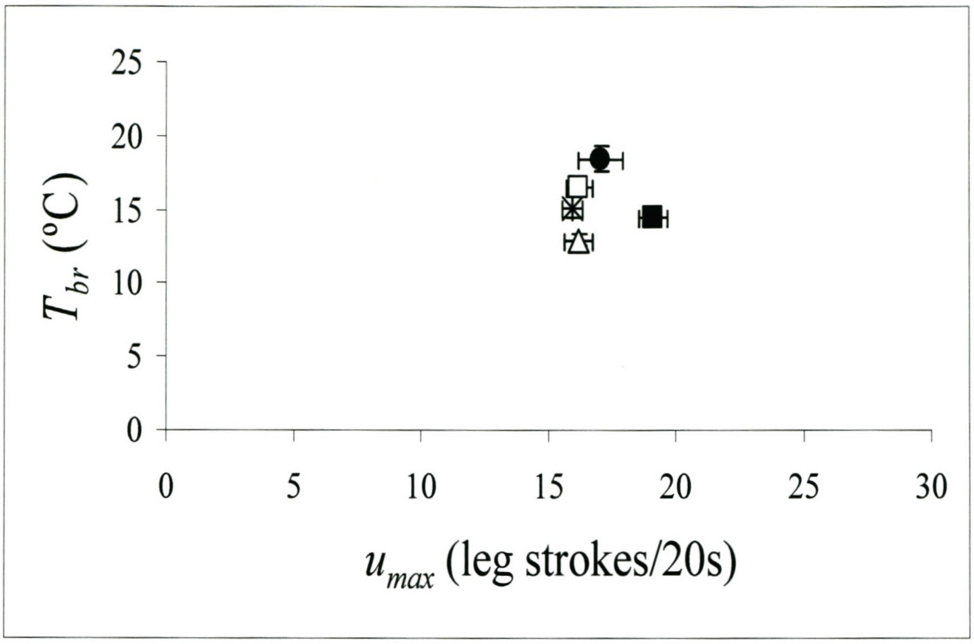


Fig. 10. Bi-plots plots of performance breadth (T_{br}) and optimum speed (u_{max}) for (A) *Halozetes marinus*, (B) *H. marionensis*, (C) *H. belgicae*, (D) *H. fulvus* and (E) *Podacarus auberti*. Plots include four acclimation temperatures: 0°C (●), 5°C (■), 10°C (Δ), 15°C (□) and field fresh (✱) individuals.

Table 4 Results of reduced major axis regression (using 10 000 bootstraps to produce the confidence intervals) of phenotypic performance breadth (T_{br}) optimum speed (u_{max}) for all species at four acclimation temperatures. Significant values, i.e. slopes ≠ 0, indicated in bold.

Species	Acclimation (°C)	R^2	Confidence $\text{Intervals}_{0.95}$
H. marinus	0	0.3600	**-1.7952 < b < -0.6487**
	5	0.0006	-2.678 < b < 2.443
	10	0.0392	-2.624 < b < 3.142
	15	0.0375	-2.663 < b < 1.081
H. marionensis	0	0.0918	-1.748 < b < 1.470
	5	0.0041	-1.263 < b < 1.195
	10	0.0107	-1.459 < b < 1.783
	15	0.2480	**-3.5809 < b < -0.9635**
H. belgicae	0	0.0104	-2.579 < b < 2.828
	5	0.0026	-3.522 < b < 4.029
	10	0.3330	**0.455 < b < 1.072**
	15	0.0955	-2.343 <b < 3.395
H. fulvus	0	0.0019	-1.551 < b < 1.388
	5	0.1610	-0.993 < b < 1.065
	10	0.0162	-1.2204 < b < 0.9913
	15	0.2600	-0.8928 < b < 0.4624
Podacarus	0	0.145	-0.985 < b < 1.392
	5	0.433	**-1.6144 < b < -0.6867**
	10	0.0311	-0.9278 < b < 0.9442
	15	0.145	-1.8330 < b < 0.8962

Table 5 Mean (±SE) performance breadth (T_{br}), optimum speed (u_{max}) and optimum temperature (T_{opt}) for specimens kept at four treatment temperatures (°C) for seven days of each of the five species under investigation. The difference row provides the mean maximum difference between any two acclimation treatments (excluding FF) within species. Superscript letters indicate significant differences between acclimation temperatures based on 95% confidence intervals derived from a generalized linear models (for T_{br} and T_{opt} a normal distribution was assumed and a log link function used; for u_{max} a Poisson distribution was assumed and a log link function was used).

		H. marinus	*H. marionensis*	*H. belgicae*	*H. fulvus*	*P. auberti*
T_{br} (°C)						
	0	13.64±0.41	11.35±0.50	12.94±0.45	15.24±0.97	18.47±0.88
	5	13.04±0.62	13.55±0.59	12.19±0.41	14.93±0.71	14.52±0.55
	10	12.98±0.33	9.94±0.38	11.71±0.88	14.34±0.77	12.97±0.57
	15	11.22±0.84	14.98±0.68	13.00±0.48	18.56±1.07	16.46±0.52
Difference		5.56 ± 0.02^{a}	6.42 ± 0.03^{c}	5.25 ± 0.02^{b}	7.85 ± 0.05^{e}	7.70 ± 0.04^{d}
$\chi^2_{(4)} = 4687.50$, $p < 0.0001$						
u_{max} (°C)						
	0	16.63±0.63	16.15±0.73	23.95±1.14	16.23±0.39	17.07±0.83
	5	14.05±0.84	15.55±0.49	20.39±1.33	15.00±0.37	19.10±0.62
	10	16.12±0.70	12.56±0.49	17.62±0.94	14.91±0.33	16.23±0.41
	15	9.44±0.63	16.79±0.61	20.23±1.28	15.07±0.45	16.23±0.58
Difference		9.22 ± 0.03^{d}	6.57 ± 0.02^{c}	11.82 ± 0.04^{e}	3.70 ± 0.02^{a}	6.27 ± 0.03^{b}
$\chi^2_{(4)} = 48236.00$, $p < 0.0001$						

Table 5 cont.

T_{opt} (°C)		*H. marinus*	*H. marionensis*	*H. belgicae*	*H. fulvus*	*P. auberti*
	0	25.00±0.0	21.00±0.46	21.58±0.55	26.75±0.75	26.50±1.09
	5	24.74±0.46	21.75±0.55	25.00±0.40	25.56±0.38	25.25±0.25
	10	24.74±0.26	21.84±0.57	20.75±0.85	24.47±0.75	22.89±0.58
	15	21.05±0.48	24.47±0.65	20.75±0.55	28.25±0.83	27.75±0.68
Difference		4.28±0.02^{a}	5.05±0.02^{b}	6.31±0.03^{d}	6.13±0.04^{c}	7.34±0.03^{e}

$\chi^2_{(4)} = 7427.70$, $p < 0.0001$

Discussion

In the case of performance breadth and optimum speed, there was no relationship between acclimation temperature and these traits for all of the species with the exception of *H. marinus*, where low temperatures led to the highest speed and widest performance breadth. Thus, the beneficial acclimation hypothesis could not be rejected (compare Fig. 2 with Figs 5 and 6). Support for the BAH is in keeping with the expectation from the majority of the literature on acclimation in labile traits (for discussion see Wilson and Franklin (2002a) and Woods and Harrison (2002)), and with theoretical predictions regarding adaptive phenotypic plasticity (Van Tienderen, 1997; Doughty and Reznick, 2004). Why *H. marinus* should differ so substantially from the species to which it is closely related, given that the values of their trait means are so similar (Figs 5-7), is not clear. What seems most likely is that the higher acclimation temperatures, especially 15°C, are deleterious and affect performance overall, as has been implied for long-term acclimation treatments of other species (see Woods and Harrison (2002) for discussion). This conclusion is partly supported by the substantial decline in all three traits at 15°C relative to the values of these traits at the other acclimation temperatures including the field-fresh treatment. It also makes intuitive sense given the very narrow range of temperatures (2-7°C, see Mélice et al., 2003) that this, the most marine of the species examined, is likely to encounter. Unfortunately, independent assessments of stress for each treatment, such as the extent of upregulation of stress proteins (Feder and Hofmann, 1999) or other thermal protectants (e.g. sorbitol Salvucci, 2000), were not undertaken, and therefore it could not be determined with certainty that the higher temperatures really were detrimental. Nonetheless, it is clear that if a deleterious acclimation hypothesis is to be tested, then independent evidence of detrimental effects will be required in addition to data on the traits of interest.

Although the predictions made by the BAH for T_{br} and for u_{max} were supported in most of the species, these predictions are no different from those of the hypothesis of compensation, or conservation of rate in the face of a temperature change (Hazel and Prosser, 1974; Huey and Berrigan, 1996; Chown and Gaston, 1999; Clarke, 2003). Moreover, in the case of T_{opt}, the prediction made by the compensation hypothesis was supported and that of the BAH rejected (again with the exception of the marine *H. marinus* where high temperature acclimation was detrimental). These findings suggest that the overall response of the more terrestrial mite species is one of compensation. Compensation, or metabolic cold "adaptation" as it is sometimes known in the context of whole-organism metabolic rates (Chown and Gaston, 1999), is well known and incontrovertible at enzyme and tissue levels in many species (reviews in Hochachka and Somero, 2002; Guderley, 2004). However, it is controversial at the whole-organism level for both theoretical and empirical reasons (Clarke, 1993; 2003). The same is true for the arthropods, where theoretical debates regarding costs and benefits mirror, and indeed contribute to those in the broader literature (see discussion in Chown and Gaston, 1999), empirical studies both support and reject the idea (reviewed in Sømme and Block, 1991; Chown and Gaston, 1999; Addo-Bediako et al., 2002), the level at which investigations should be undertaken remains contentious (Chown et al., 2003; Hodkinson, 2003), and where there has been vigorous criticism of the generally *a posteriori*, blanket adaptationist approach that has characterized many previous investigations (Huey and Berrigan, 1996).

Here, an explicitly *a priori*, strong inference approach was adopted, and the data are unequivocal regarding the presence of compensation at the intra-specific level, the least contentious of the levels at which whole-organism compensation can be investigated (Hodkinson, 2003). Thus, there is strong support for compensation, but whether it is beneficial is much more difficult to ascertain. The predictions of the BAH suggest that this might be the case for T_{br} and u_{max}, but perhaps not so for T_{opt}. Clearly, there are several beneficial scenarios that could be outlined on the basis of the generally low (by global standards) temperature variation of the habitats occupied by these mites (see Table 6, Appendix 2), and the absence of opportunities to make use of frequent periods of high temperature when activity can be maximized for mate-finding, feeding, reproduction and growth (see Gotthard and Nylin, 1995; Chown, 1997; Gotthard et al., 2000; Addo-Bediako et al., 2002). In other words, it might be argued that the generally low and constant temperatures across all seasons in the habitats occupied by these species promote the conservation of rate in the face of temperature declines. Indeed this would also seem plausible in the context of the typically "slow" strategies of Antarctic insects in general (Crafford et al., 1986; Convey, 1996) and Antarctic ameronothrid mites in particular (Convey, 1994). However, there are reasons why these arguments might not apply. First, T_{opt} for all of the species is considerably higher than the temperatures routinely encountered in the habitats they occupy (Table 6). Moreover, when temperatures are occasionally this high, it is on dry, sunny days when the mites tend not to be active. Such a mismatch between optimum temperatures and likely habitat temperatures has been noted for other species (Hertz et al., 1983; Bennett, 2004; for arthropods see Abushama and Al-salameen, 1989; Ward and Seely, 1996), and it would have to be more carefully explored in these mites too. Second, even if the previous discrepancy could be explained, it would have to be demonstrated that beneficial acclimation or compensation is an instance of adaptive phenotypic plasticity. Several conditions need to be satisfied to do so.

First, the response would have to be shown to be repeatable, or that there is consistent variation among individuals. The repeatability analysis undertaken here cannot be used to assess this condition because it was used as much to investigate measurement reliability as variation among individuals (Lessells and Boag, 1987; Falconer and Mackay, 1996).

Nonetheless, the generally high values for the intraclass correlation coefficient for u_{max} suggest that the condition, for this trait at least, is satisfied. Second, a genetic basis for the plasticity would have to be demonstrated. This is a controversial topic, but one in which there is some convergence on a consensus view (see Via et al., 1995; Sarkar, 2004). However, for the traits and species examined here such information is not available, although aspects of locomotor performance are known to be heritable in other arthropods (e.g. Gilchrist, 1996). Finally, a relationship between variation in the response and fitness would have to be demonstrated. The question of the fitness consequences of locomotor performance is also controversial, with studies both supporting and rejecting the idea (Heinrich and Bartholomew, 1979; Scott et al., 1997; Hoffmann and Hewa-Kapuge, 2000; Gibert et al., 2001; Angilletta et al., 2002). In mites, the same benefits of improved locomotion speed for oviposition, mate finding and retention, and predator avoidance could be envisaged. However, whether this is the case for the generally slow oribatids (9-24 legstrokes/20s is not rapid movement by any standards) remains to be fully investigated.

In the above context, it is nonetheless worth noting that in several species, locomotor performance has an effect on a variety of other fitness-related traits, such as mate-finding, fecundity, and predator avoidance (Huey and Dunham, 1987; Semlitsch et al., 1999; Van Damme and Vanhooydonck, 2001). Thus, it can be considered a single trait with a wide range of effects on other traits. The same is true of traits such as stress protein expression (see review in Feder and Hofmann, 1999 and data in Krebs and Feder, 1998a, 1998b). Indeed, it is widely appreciated that traits can be linked both at the genetic and performance levels in a wide variety of ways (e.g. Falconer and MacKay, 1996). It thus seems something of an unrealistic goal to suggest that acclimation studies should focus on the fitness effects on single traits (Woods and Harrison, 2002).

The expectation that the marine species should show much less flexibility than their vegetation-bound counterparts was only supported in the case of T_{br} and T_{opt}. *H. marinus* is exposed to a fairly constant thermal environment where the range of temperatures is small (Table 6), the lack of flexibility in T_{br} and T_{opt} can therefore be expected as a constant thermal environment should not induce much change in these traits (Spicer and Gaston, 1999). A possible reason for the greater flexibility in T_{br} and T_{opt} in the terrestrial species may be due to the occasional exposure to high temperatures (*ca.* 33 °C) that may be experienced in *Poa cookii* tillers (Chown and Crafford, 1992). However, the epilithic species may also experience extended periods where they are not exposed to wave action, as such they may experience warmer microclimate conditions as the black rock substrate on which they occur warms considerably. Admittedly if the time of day where the most extreme conditions occur coincide with high tide these species will be submerged and will then not be exposed to these conditions (Helmuth et al., 2002). Nonetheless, as tides rise and fall the change from marine to terrestrial conditions, and vice versa, can place extreme demands on the physiology of intertidal organisms (Denny and Paine, 1998). This demand on physiology may lead to a more stable T_{br} and T_{opt} as the changes in the thermal conditions experienced by the epilithic species are frequent and intense. These species require both the T_{br} and T_{opt} traits to encompass the variable thermal conditions, and rapid adjustment of these traits to the changing conditions may not be possible. Why *H. marinus* shows large flexibility in u_{max} relative to the terrestrial species may stem from the effects of high temperatures. The low locomotor performance of *H. marinus* at high acclimation temperatures may have induced the large level of flexibility seen in this trait, which suggests that the flexibility in this trait is not beneficial.

In conclusion, this study could not reject the beneficial acclimation hypothesis for two of three labile traits in four of five species of oribatid mites. However, here, beneficial acclimation is equivalent to compensation, which also characterized the response of the third trait in the four species. Such compensation can be considered an absence of phenotypic plasticity, which is nonetheless adaptive. Such an outcome has been predicted in models of the response of traits to stable environments under certain conditions (Van Tienderen, 1997; Berrigan and Scheiner, 2004). In addition the flexibility in the three traits measured varied between species from the different habitats but not always in the manner that was expected, suggesting that flexibility in these traits are not always influenced by short term thermal variation experienced in certain microhabitats.

Table 6 Sea surface (SS) temperature (°C) and microhabitat temperature (°C) of two terrestrial sites (0m and 200m a.s.l.) for two years. Mean minimum (Mean min) and maximum (Mean max) temperatures, absolute minimum (Abs min) and maximum (Abs max) temperatures, and temperature range are given.

	Year	Mean (°C)	Abs. Min	Abs. Max	Mean Min	Mean Max	Range
SS							
	2002	5.4	3.7	8.5	4.9	6.2	4.8
	2003	5.4	3.7	8	4.7	6.0	4.3
0m							
	2002	5.3	0.5	22.5	3.8	7.1	22
	2003	7.0	1	22	5.1	8.6	21
200m							
	2002	4.4	-5	18.5.	2.2	7.0	23.5
	2003	5.2	-6	19	3.5	6.6	25

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Chapter 3

Thermal limits of five oribatid mite species: testing the beneficial acclimation hypothesis and its alternatives

Introduction

The way in which organisms interact with their environment is a function of, and also determines, life history, which in turn influences abundance and distribution (Holt et al., 2002). In consequence understanding the interactions between the environment and the response of organisms has been, and continues to be, a major theme in ecology and evolutionary physiology (Bartholomew, 1987; Feder et al., 2000; Chown and Nicolson, 2004). This interest has increased especially because of rapidly changing modern climates (IPCC, 2001) and the demonstration (Parmesan et al., 1999, Walther et al., 2002; Parmesan and Yohe, 2003) and expectation (Thomas et al., 2004) that these changes will have a fundamental influence on species and on human attempts to conserve them. One of the most significant questions, from a physiological perspective, is the extent and nature of responses by species to the changing thermal environment. Are species likely to adapt, will they simply move and what is the role of phenotypic flexibility, if any, likely to be (Davis and Shaw, 2001; Bale et al., 2002; Chown and Nicolson, 2004)? Responses to the thermal environment could occur via adaptation of the basal response, via phenotypic flexibility, or via an adaptive change in the latter with the extent of the response being determined by the interaction of these. From a physiological perspective, phenotypic flexibility is of considerable interest, because it has been commonly documented yet the thermal benefits thereof remain controversial (Woods and Harrison, 2002; Wilson and Franklin, 2002)

Phenotypic flexibility (or plasticity) can be defined as the expression of different phenotypes by an organism depending on the biotic or abiotic environment, in a way that may alter that organism's fitness (Kingsolver and Huey, 1998; Agrawal, 2001). Phenotypic plasticity in the form of acclimation has long been considered beneficial to organisms (see Prosser, 1986; Spicer and Gaston, 1999; Chown and Nicolson, 2004), the reasoning being that an animal exposed to a given temperature is thought to perform better subsequently at that temperature than others. This improved performance after pre-exposure was termed the Beneficial Acclimation Hypothesis (BAH) and defined by Leroi et al. (1994) as "…acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment".

Despite its intuitive appeal, the BAH has been rejected by several studies (Leroi et al., 1994; Bennett and Lenski, 1997; Gibbs et al., 1998; Woods, 1999; Woods and Harrison, 2001). Leroi et al. (1994) observed in *Escherichia coli* that acclimation to 32°C relative to 41.5°C enhanced competitive fitness at 32°C, but prior acclimation to 41.5°C relative to 32°C reduced competitive fitness at 41.5°C, thus rejecting the BAH. Gibbs et al. (1998) found no benefits of thermal acclimation of cuticular lipid composition and water loss rates among adult *Drosophila mojavensis*: mean hydrocarbon chain length increased after acclimation but water loss rates were unaffected, suggesting that apparent adaptive changes in cuticular lipids do not result in reduced rates of water loss, as had been previously speculated. Woods and Harrison (2001) found that *Manduca sexta* larvae reared from the first instar on low-water (69%) or high-water (80%) artificial diets, then transferred in the early fifth instar to either the same or opposite diets, responded to short-term hydric stress by minimizing water excretion rates through increased rectal water re-absorption, and long-term hydric stress by significant reduction of evaporative water loss (Woods and Harrison, 2001). Therefore, short-term and long-term changes appeared beneficial. However, animals reared on low water diets grew less rapidly, but made apparently beneficial changes to an important component of the water balance physiology. The authors reasoned that different physiological processes and levels of biological organization are affected in differing ways by environmental change.

The rejection of the BAH has resulted in several alternative hypotheses. The Colder is Better (CIB) hypothesis predicts that organisms raised at cooler temperatures have a higher significant fitness across all temperatures than organisms that are raised at higher or intermediate temperatures (Huey et al., 1999). Hotter is Better (HIB) is the reciprocal of the CIB, i.e. organisms raised at higher temperatures will have an overall higher relative fitness across all temperatures than organisms raised at cooler or intermediate temperatures (Huey et al., 1999; Wilson and Franklin, 2002). The third competing hypothesis is the Deleterious Acclimation Hypothesis (DAH) which suggests that chronic acclimation will always have a deleterious effect owing to its negative effect on physical processes in aggregate (see above and Loeschcke and Hoffmann, 2002). Woods and Harrison (2001) suggested that the BAH will not be supported whenever chronic environmental exposure negatively affects physiological processes in aggregate, regardless of the potential adaptive or maladaptive value expressed in a particular trait, because performance traits represent the aggregate outcome of mechanistic processes or traits.

Nonetheless, the negative findings regarding the BAH have subsequently been questioned, because the large majority thereof can be construed as studies of developmental plasticity, rather than acclimation (Wilson and Franklin, 2002). By definition, developmental plasticity refers to those alterations made as a consequence of different developmental environments, whereas acclimation responses are reversible, facultative changes to the phenotype (Wilson and Franklin, 2002). Therefore the BAH may still be supported if the functional benefits of thermal acclimation are investigated.

It has also been suggested that the response to temperature differs in marine and terrestrial species. In marine species, upper and lower lethal temperatures are considered coupled (Pörtner, 2001). The coupling is thought to be due to the adjustment of mitochondrial densities (due to temperature changes) and aerobic capacity (Pörtner, 2001).

These changes in densities and functional capacities are related to shifts in the oxygen-limited thermal window. Resistance adaptation to cold results in an increase in mitochondrial aerobic capacity thereby eliciting a downward shift in low temperature thresholds, whereas the reduction of mitochondrial density observed during warming is thought to reduce the baseline oxygen demand which allows the high temperature thresholds to shift to higher values (Pörtner, 2001). However, the coupling of the upper and lower thermal limits found in marine species is not evident in terrestrial insect species (Chown, 2001; Klok and Chown, 2003; Kimura, 2004). This lack of coupling has been attributed to differences in oxygen availability in marine and terrestrial environments, and a highly efficient oxygen delivery system in the insects: the tracheae. Thus, instead of a dual system of oxygen uptake and delivery, there is a single system which is likely to minimize the mismatch between supply and demand that lies at the heart of Pörtner's (2001) oxygen limitation of thermal tolerance hypothesis, at least at the upper end of the scale (see Klok et al. 2004; Sinclair et al. 2004). Thus, the critical temperatures that affect fitness in terrestrial species are more likely to be set by stress responses than by aerobic scope.

Therefore, in this study, the BAH is investigated in three traits; supercooling points, upper and lower lethal temperatures using a strong inference approach (see Huey & Berrigan 1996) and using mite species from marine and terrestrial habitats. In particular, the predictions are made that the response to acclimation effects in all species will be beneficial, and that marine species will show reduced flexibility relative to terrestrial species because the of the narrow range of temperatures experienced in the marine environments compared to that of terrestrial environments (Schultze, 1971; Chown and Crafford, 1992).

Materials and methods

Study site and animals

Samples were collected on sub-Antarctic Marion Island (46°54'S, 37°45'E). The island is an isolated volcanic island in the Southern Ocean and is the larger of the two islands in the Prince Edward Island group, situated 1770 km south east of South Africa. It has a low mean annual air temperature (*c.* 5.7°C), high humidity (83%), and an annual precipitation of approximately 1900 mm (which is distributed evenly throughout the year) (Smith, 2002). The island is also characterized by a high degree of cloudiness and strong, predominantly westerly winds, which reach gale force proportions throughout much of the year (Schulze, 1971).

Five species were investigated: *Halozetes marinus*, *H. marionensis*, *H. belgicae*, *H. fulvus* and *Podacarus auberti*. All five species are ecologically separated in the marine and terrestrial environments. *Halozetes marinus* Engelbrecht and *H. marionensis* Engelbrecht are found in the marine littoral zone occurring respectively in the red seaweeds in the lower shore and in the black lichen *Verrucaria* sp. which dominates the upper shore (Mercer et al., 2000). *H. marinus* also occurs in thick mats of filamentous algae on sheltered shores (Mercer et al., 2000; pers observation). *H. belgicae* Michael occurs in *Turgidosculum complicatulum* (formerly *Mastodia tesselata*) and *Caloplaca* sp. in the supra-littoral zone. *H. belgicae* and *H. marionensis* inhabit saltwater and brackish/freshwater pools in the supra-littoral zone (Marshall et al. unpublished data). *H. fulvus* and *Podacarus auberti* are predominantly found in terrestrial environments on moss (e.g. *Sanionia uncinata*) in mires and *Poa cookii* respectively (Mercer et al., 2000; Barendse et al., 2002) (Fig. 1).

Specimens were collected at Transvaal Cove, Macaroni Bay, Archway, Duikers Point River, and the area immediately around the Entomology laboratory at the scientific station. All of the sites are situated on the East coast of the Island and the distance from the northern most site (Duikers Point) to the southern most site (Archway) is approximately 4 km. *H. marinus* was collected mostly from Duikers point and Archway, with occasional collection from Transvaal Cove, *H. marionensis* and *H. belgicae* were sampled at Macaroni Bay, *H. fulvus* was sampled from moss in mires within the vicinity of the Entomology laboratory at the scientific station and *Podacarus auberti* was sampled from *Poa cookiii* tussocks at Transvaal Cove.

Acclimation

Plant samples were collected from the field, placed into plastic bags and returned to the laboratory within two hours to extract animals. *Halozetes marionensis* was the only species to be collected directly from the rock-substrate on which it occurs. Specimens were removed from the rock and placed, with a sample of the vegetation on which they feed, into vials containing moistened Plaster-of-Paris substrate and returned to the laboratory within two hours. Specimens in the laboratory were kept in vials with a moistened Plaster-of-Paris substrate, and housed in preset incubators at one of four acclimation temperatures (0°C, 5°C, 10°C, 15°C, ± 1°C in each case) for seven days for a set of experiments. Animals were fed on the plant material from which they were collected. Day length was set at 14:10 light-dark cycles (L:D) for the 10°C and 15°C incubators and at 9:15 L:D for the 0°C and 5°C incubators. All experiments were repeated on field fresh animals, which were animals collected from the field and used within 24 h after collection (animals held under ambient conditions during this period).

Supercooling point (crystallisation temperature)

Supercooling point experiments were carried out with a sample of at least 20 individuals for each acclimation temperature for each species. Animals were attached to the end of a thermocouple (Type T copper-constantan 40-gauge) with silicon grease and then placed in the end of a pipette tip. The pipette tip was placed into a sealed test tube and submerged in a Grant LTC 12 water bath. The thermocouples were attached to a Campbell CR-10 (Campbell Scientific, Inc., England) or Pico (Pico Technology limited, United Kingdom) data logger, and measurements taken every second.

Many supercooling point experiments have used a cooling rate of 1°C. min^{-1} (Chown and Nicolson, 2004), although a rate of 0.01°C.min^{-1} has been observed as being more common in the field (Sinclair, 2001). Due to the fact that cooling rates affect physiological processes at low temperatures, with quicker cooling rates resulting in lower insect survival (Kelty and Lee, 1999; Sinclair, 2001), a cooling rate of 0.1°C.min^{-1} (a compromise cooling rate) was used. Animals were initially held at 0°C for 15 minutes for those animals acclimated at 0 °C, and at 5 °C for field fresh animals and those acclimated at 5, 10 and 15 °C, after which the animals were cooled to -30°C. The lowest temperature reached before the onset of an exotherm was taken as the SCP for that individual (Lee, 1991; Vannier, 1994). Following its exotherm, an individual was removed from the water bath and then given an opportunity to recover at its original acclimation temperature (5°C for FF animals). Survival was checked at 24 h and 48 h. All field fresh data were analysed separately from the data collected during the acclimation trials. The SCP data from the different treatment experiments were not normally distributed. Therefore the treatment effects within and between species were investigated using a generalized linear model assuming a normal distribution and using an identity link function (Quinn and Keough, 2002).

Lower and Upper Lethal Temperatures

Super cooling points on their own provide little insight into cold hardiness. Rather, it is the combination of SCP's and other thermal hardiness techniques that provide relevant information on the cold hardiness of invertebrates (Baust and Rojas, 1985; Bale, 1993). Therefore lower lethal temperatures were determined along with SCPs. For upper and lower lethal temperatures, five replicates of 10 animals were used for each temperatures for all species except for *H. fulvus* for which five animals were used in each replicate. For the lower lethal limit experiments a wet and a dry treatment was used. For the wet treatment, animals were placed onto moist filter paper during the course of the experiment, whilst in the dry treatment the animals were placed above dry silica gel. The animals were not placed directly on the silica gel to avoid possible damage to their cuticles. Animals in the upper lethal limits experiments were placed on moistened filter paper only because the intention was not to measure desiccation resistance.

The animals were placed into a 1.5 ml eppendorf vial and the vial was submerged in a water bath at a given temperature for one hour. The animals were then removed and transferred to a recovery vial at the original acclimation temperature. For field-fresh animals recovery temperatures were set to 5°C. After 24 h and 48 h animals were scored for survival. Survival was considered as coordinated movement, while twitching or uncoordinated movement indicated a moribund state, and no movement indicated mortality. Survival is represented as a proportion (number of individuals surviving out of a batch of 5 or 10 animals, depending on the treatment). The temperature was then lowered (in the case of the LLT experiments) or increased (in the case of ULT experiments) by 1°C increments and the assessment repeated with new animals. Experiments were continued until survival varied between 0 and 1. Field fresh (FF) data were analysed separately from the acclimation temperatures. For the LLT and ULT mortality data a logistic regression was used to calculate LT50 values. This procedure was only applied where the logistic regression was significant based on the Wald statistic (Quinn and Keough, 2002). The treatment effects within each species were investigated using an unequal N honest significant difference test following an analysis of variance. Where data was not normally distributed treatment effects within species were investigated using a generalized linear model assuming a normal distribution and using an identity link function (Quinn and Keough, 2002).

Maximum difference

To test the prediction that the species from the less variable marine environment would show a reduced flexibility relative to the species from the more variable terrestrial environment, the largest difference in each of the traits across the four acclimation temperatures for each of the species was compared. In this case a mean and variance for this acclimation-associated difference in a given trait was produced by randomly drawing values for each trait for each acclimation temperature from the data available for each individual at each temperature, and calculating the largest absolute difference between them. This was repeated 10 000 times to obtain the mean and variance for each species and these were compared using a Tukey honest significant difference test following an analysis of variance (in the case of LLT dry and ULT) and where data was not normally distributed a generalized linear model with a normal distribution and identity link function was used (in the case of SCP and LLT wet).

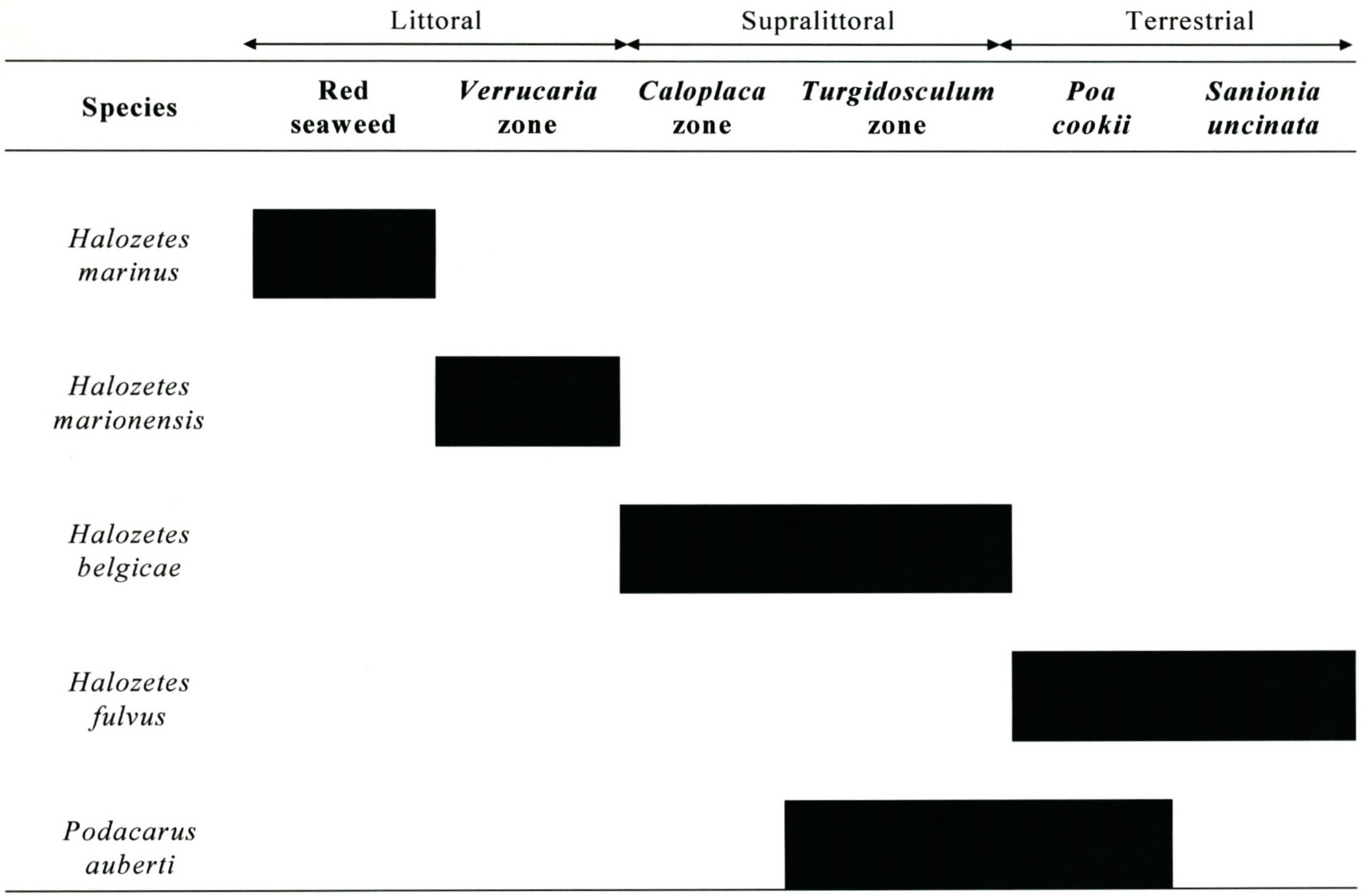


Fig. 1. Diagramatic representation of the ranges of the five species of oribatid mites along the rocky shore and terrestrial habitats.

Results

Supercooling point (crystallisation temperature)

No individuals survived freezing. The supercooling points of most species were lower than the lower lethal temperature calculated, suggesting some pre-freeze mortality (Fig. 2), the only exception being *H. marionensis* in which the supercooling point and the lower lethal limit value of the dry treatment at the 10 °C acclimation were similar (Fig. 2b).

The SCP of *H. marionensis* was lower than that of the other species, which did not differ from each other (Wald $X^2_{(4)} = 29.560$, $p < 0.0001$) (Fig. 3, Table 1). There were generally no consistent differences in SCP associated with acclimation temperature for most of the species (Fig. 2, Table 1). The exceptions were *H. marinus* and *H. marionensis*, where *H. marionensis* showed a clear trend of increasing SCPs with increasing acclimation temperature (Fig. 2b, Table 1), and *H. marinus* a significant increase in SCP at the highest acclimation temperature (Fig 2a, Table 1).

Lower and Upper Lethal Temperatures

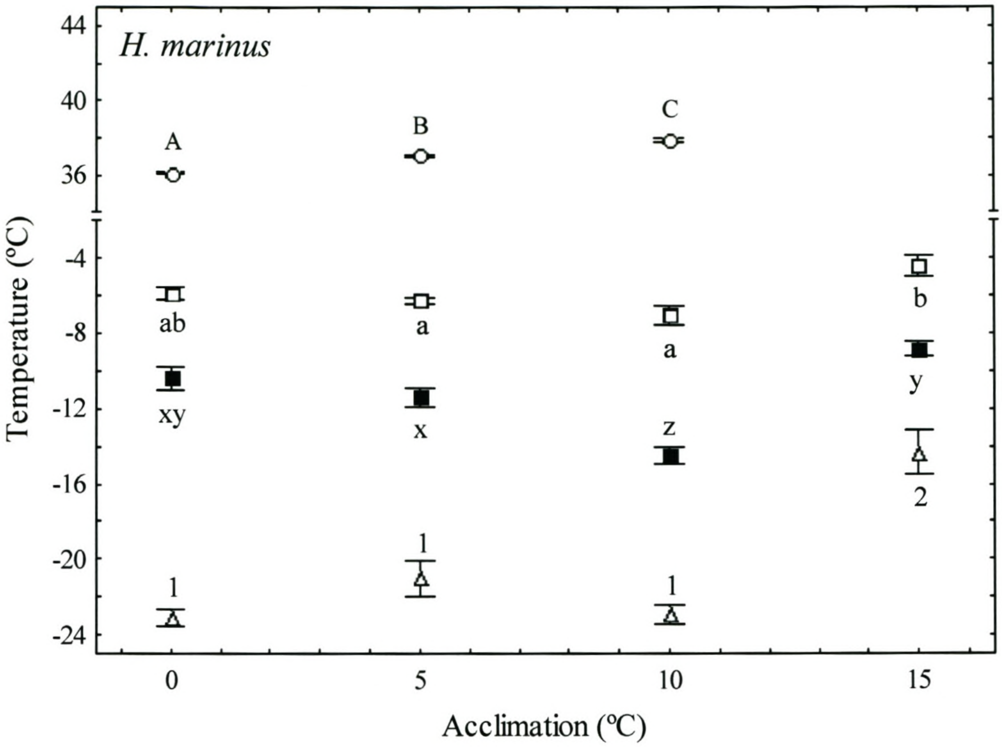
Differences in field fresh lethal temperatures were found between all species (Table 2 - 3). In the wet treatment experiment, significantly lower LLTs were found in the two intertidal species (*H. marinus*, -6.52 °C and *H. marionensis*, -6.39 °C) when compared to the terrestrial species (*H. fulvus*, -5.43 and *Podacarus auberti*, -5.51). However, *H. belgicae,* the supralittoral species, had a significantly higher LLT (-4.21 °C) when compared to all the other species. The ULTs showed the same trends as that of the wet treatment LLT experiment with significantly lower ULTs in *H. marinus* (37.5°C) and *H. marionensis* (36.9 °C) when compared with *H. fulvus* (38.3 °C) and *P. auberti* (38.9 °C). Again, *H. belgicae* (40.55 °C) showed the highest ULT of all the species. In the case of the dry treatment LLT experiment, significant differences in lower lethal temperatures between the species were found but they were not consistent, with *H. marionensis* (-13.36 °C) and *H. fulvus* (-13.38) showing the lowest temperatures and *P. auberti* (-5.96 °C) the highest (Table 2).

Although acclimation had a significant effect on LLTs, the effects were generally not consistent for any of the species (Fig. 2, Table 3). The possible exception is *H. marionensis* where LLT increased significantly with increasing acclimation temperature (Fig. 2b). In the case of the ULTs, only *H. marinus* showed a consistent difference associated with acclimation temperature (Fig. 2a). *H. belgicae* and *H. fulvus* showed significantly higher ULTs at the highest acclimation temperature (Fig. 2c - d), with the opposite was found for *P. auberti* where a significant decrease in ULT was found at the lowest acclimation temperature (Fig. 2e). No correlation was found between ULT and LLT (Table 4). However, the correlation was only calculated for *H. marinus*, *H. belgicae*, *H. fulvus* and *P. auberti* because they were the only species for which ULT data were collected for three or more acclimation temperatures.

Maximum difference

Differences in the extent of plasticity of the traits examined between species were significant (Table 5). Over the 15°C range of acclimation temperatures, SCP differed by 11.3 to 18.5 °C, LLT wet treatment by 0.9 to 2.8°C,and LLT dry treatment by 1.9 to 5.7 °C. ULT differed by 2.7 to 4.2 °C over the 15°C acclimation range. However, this later range was only calculated for *H. belgicae*, *H. fulvus* and *P. auberti* because they were the only species for which ULT data were collected across all acclimation temperatures. Although differences between species in the range of plasticity were apparent, these were not clearly interpretable, with the possible exception of the LLT dry treatment where plasticity was less in the terrestrial species compared to the marine ones.

A


H. marinus
Temperature (ºC)
Acclimation (ºC)
44
40
36
-4
-8
-12
-16
-20
-24
0
5
10
15
A
B
C
ab
a
a
b
xy
x
z
y
1
1
1
2

B

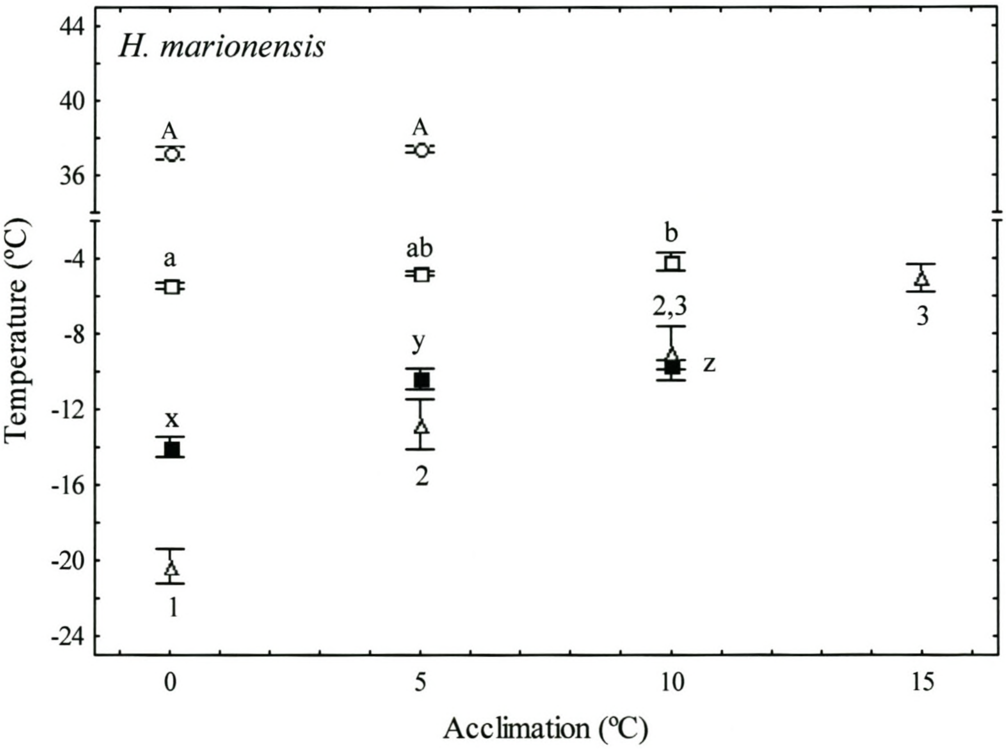
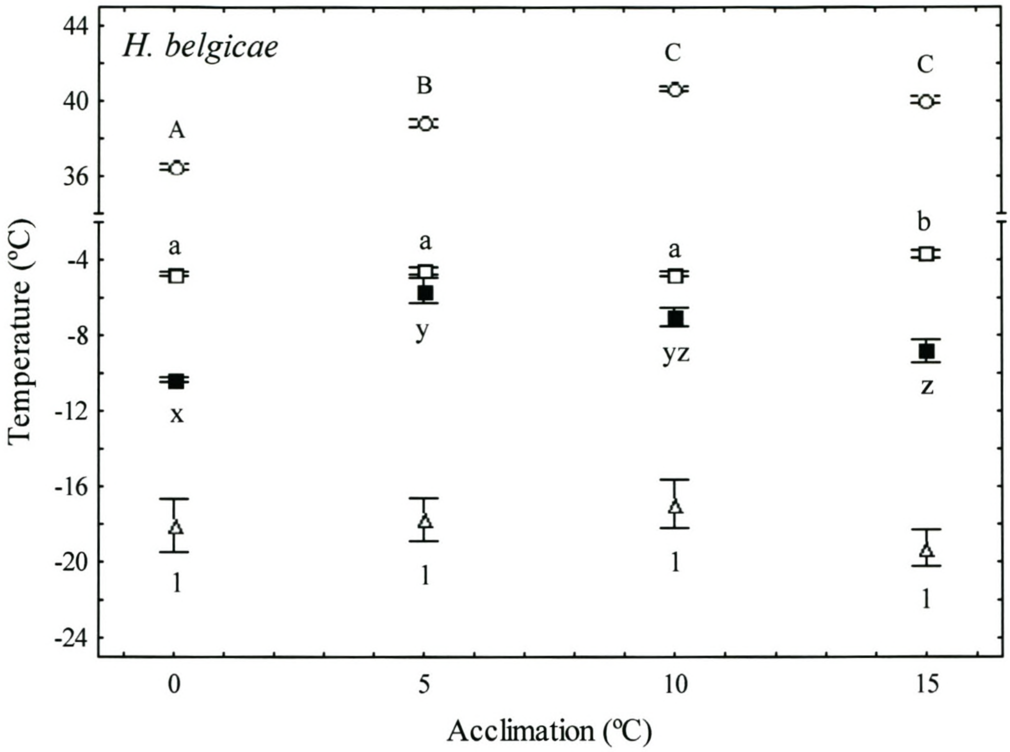

H. marionensis
Temperature (ºC)
Acclimation (ºC)
44
40
36
-4
-8
-12
-16
-20
-24
0
5
10
15
A
A
a
ab
b
x
y
z
1
2
2,3
3

Fig. 2 a - b

C


H. belgicae
Temperature (ºC)
Acclimation (ºC)
44
40
36
-4
-8
-12
-16
-20
-24
0
5
10
15
A
B
C
C
a
a
a
b
x
y
yz
z
1
1
1
1

D

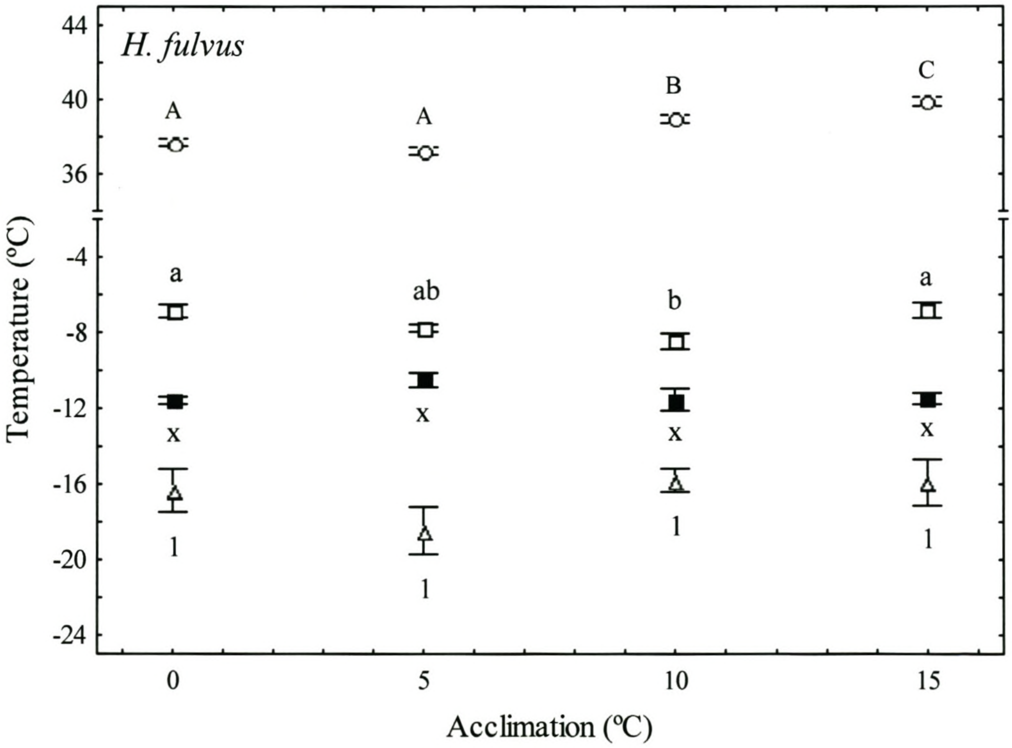

H. fulvus
Temperature (ºC)
Acclimation (ºC)
44
40
36
-4
-8
-12
-16
-20
-24
0
5
10
15
A
A
B
C
a
ab
b
a
x
x
x
x
1
1
1
1

Fig. 2 c – d

E

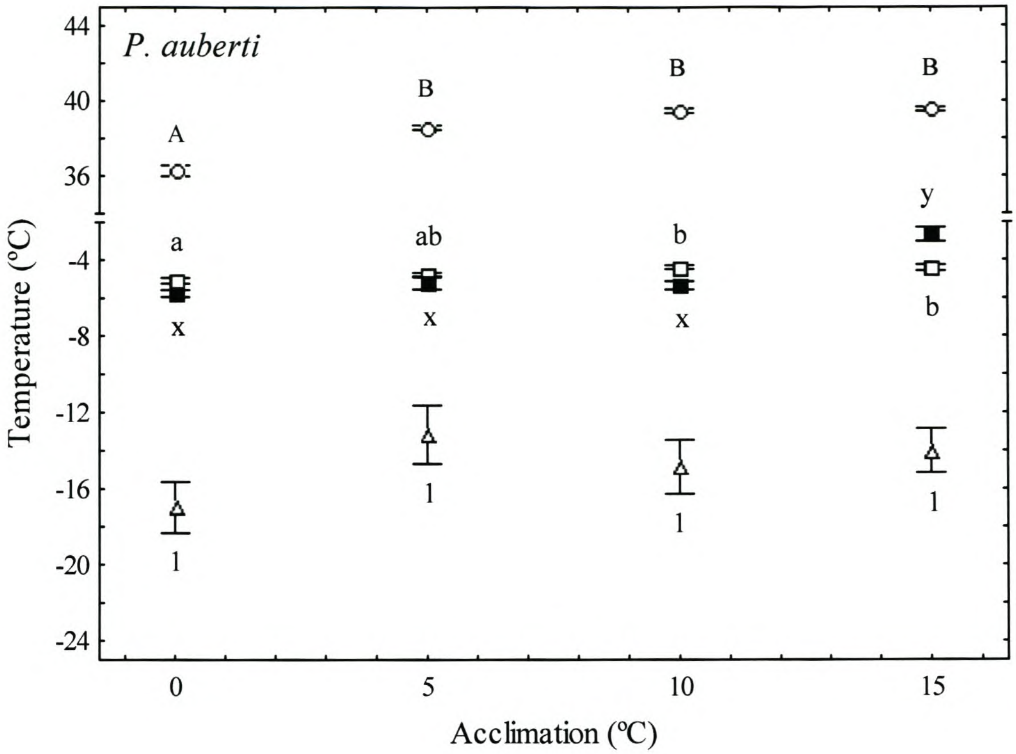


Fig. 2 Thermal limits at four acclimation temperatures (means ± S.E.) for (A) *H. marinus*, (B) *H. marionensis*, (C) *H. belgicae*, (D) *H. fulvus* and (E) *Podacarus auberti* (SCP (Δ); LLT50 wet (□); LLT50 dry (■) and ULT50 (○)). Letters and numbers denote significant differences between acclimation treatments for each thermal limit based on unequal N honestly significant difference test following an ANOVA or 95% confidence intervals following a generalized linear model assuming a normal distribution with an identity link function. Results of the analyses can be found in Table 3.

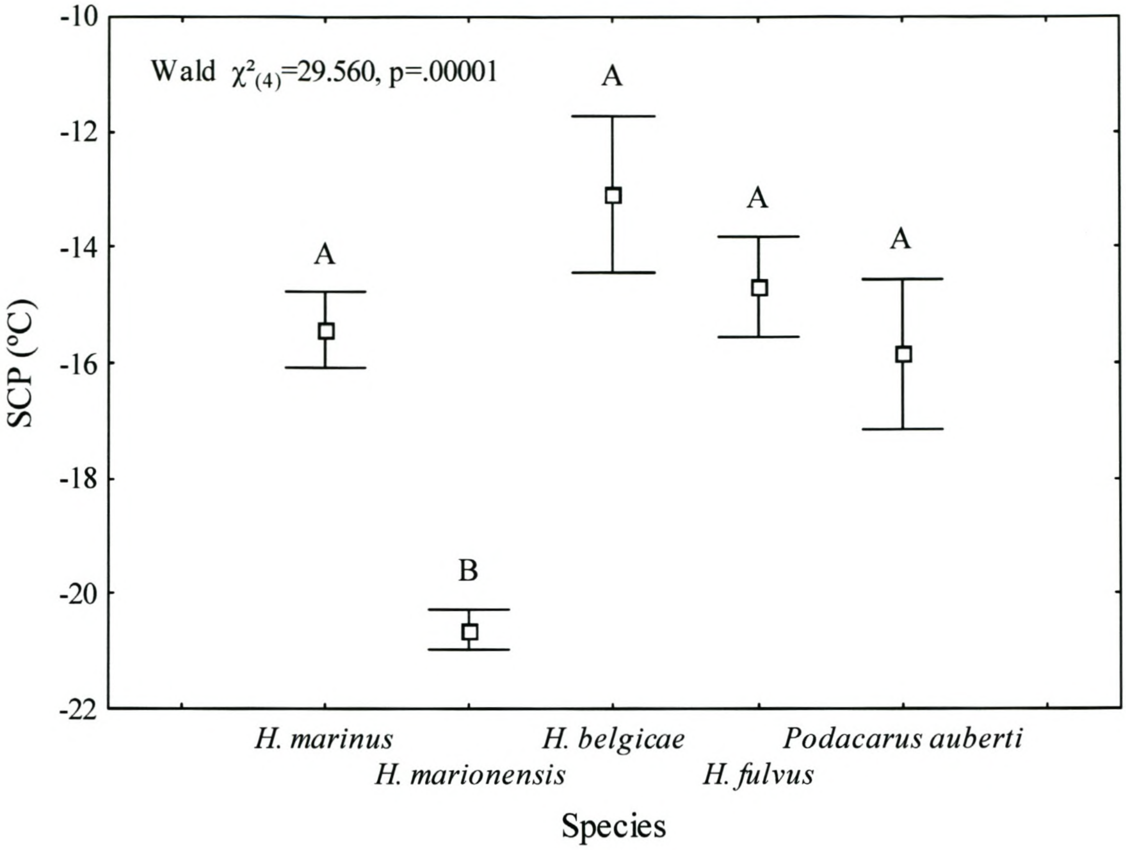


Fig. 3 Mean (± S.E.) supercooling points (SCP) for field fresh animals for *Halozetes marinus*, *H. marionensis*, *H. belgicae*, *H. fulvus* and *Podacarus auberti*. Letters denote significant differences between acclimation treatments based on 95% confidence intervals following a generalized linear model assuming a normal distribution with an identity link function.

Table 1 Summary statistics of mean supercooling points (°C ± SE) between each species for field fresh (FF) individuals, and between each acclimation temperature within each species. Differences between groups as indicated by different letters are based on 95% confidence intervals following a generalized linear model assuming a normal distribution with an identity link function.

FF		n	Mea± SE	Median	Minimum	Maximum	Range
H. marinus	a	33	-15.± 0.7	-14	-23	-11	12.0
H. marionensis	b	26	-20.± 0.4	-20.25	-25.9	-18.2	7.7
H. belgicae	a	28	-13.± 1.3	-9.55	-24	-3.6	20.4
H. fulvus	a	23	-14.± 0.9	-15.7	-20.5	-5.3	15.2
Podacarus auberti	a	33	-15.± 1.3	-19.2	-24.3	-2.9	21.4
Wald $\chi^2_{(4)} = 29.560$, $p < 0.0001$							
H. marinus							
0°C	a	39	-23.± 0.5	-23.7	-27.8	-13.5	14.3
5°C	a	34	-21.± 0.9	-22.8	-25.9	-3.9	22.0
10°C	a	34	-22. 0.5	-23.1	-27.6	-14.0	13.6
15°C	b	37	-14.± 1.2	-15.2	-26.8	-2.6	24.2
Wald $\chi^2_{(3)} = 73.549$, $p < 0.0001$							
H. marionensis							
0°C	a	36	-20.± 0.9	-21.3	-26.4	-4.5	21.9
5°C	b	39	-12.± 1.3	-10.4	-25.4	-2.8	22.6
10°C	bc	25	-9.± 1.4	-8.4	-23.4	-2.0	21.4
15°C	c	28	-5.± 0.7	-3.7	-22.6	-2.8	19.8
Wald $\chi^2_{(3)} = 98.445$, $p < 0.0001$							

Table 1 cont.

H. belgicae		n	Mean ± SE	Median	Minimum	Maximum	Range
0°C	a	31	-18.± 1.4	-22.2	-25.9	-3.2	22.7
5°C	a	38	-17.± 1.1	-20.6	-27.5	-4.8	22.7
10°C	a	44	-16.± 1.3	-20.4	-26.6	-2.6	24.0
15°C	a	27	-19.± 1.0	-20.8	-24.8	-3.2	21.6
Wald $\chi^2_{(3)} = 1.7577$, $p = 0.624$							
H. fulvus		n					
0°C	a	20	-16.± 1.1	-17.2	-25.2	-3.8	21.4
5°C	a	25	-18.± 1.2	-20.0	-25.6	-3.4	22.2
10°C	a	34	-15.± 0.6	-15.2	-23.4	-10.2	13.2
15°C	a	26	-15.± 1.2	-15.4	-25.0	-4.4	20.6
Wald $\chi^2_{(3)} = 4.561$, $p = 0.207$							
P. auberti							
0°C	a	31	-17.± 1.3	-20.1	-24.4	-2.6	21.8
5°C	a	32	-13.± 1.5	-11.4	-26.1	-3.4	22.7
10°C	a	32	-14.± 1.4	-16.5	-25.1	-3.4	21.7
15°C	a	43	-14.± 1.2	-10.1	-26.0	-3.7	22.3
Wald $\chi^2_{(3)} = 4.213$, $p = 0.239$							

Table 2 Summary statistics for lower (A) and upper (B) lethal limits (°C ± SE) for field fresh animals between species. Letters refer to significant differences obtained using an unequal honest significant difference test following an ANOVA.

A	LLT50	SE
Wet treatment		
Halozetes marinus	-6.52^{a}	0.20
H. marionensis	-6.39^{a}	0.26
H. belgicae	-4.21^{b}	0.16
H. fulvus	-5.43^{c}	0.09
Podacarus auberti	-5.51^{c}	0.18
$F_{(4, 19)} = 17.303$, $p < 0.0001$		
Dry treatment		
H. marinus	-9.80^{a}	0.06
H. marionensis	-13.36^{b}	0.05
H. belgicae	-9.34^{a}	0.06
H. fulvus	-13.38^{b}	0.05
P. auberti	-5.96^{c}	0.10
$F_{(4, 18)} = 58.654$, $p < 0.0001$		

B	ULT50	SE
H. marinus	37.46^{a}	0.36
H. marionensis	36.91^{a}	0.68
H. belgicae	40.55^{b}	0.24
H. fulvus	38.25^{c}	0.61
P. auberti	38.90^{d}	0.40
$F_{(4, 19)} = 103.65$, $p < 0.0001$		

Table 3 Results of the analysis of the lower and upper lethal temperatures (LLT and ULT) and SCPs between the four acclimation temperatures for each species following an ANOVA (*) or a generalized linear model assuming a normal distribution with an identity link function (†) (see Fig. 2).

H. marinus	Test statistic	P
SCP	$\chi^2_{(3)} = 73.55^\dagger$	< 0.0001
LLT wet	$F_{(3, 14)} = 7.38$*	< 0.005
LLT dry	$F_{(3, 16)} = 22.83$*	< 0.0001
ULT	$F_{(2, 11)} = 100.02$*	< 0.0001
H. marionensis		
SCP	$\chi^2_{(3)} = 98.45^\dagger$	< 0.0001
LLT wet	$\chi^2_{(2)} = 10.93^\dagger$	< 0.005
LLT dry	$F_{(2, 12)} = 25.64$*	< 0.0001
ULT	$F_{(1, 8)} = 0.28$*	0.609
H. belgicae		
SCP	$\chi^2_{(3)} = 1.76^\dagger$	0.624
LLT wet	$F_{(3, 16)} = 9.06$*	< 0.001
LLT dry	$F_{(3, 15)} = 13.83$*	< 0.0005
ULT	$\chi^2_{(3)} = 410.86^\dagger$	< 0.0001
H.fulvus		
SCP	$\chi^2_{(3)} = 4.56^\dagger$	0.207
LLT wet	$F_{(3, 16)} = 5.15$*	< 0.05
LLT dry	$F_{(3, 16)} = 1.74$*	0.199
ULT	$F_{(3, 12)} = 28.65$*	< 0.0001
P. auberti		
SCP	$\chi^2_{(3)} = 4.21^\dagger$	0.239
LLT wet	$F_{(3, 15)} = 6.54$*	< 0.005
LLT dry	$\chi^2_{(3)} = 93.48^\dagger$	< 0.0001
ULT	$\chi^2_{(3)} = 269.35^\dagger$	< 0.0001

*Normally distributed, ANOVA used

†Non-normally distributed, generalized linear model used

Table 4 Pearson's product moment correlation between upper lethal temperature (ULT) and lower lethal temperature (LLT) within each mite species across three or more acclimation temperatures.

Species	Pearsons product moment (r)	p- value
Halozetes marinus	-0.95	> 0.05
H. belgicae	0.54	> 0.07
H. fulvus	-0.59	> 0.08
Podacarus auberti	0.59	> 0.09

Table 5 Mean (ºC ±SE) SCP, LLT and ULT for individuals acclimated at four temperatures (ºC). The difference row provides the mean maximum difference of 10 000 random calculations of largest absolute difference from the data available (refer to text) between any two acclimation treatments within a species. Letters refer to significant differences when comparing the difference values between species obtained using Tukey honest significant difference test following an ANOVA (*), or based on 95% confidence intervals following a generalized linear model assuming a normal distribution with a log link function (†) for non-normally distributed data.

SCP	*H. marinus*	*H. marionensis*	*H. belgicae*	*H. fulvus*	*P. auberti*
0°C	-23.1±.5	-20.3±0.9	-18.1±1.4	-16.3±1.1	-17.0±1.3
5°C	-21.0±0.9	-12.8±1.3	-17.8±1.1	-18.5±1.2	-13.2±1.5
10°C	-22.9±0.5	-9.0±1.4	-16.9±13	-15.8±0.6	-14.9±1.4
15°C	-14.4±1.2	-5.0±0.7	-19.3±1.0	-15.9±1.2	-14.0±1.2
Difference	12.47±0.07^{b}	18.46±0.04^{e}	14.01±0.06^{c}	11.29±0.04^{a}	16.24±0.05^{d}

† $\chi^2_{(4)} = 11019.00, p < 0.0001$

LLT (wet)					
0°C	-5.87±0.09	-5.43±0.13	-4.73±0.15	-6.86±0.08	-5.08±0.14
5°C	-6.25±0.15	-4.78±0.15	-4.56±0.2	-7.76±0.09	-4.75±0.19
10°C	-7.04±0.26	-4.17±0.24	-4.72±0.18	-8.46±0.06	-4.37±0.28
15°C	-4.42±0.16	-	-3.69±0.1	-6.80±0.09	-4.42±0.3
Difference	2.76±0.01^{e}	1.41±0.01^{bc}	1.25±0.004^{c}	2.19±0.01^{d}	0.87±0.003^{a}

† $\chi^2_{(4)} = 28101.\ 00, p < 0.0001$

Table 5 cont.

	H. marinus	*H. marionensis*	*H. belgicae*	*H. fulvus*	*P. auberti*
LLT (dry)					
0°C	-10.37±0.05	-13.98±0.05	-10.33±0.04	-11.58±0.06	-5.75±0.04
5°C	-11.38±0.04	-10.38±0.04	-5.60±0.05	-10.50±0.05	-5.21±0.05
10°C	-14.47±0.06	-9.63±0.04	-7.01±0.05	-11.54±0.05	-5.33±0.05
15°C	-8.84±0.07	-	-8.83±0.05	-11.46±0.07	-2.66±0.07
Difference	5.73±0.01^{e}	4.57±0.01^{c}	4.91±0.01^{d}	1.94±0.01^{a}	3.26±0.01^{b}

*$F_{(4,\ 49995)} = 19573.0$, $p < 0.0001$

ULT					
0°C	36.10±0.41	37.20±0.16	36.52±0.21	37.70±1.08	36.28±0.08
5°C	37.05±0.76	37.41±0.14	38.86±0.18	37.25±0.43	38.60±0.5
10°C	37.89±0.69	-	40.68±0.16	38.98±0.34	39.48±0.32
15°C	-	-	40.07±0.2	39.91±0.28	39.54±0.3
Difference	1.80±0.002^{b}	0.67±0.004^{a}	4.16±0.004^{e}	2.71±0.01^{c}	3.37±0.01^{d}

*$F_{(4,\ 49995)} = 85852.0$, $p < 0.0001$

Discussion

Thermal tolerances

All of the mite species that were investigated here are freeze-intolerant, a strategy that is almost universal in mites and collembola (Block, 1982; Cannon and Block, 1988; Convey, 1996; Sinclair et al., 2003). However, in all species the supercooling point (SCP, or crystallization temperature) was lower than and thus not equivalent to the lower lethal temperature. This suggests that there was pre-freeze mortality in all of the species investigated. Pre-freeze mortality has been observed in several species of insects and other arthropods (Baust and Rojas, 1985; Bale, 1987, 1993; Koštal et al., 2004), and its occurrence here is therefore not necessarily surprising. However, differences in the experimental protocol used to calculate SCP and the one followed for LLT may have contributed to the observed pre-freeze mortality. The SCP experiments used a cooling rate of 0.1°C/min whereas in the lower lethal temperature experiments the animals were submerged at subzero temperatures for an hour immediately after removal from their acclimation temperature (essentially 'plunge' experiments). Therefore chilling-injury may have occurred as a result of rapid cooling, may well have induced mortality above the freezing point. The rate at which insects are cooled has been shown to affect both the temperature at which they freeze and the survival of freezing (Bale, 1993; Sinclair and Chown, 2003; Chown and Nicolson, 2004), although the effect on SCP is generally small. Whether the protocol adopted in the LLT experiments affected the freezing point, likelihood of chilling injury, or both, is impossible to tell and deserves further exploration. In the absence of this information it seems safest to propose that there is at least some pre-freeze mortality in the species examined here. Therefore, they should be considered moderately chill-tolerant rather than freeze-avoiding (see Bale, 1996). This level of chill tolerance is typical of several other arthropod species on Marion Island, and includes the moderately chill-tolerant adult kelp flies *Paractora dreuxi* (Klok and Chown, 2001), the weevil *Palirhoeus eatoni* (Van der Merwe et al., 1997) and psocid *Antarctopsocus jeanneli* (Slabber and Chown, 2004). Moreover, it is in keeping with the generally high subzero microclimate temperatures which the mite species are likely to encounter (Table 6, Appendix 2).

Relative to their LLTs, the microhabitat temperatures encountered by *H. marinus*, *H. marionensis* and *H. belgicae* are unlikely to reach sub-lethal levels. Over the period for which microclimate data are available it is clear that these temperatures never fell below freezing and always lay within the lower lethal temperatures of the species concerned. This was not the case for *H. fulvus* and *P. auberti* (both inland terrestrial species), which might experience ambient temperatures that lie below their LLTs (Table 6) albeit infrequently and only for short periods. This might explain the absence of *P. auberti* from the highest altitude sites on Marion Island (Barendse et al., 2002), although in the case of *H. fulvus* this seems unlikely given that they occur in abundance at 800 m asl. Perhaps this species has sufficiently well developed behavioural regulation to ensure that the most severe thermal environments are avoided (see e.g. Hayward et al., 2001).

The upper lethal temperatures of all five mite species are much higher than some invertebrate species on Marion Island. For example, the ULT of six weevil species that occur on the island vary between 31 °C and 34 °C (Van der Merwe et al., 1997) and that of the psocid *Antarctopsocus jeanneli* (Slabber and Chown, 2004) is 29.5 °C. However, the ULT values in this study fall in the same range as the CTmax values for many of the species on the island (35.5 °C – 39.7 °C) (Klok and Chown, 1997; Klok and Chown, 1998; Klok and Chown, 2001). In addition, the upper lethal temperatures for all mite species investigated here are high enough to ensure tolerance of the absolute maximum temperatures likely to be experienced by these species in their thermal environments including the uncommonly high micro-climate temperatures that may occasionally occur. Specifically, the ULTs of *H. fulvus* and *P. auberti* will ensure survival of the uncommonly high temperatures (*ca*. 33 °C) that can occur in *Poa cookii* tillers (Chown and Crafford, 1992). *H. marionensis* and *H. belgicae* might also experience extended periods when they are not exposed to wave action. Thus they might also encounter high temperature microclimate conditions because the black, basalt substrate on which they occur warms considerably. Admittedly if the time of day where the most extreme conditions occur coincides with high tide the species will be submerged and will not be exposed to such extreme conditions (Helmuth et al., 2002). However, high tides do not always coincide with the warmest parts of the day at Marion Island (pers. observation). In addition, as tides rise and fall the change from marine to terrestrial conditions, and vice versa, can place extreme demands on the physiology of intertidal organisms (Denny and Paine, 1998). Therefore, the intertidal species may well have upper lethal limits that barely exceed microhabitat temperatures. Unfortunately, attempts to record microhabitat temperatures in the intertidal over a significant period were thwarted by continual loss of the microloggers used to do so.

Responses to acclimation

Owing to the small and idiosyncratic changes effected to all three thermal tolerance variables examined (SCP, LLT, ULT) with acclimation, it is clear that there is very little support for the beneficial acclimation hypothesis (BAH). The only exceptions were small changes in the ULT of *H. marinus*, and the SCP and LLT of *H. marionensis*, which can be interpreted as supporting the BAH. Moreover, the alternatives to the BAH were not supported either, in the sense that acclimation did not appear to be deleterious, nor did warmer or colder conditions appear to favour survival (see Huey and Berrigan 1996; Woods and Harrison 2002). In addition, in the case of the SCP and LLT of *H. marionensis* it is not possible to distinguish the "colder is better" from the BAH hypothesis, whereas for ULT in H. marinus it is not possible to distinguish the BAH from "warmer is better", because these hypotheses make similar predictions. Therefore, this study provided no support for the BAH, in keeping with a variety of recent conclusions (Gibbs et al., 1998; Woods, 1999; Woods and Harrison, 2001). Importantly, the acclimation trials undertaken here were not of developmental plasticity. That is, acclimation did not take place over the full development period of the immature stages, but was rather restricted to a short period in the adult stage. Thus, the criticism that investigations of the BAH have more to do with the extent of developmental plasticity than with what physiologists consider acclimation to be (see Wilson and Franklin, 2002 for discussion) do not apply here. Rather, it appears that in these five mite species beneficial acclimation does not take place. Indeed, acclimation responses typically appear to be rather poorly developed, with the exception perhaps of supercooling point, which appears to be highly malleable. This is not surprising, given that supercooling points have been shown to be sensitive to acclimation in a wide variety of other terrestrial arthropods (e.g. Sømme and Block, 1982; Cannon and Block, 1988; Block, 1990; Klok and Chown, 1998; Worland et al., 2000).

The acclimation trials also revealed little in the way of an association between ULT and LLT ($p > 0.05$ in all cases, see Table 4). For marine species such a finding is unusual because tolerances are generally coupled such that a decline in ULT is associated with a decline in LLT and *vice versa* (see discussion in Pörtner, 2001). However, the decoupling of acclimation responses to upper and lower lethal is in keeping with findings for insects (Chown, 2001; Klok and Chown, 2003). Based on several recent mechanistic studies (Košťál et al., 2004; Klok et al., 2004; Sinclair et al., 2004), Chown and Nicolson (2004) suggested that this difference between marine and terrestrial species may well be a consequence of the fact that terrestrial insects have considerably better developed oxygen delivery systems than marine invertebrates. In consequence, any alterations effected to mitochondrial density or functioning to enhance aerobic capacity at low temperatures are unlikely to affect performance (via a mismatch between mitochondrial oxygen demand and circulatory supply) at high temperatures. Thus, tracheated arthropods are unlikely to show a strong coupling between changes in upper and lower lethal temperatures either through space or with acclimation (reviews in Chown, 2001; Chown and Nicolson, 2004; see also Kimura, 2004). The ameronothrid mites, like other oribatids, have a well developed tracheal system, which may well explain the similarity of their responses to insects, and might thus provide further support for Chown and Nicolson's (2004) mechanistic hypothesis underlying the variation in the coupling of upper and lower lethal temperature limits in arthropods. By contrast, the lack of difference between the marine and terrestrial mite species, in terms of the extent of their plasticity, is a little more difficult to explain. However, these mites are all air breathing, and are very closely related, which might explain this lack of variation. Moreover, the environments they occupy might be more similar than we have presumed owing to microhabitat selection. This idea deserves further exploration.

In conclusion, this study has shown that the thermal tolerances of these ameronothrid mites species are typical both of the group to which they belong and the area (the sub-Antarctic) in which they are found. More importantly, it has provided little support for the beneficial acclimation hypothesis, and indeed for acclimation of any kind in upper and lower lethal limits. Although such lack of flexibility is unusual for animals from temperate environments (Chen et al., 1990), it might be expected on Marion Island where there is a limited range of temperatures (see Table 6 and Smith, 2002), and where microhabitat selection might further reduce this range.

Table 6 Sea surface (SS) temperature (°C) and microhabitat temperature (°C) of two terrestrial sites (0m and 200m a.s.l.) for two years. Mean minimum (Mean min) and maximum (Mean max) temperatures, absolute minimum (Abs min) and maximum (Abs max) temperatures, and temperature range are given.

	Year	Mean (°C)	Abs. Min	Abs. Max	Mean Min	Mean Max	Range
SS							
	2002	5.4	3.7	8.5	4.9	6.2	4.8
	2003	5.4	3.7	8	4.7	6.0	4.3
0m							
	2002	5.3	0.5	22.5	3.8	7.1	22
	2003	7.0	1	22	5.1	8.6	21
200m							
	2002	4.4	-5	18.5.	2.2	7.0	23.5
	2003	5.2	-6	19	3.5	6.6	25

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Chapter 4

Conclusions

This study sought to explicitly investigate the effects of acclimation on thermal tolerance and locomotor performance of five mite species. The data presented here provide support for the BAH in two of the three performance traits measured (performance breadth - T_{br}; and optimum speed - u_{max}), with the third trait (optimum temperature - T_{opt}) supporting the compensation hypothesis. The support is in keeping with the expectations from the majority of the literature on acclimation in labile traits (Wilson and Franklin, 2002; Woods and Harrison, 2002), and with theoretical predictions regarding adaptive phenotypic plasticity (Van Tienderen, 1997; Doughty and Reznick, 2004). However, the predictions made by the BAH were the same for the compensation hypothesis in the case of both T_{br} and u_{max}, suggesting that the overall response is one of compensation. Such an outcome that has been predicted in models of the response of traits to stable environments under certain conditions (Van Tienderen, 1997; Berrigan and Scheiner, 2004). Whether this compensation is beneficial might be the case for T_{br} and u_{max} as support for the BAH was shown, but it may not be so for T_{opt} where this support was not shown. Responses to acclimation were evident in the lethal temperature traits, but as was the case with T_{opt}, few showed support for the BAH. In addition, support for the alternative hypotheses was also lacking, although there may have been partial support for the detrimental acclimation hypothesis. However, a definitive conclusion could not be reached because, if a deleterious acclimation hypothesis is to be tested, then independent evidence of detrimental effects (such as assessments of stress proteins – for review see Feder and Hofmann, 1999) will be required in addition to data on the traits of interest.

The study also sought to investigate variation amongst individuals in the three performance traits. The repeatability values were calculated largely as an assessment of measurement reliability, although they also provided a measure of within- relative to between individual variation. In the latter case, high repeatability (i.e. low within-individual variation) provides an indication that there is repeatable variation between individuals, which is a requirement of natural selection (Bech et al., 1999). Although the analysis undertaken here was not specifically designed to test this condition, the high values for the intraclass correlation coefficient for u_{max} suggest that the condition for this trait was satisfied. This may indicate that the plasticity shown could well be adaptive.

Evidence for the decoupling of lethal temperatures in the mites species investigated here supports the notion that these traits are decoupled in tracheated species, as was suggested by Chown (2001) for insects. In addition the extent of phenotypic plasticity of lethal temperatures was investigated, with predictions of increased flexibility in more thermally variable environments. These predictions were met for T_{br} and T_{opt} in the locomotor performance traits. However results for the lethal temperature traits were not always clear, with high flexibility being found in both the epilithic and supralittoral species relative to the vegetation bound species. This suggests that flexibility in these traits is not always influenced by short term thermal variation.

These outcomes of this study raise several issues which should be addressed in future research:

- The response of an organism to acclimation is a complex one. To fully understand the effects of acclimation, future studies testing the beneficial acclimation hypothesis should not only adopt a strong inference approach (Huey et al., 1999), but the covariation of traits should also be incorporated into predictions regarding the effects of acclimation. Furthermore, the relative position of each acclimation trial along a performance curve should be examined.

- Repeatability of performance traits should be assessed where possible. This will provide an assessment of whether the traits fulfil one of the three prerequisites for natural selection. In addition studies should also investigate trait heritability and the the fitness consequences of trait variation to determine whether the other prerequisites of natural selection are met.

- Cellular level responses to thermal acclimation in terrestrial and intertidal mite species are poorly understood, and although these responses were not tested in this study, the degree to which these responses affect critical temperature thresholds needs requires attention, as the contribution of these responses vs. organism-level responses will provide insight into the processes determining lethal limits.

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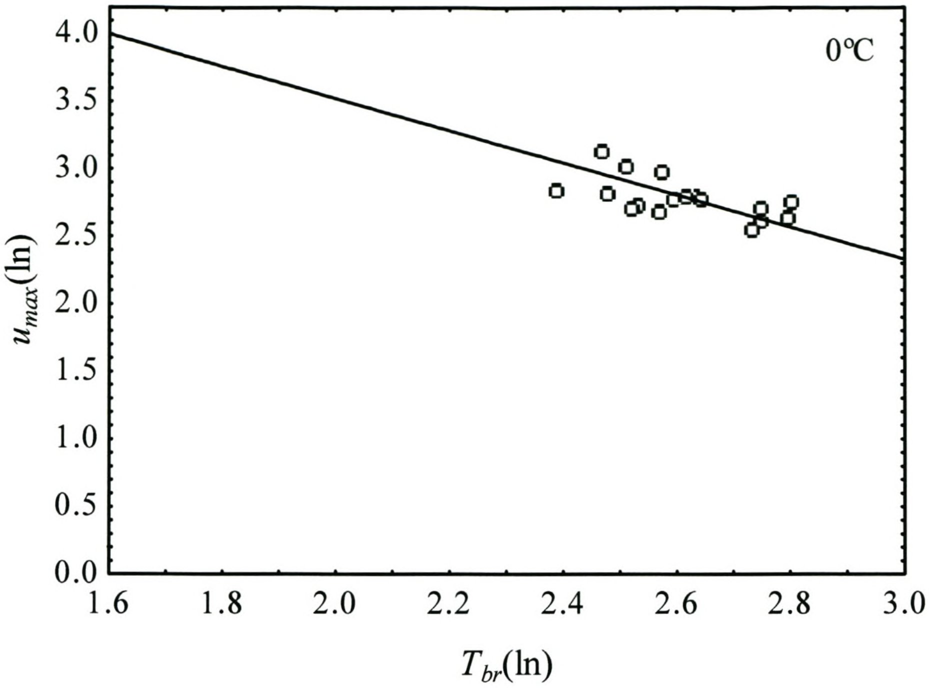
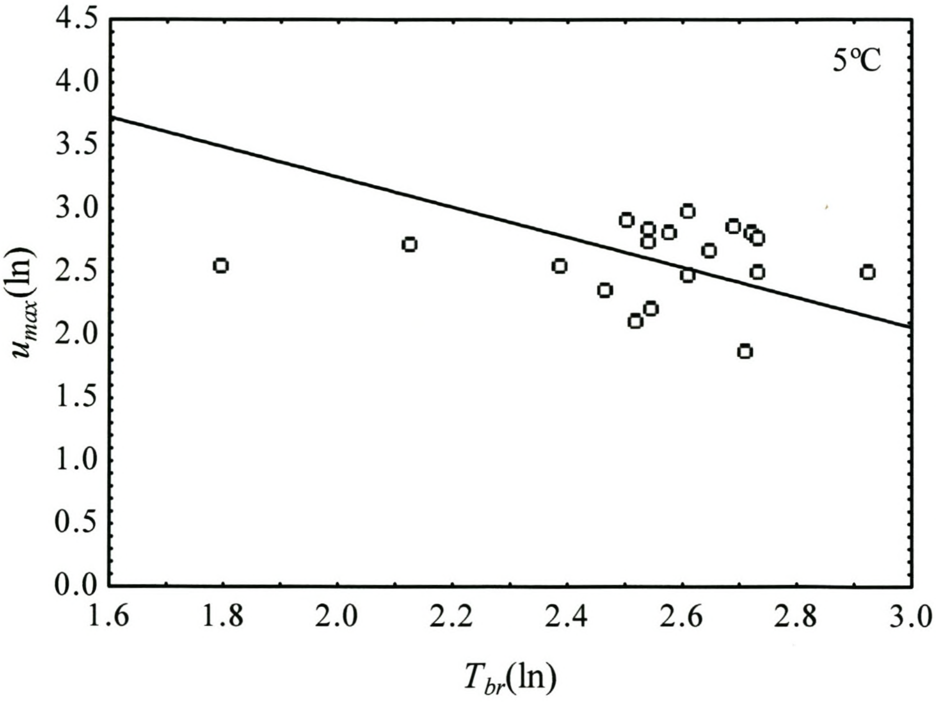
Appendix 1

Reduced major axis regressions

A


0℃
u_{max}(ln)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
T_{br}(ln)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u_{max}(ln)
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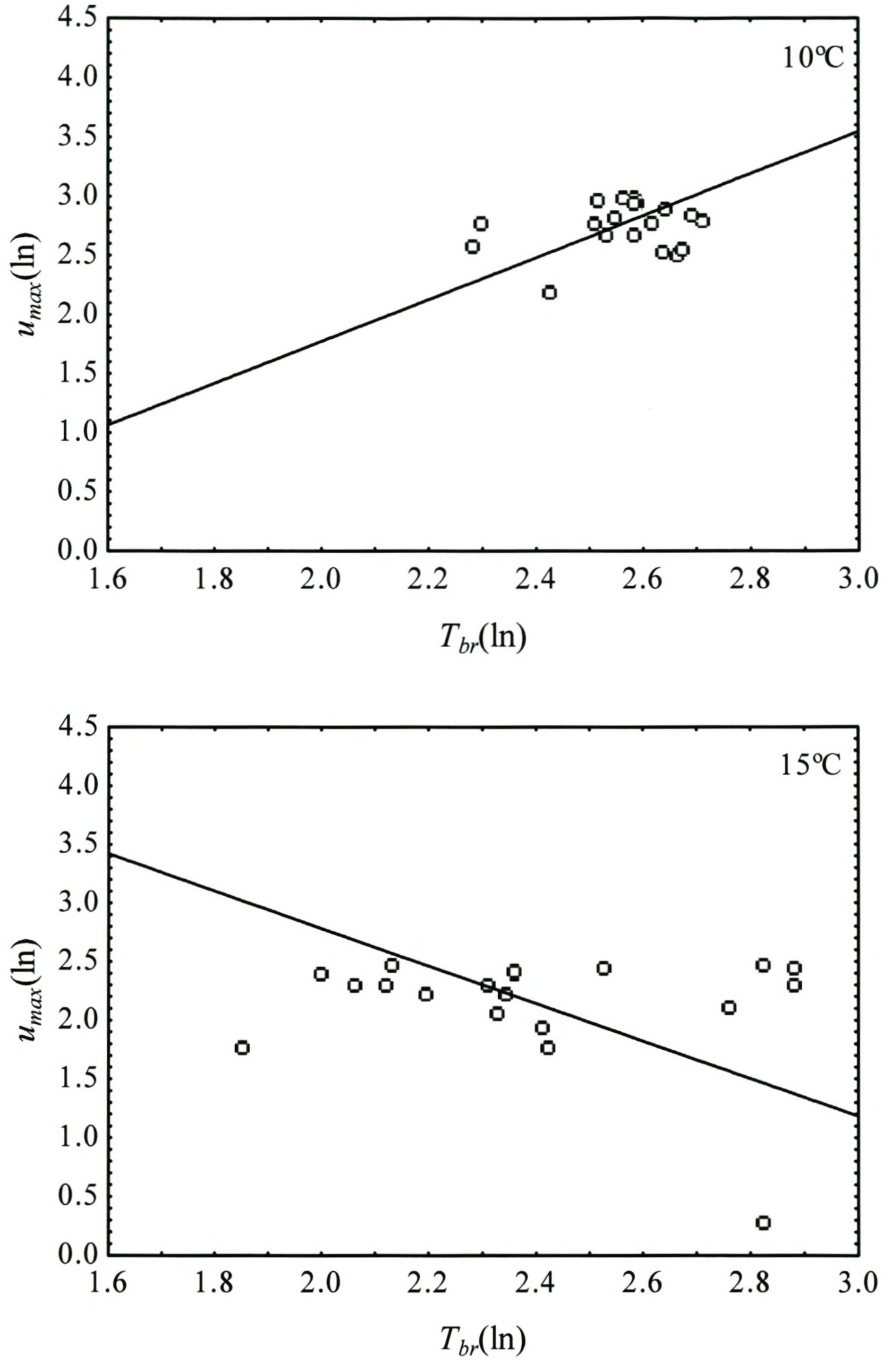
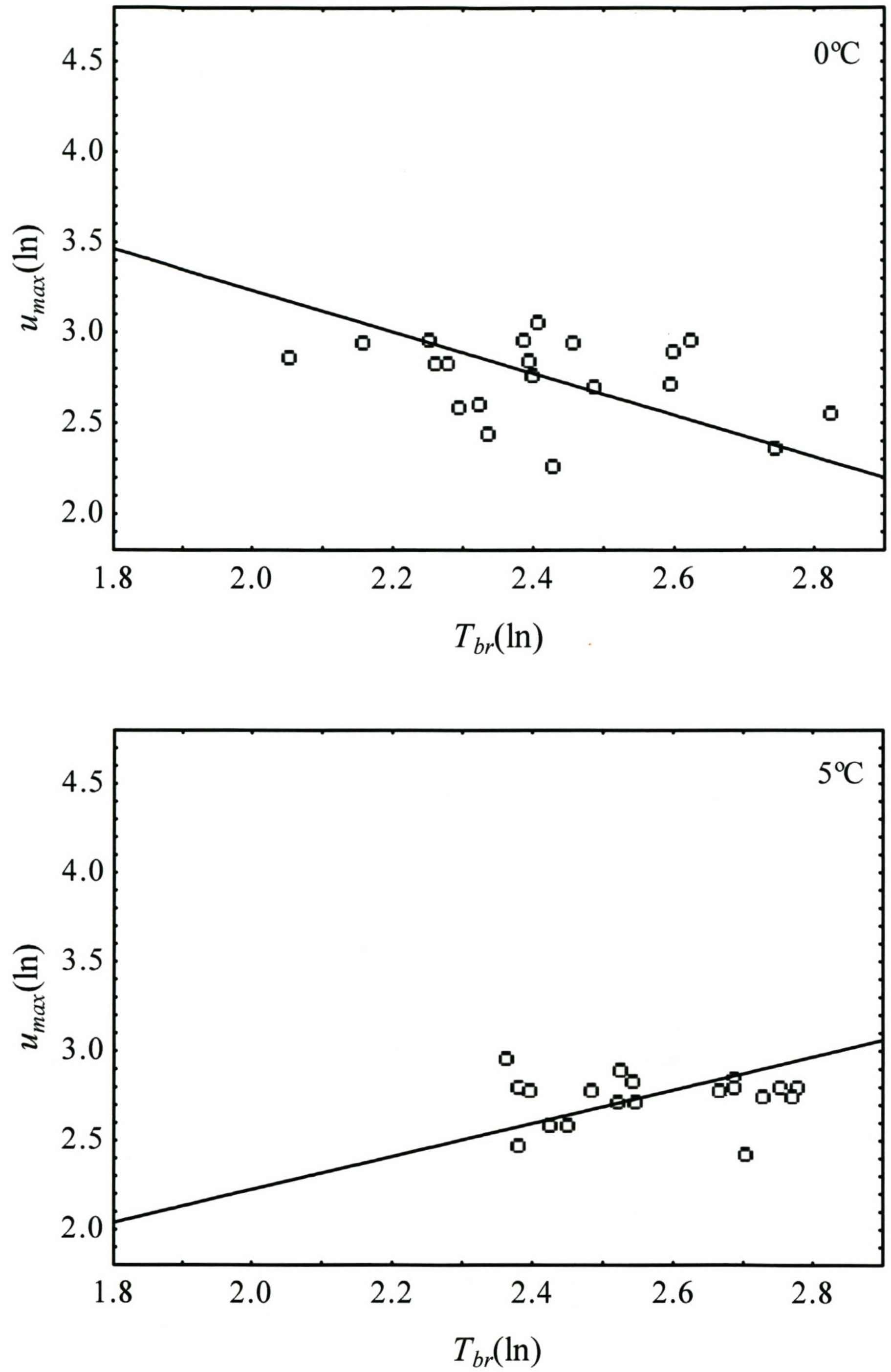
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
T_{br}(ln)



Appendix 1A Phenotypic performance breadth (T_{br}) versus optimum speed (u_{max}) at 0°C, 5°C, 10°C and 15°C acclimation temperatures in *Halozetes marinus*.

B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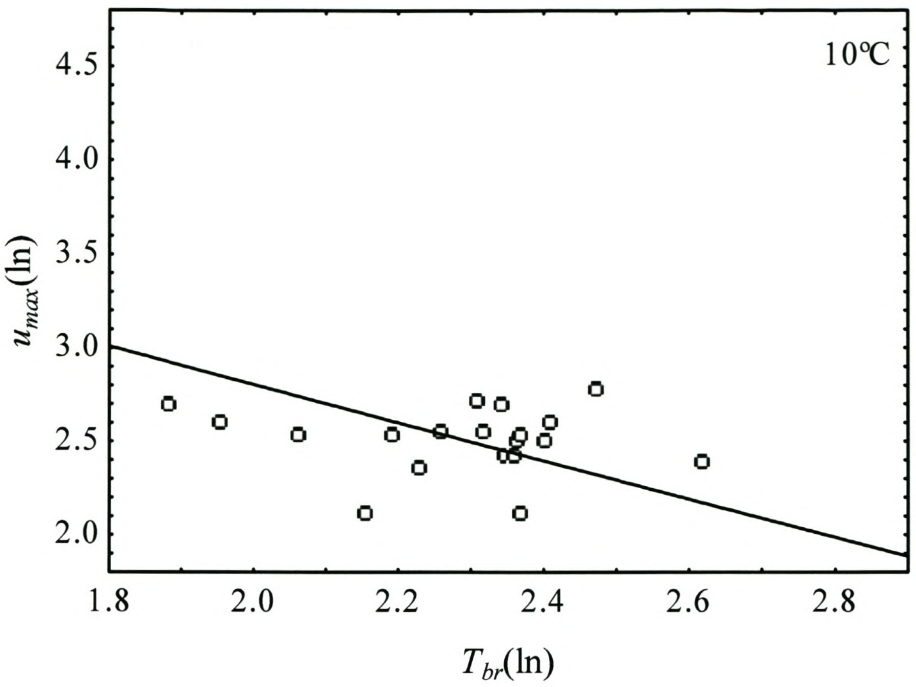
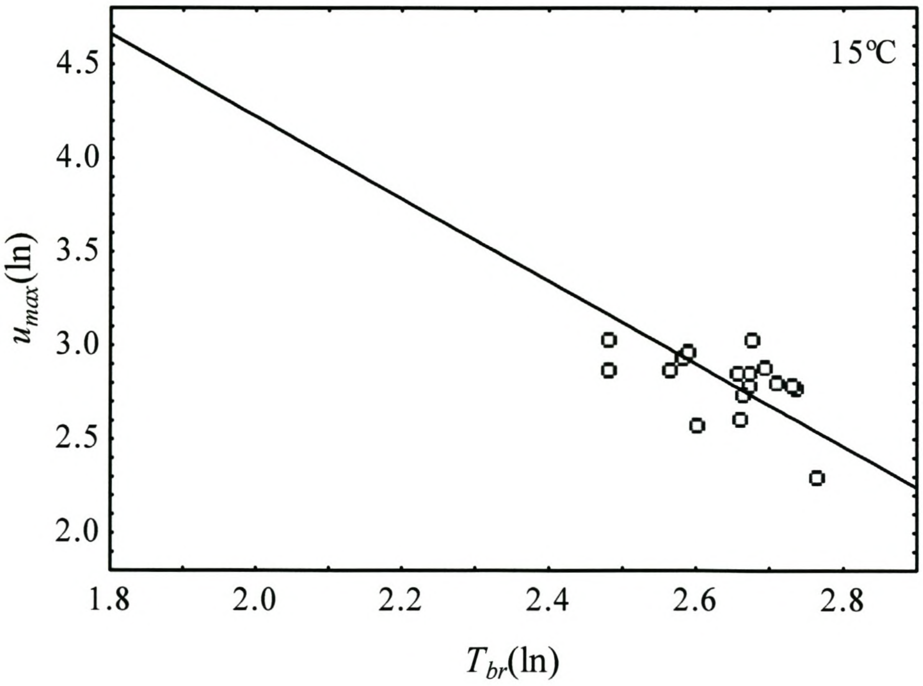
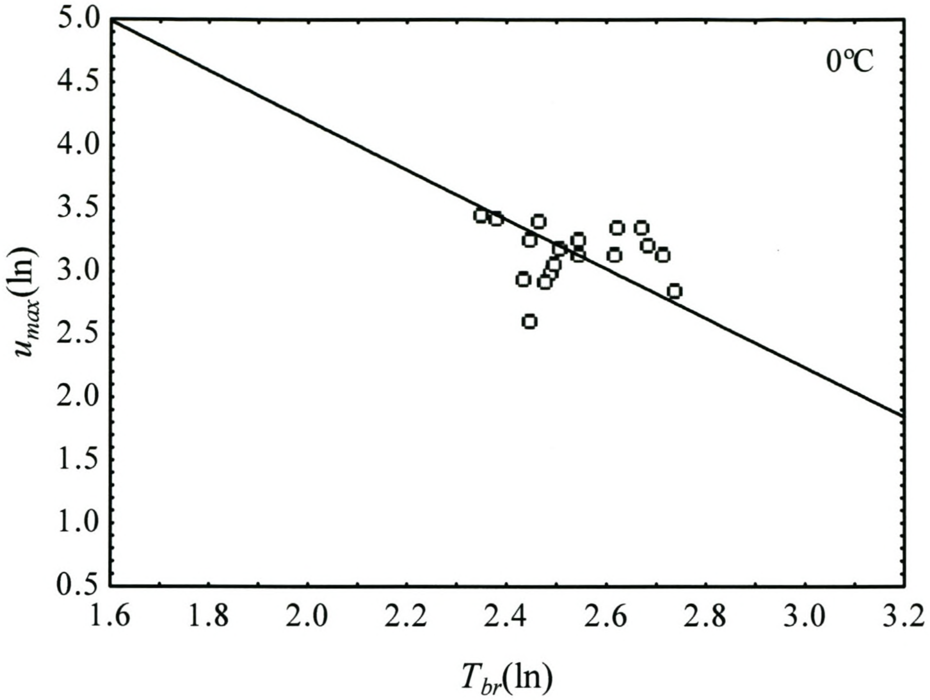
4.0
3.5
3.0
2.5
2.0
u_{max}(ln)
1.8
2.0
2.2
2.4
2.6
2.8
T_{br}(ln)
5ºC
4.5
4.0
3.5
3.0
2.5
2.0
u_{max}(ln)
1.8
2.0
2.2
2.4
2.6
2.8
T_{br}(ln)





Appendix 1B Phenotypic performance breadth (T_{br}) versus optimum speed (u_{max}) at 0°C, 5°C, 10°C and 15°C acclimation temperatures in *H. marionensis*.

C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4.5
4.0
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3.0
2.5
2.0
1.5
1.0
0.5
u_{max}(ln)
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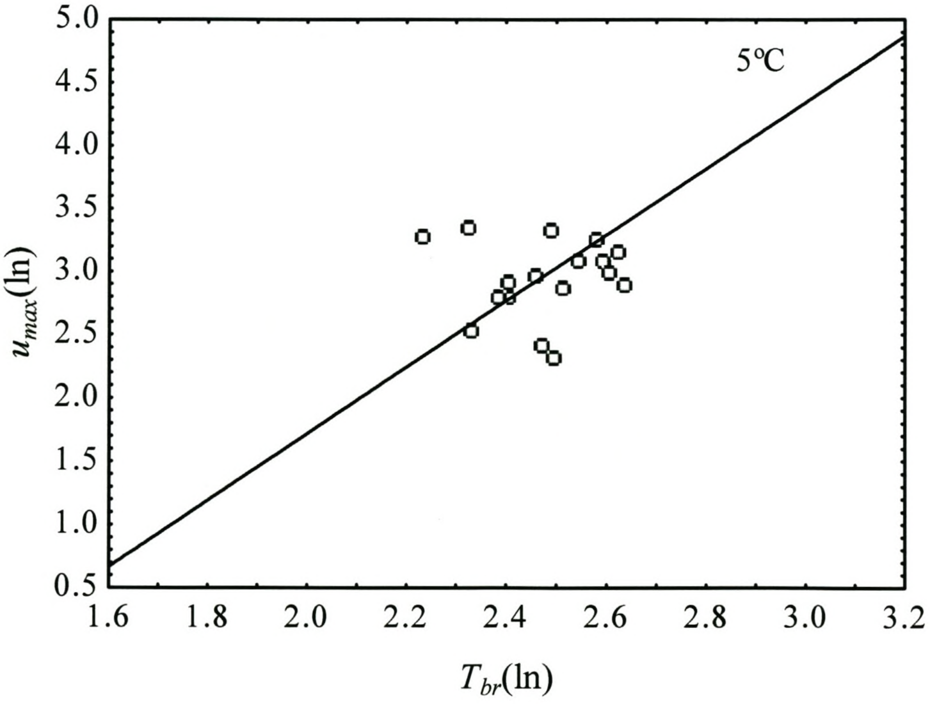
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
T_{br}(ln)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0.5
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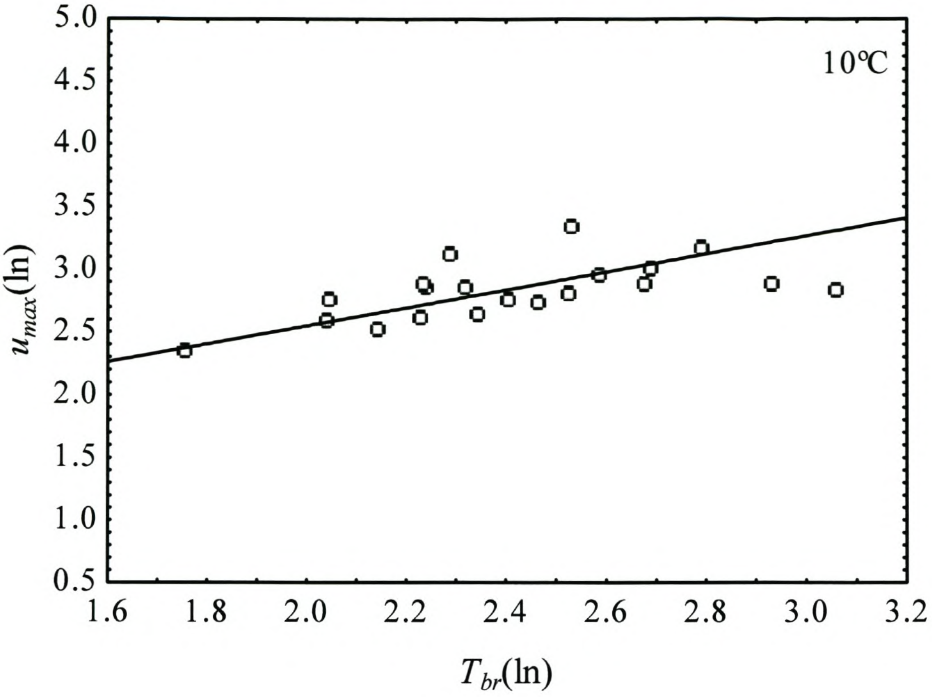
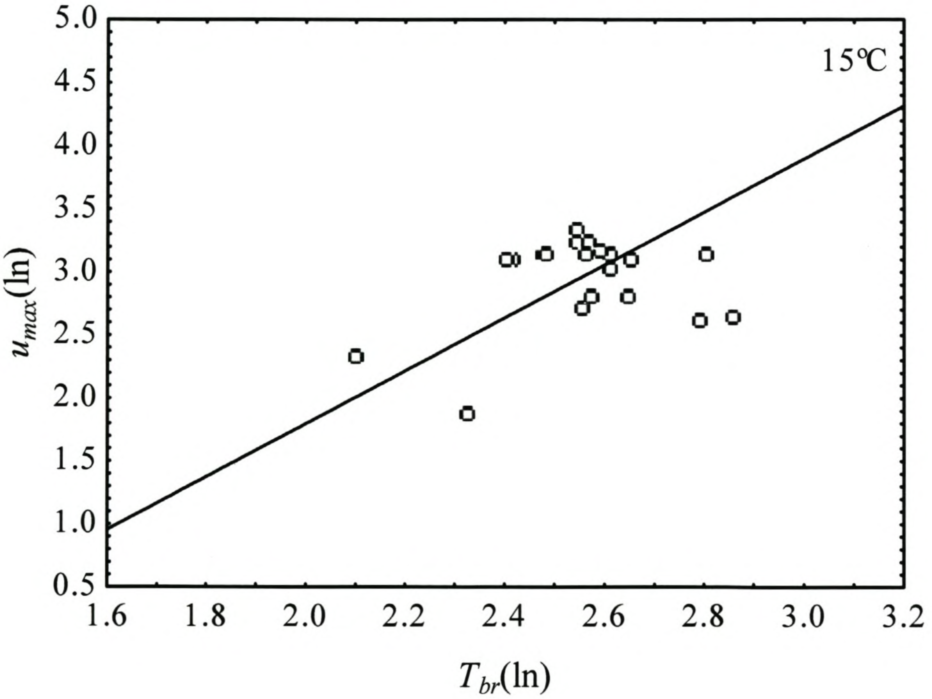
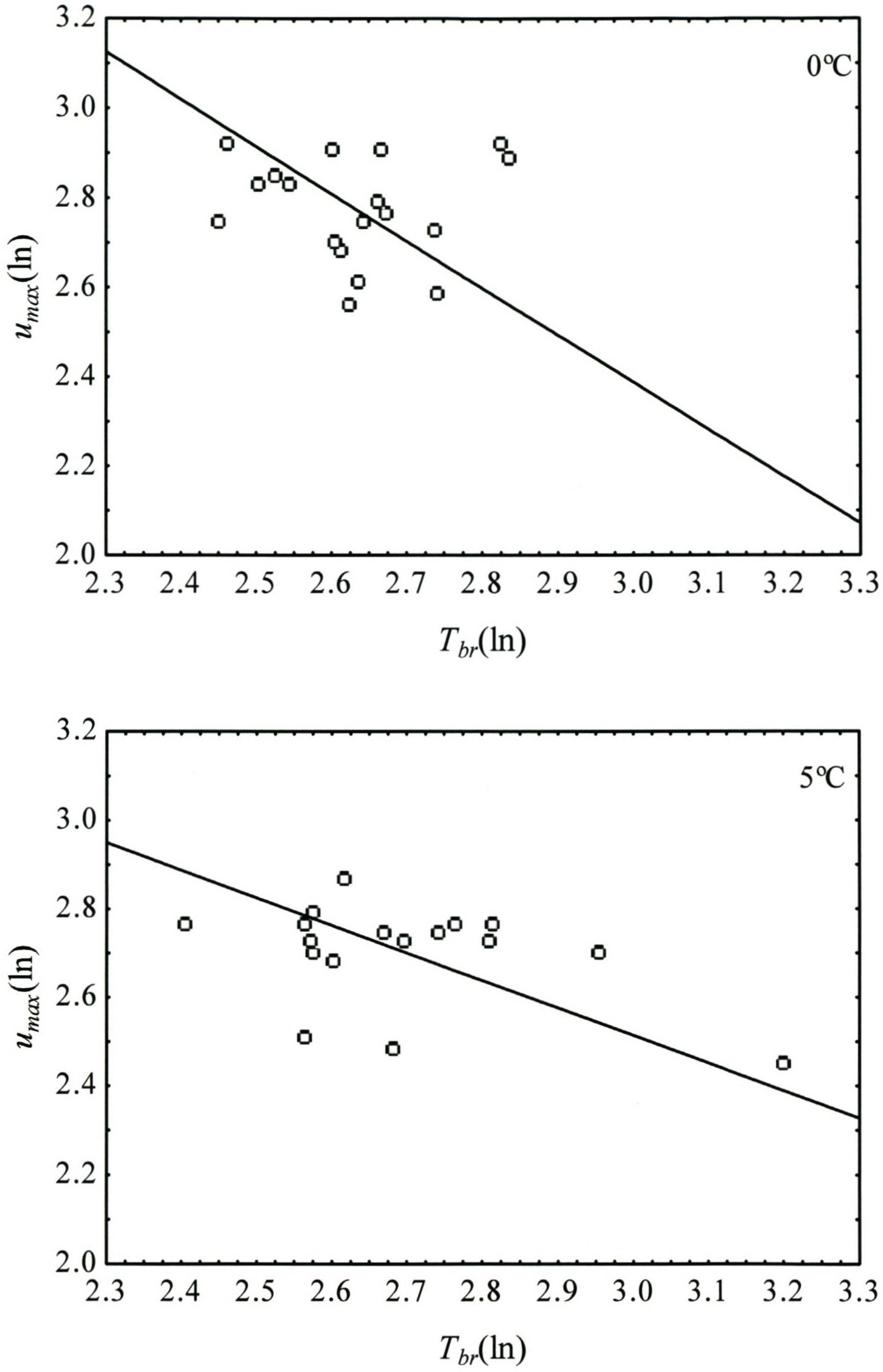
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
T_{br}(ln)





Appendix 1C Phenotypic performance breadth (T_{br}) versus optimum speed (u_{max}) at 0°C, 5°C, 10°C and 15°C acclimation temperatures in *H. belgicae*.

D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2.8
2.6
2.4
2.2
2.0
u_{max}(ln)
2.3
2.4
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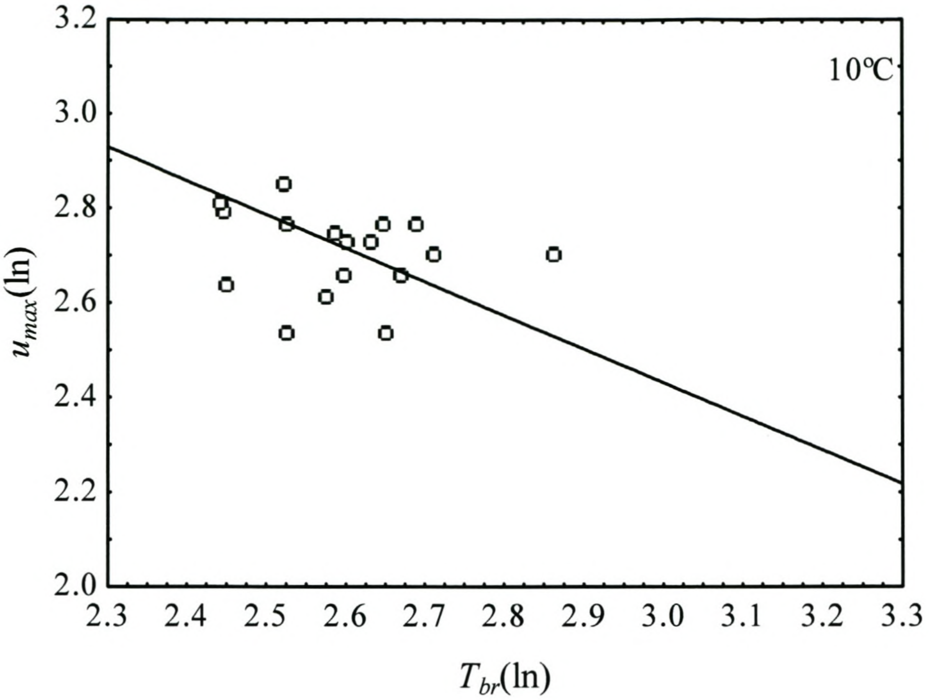
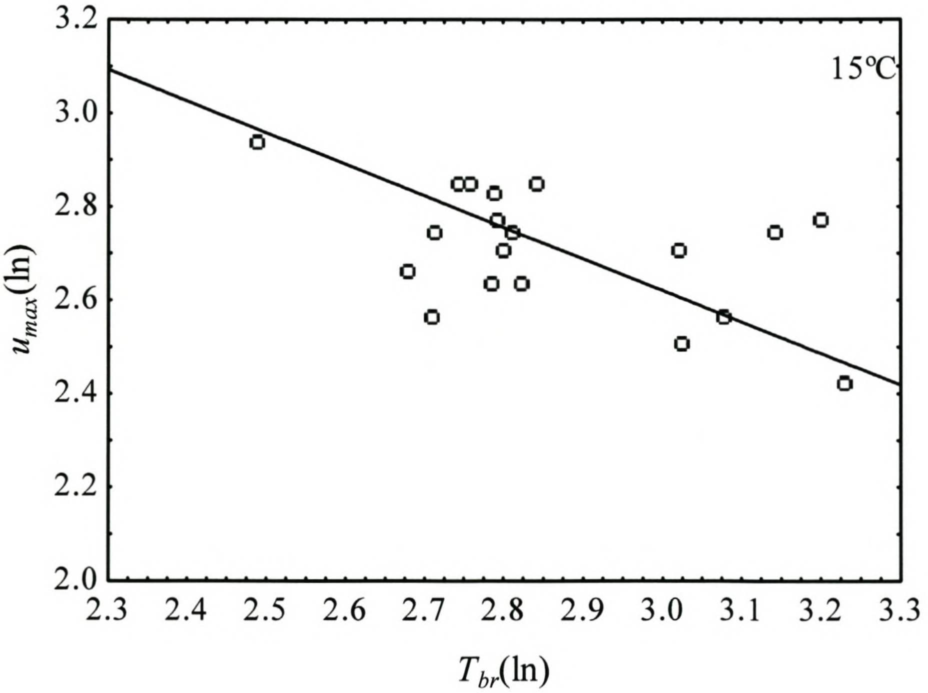
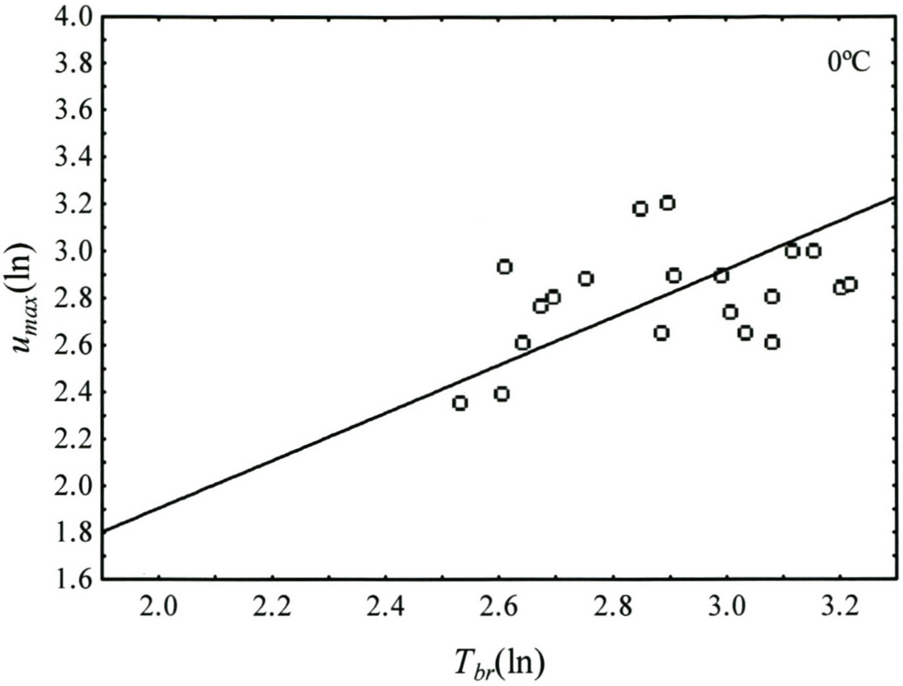
2.6
2.7
2.8
2.9
3.0
3.1
3.2
3.3
T_{br}(ln)
5ºC
3.2
3.0
2.8
2.6
2.4
2.2
2.0
u_{max}(ln)
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0
3.1
3.2
3.3
T_{br}(ln)





Appendix 1D Phenotypic performance breadth (T_{br}) versus optimum speed (u_{max}) at 0°C, 5°C, 10°C and 15°C acclimation temperatures in *H. fulvus*.

E


0ºC
u_{max}(ln)
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2.0
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1.6
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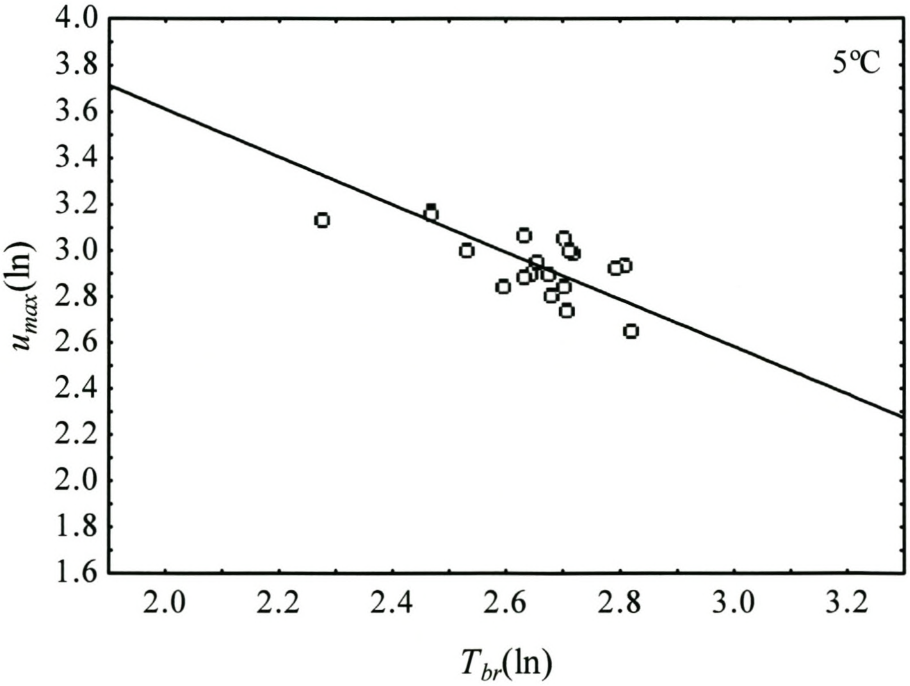
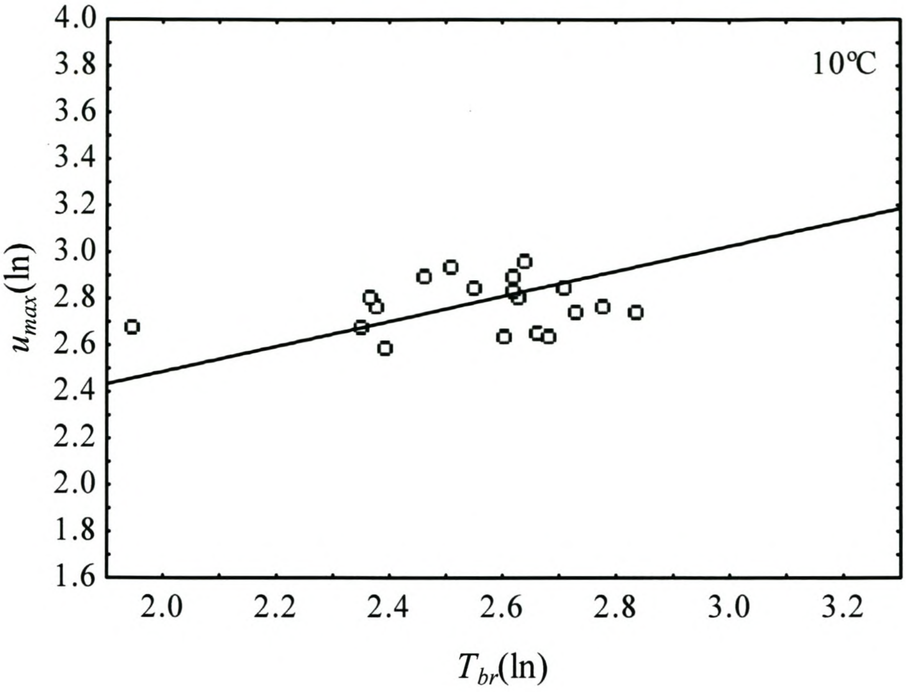
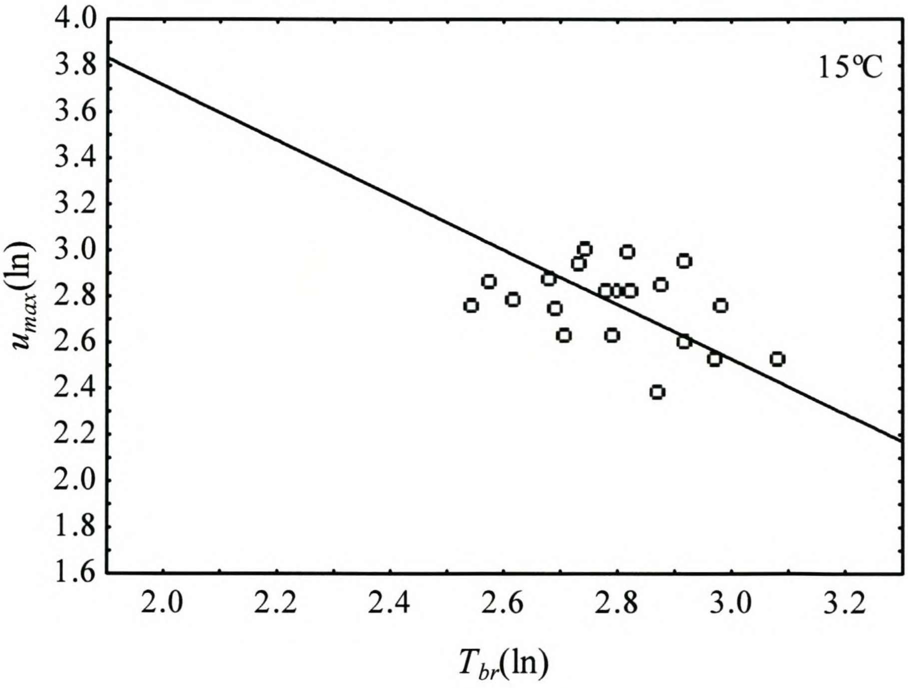
2.4
2.6
2.8
3.0
3.2
T_{br}(ln)


5ºC
u_{max}(ln)
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
2.0
2.2
2.4
2.6
2.8
3.0
3.2
T_{br}(ln)





Appendix 1E Phenotypic performance breadth (T_{br}) versus optimum speed (u_{max}) at 0°C, 5°C, 10°C and 15°C acclimation temperatures in *Podacarus auberti*.

Appendix 2

Microhabitat temperature tables

Appendix 2A Monthly microhabitat temperature (°C) for two altitudinal sites, 0m and 200m a.s.l., on Marion Island collected over a two year period. Mean minimum (Mean min) and maximum (Meanmax) temperatures, absolute minimum (Abs min) and maximum (Abs max) temperatures, temperature range at each altitudinal site, and standard deviation (SD) and standard error (SE) are given (Data logged at hourly intervals).

Year	Month	Mean (°C)	Abs. Min	Abs. Max	Mean Min	Mean Max	Range	SD	SE
0m									
2002	May	5.3	1.5	11	3.2	7.6	9.5	2.2	0.10
	June	3.4	0.5	7.5	1.6	5.2	7	1.9	0.07
	July	3.8	0.5	8	2.5	5.3	7.5	2.1	0.08
	August	4.7	1	9	3.5	6.0	8	2.0	0.07
	September	4.0	0.5	10	2.8	5.6	9.5	2.1	0.08
	October	6.1	2	11.5	4.7	7.8	9.5	1.7	0.06
	November	6.7	2	11	5.4	8.1	9	1.9	0.07
	December	8.7	3.5	22.5	6.3	11.4	19	3.6	0.13
2003	January	10.6	3.5	22	7.5	14.3	18.5	3.2	0.12
	February	9.6	5	17	7.4	12.6	12	2.3	0.09
	March	9.5	5	16.5	7.4	12.1	11.5	2.3	0.10
	April	-	-	-	-	-	-	-	-
	May	9.1	3.5	18.5	4.4	5.7	15	4.1	0.22
	June	4.2	1.5	7.5	3.1	5.2	6	1.4	0.05
	July	3.4	1	6	2.6	4.2	5	1.2	0.04
	August	4.2	2	6.5	3.5	4.9	4.5	1.3	0.05
	September	4.8	2.5	7.5	3.9	5.7	5	1.3	0.05
	October	5.8	2	16.5	4.1	8.3	14.5	2.2	0.08

Appendix 2A cont.

Year	Month	Mean (°C)	Abs. Min	Abs. Max	Mean Min	Mean Max	Range	SD	SE
	November	7.7	2.5	19	5.3	11.5	16.5	2.4	0.09
	December	8.2	4	16	6.7	10.0	12	2.0	0.07
2004	January	10.4	6	18.5	8.5	12.9	12.5	2.1	0.08
	February	10.7	7.5	15.5	9.2	12.4	8	1.4	0.05
	March	9.1	6.5	14.5	8.0	10.1	8	1.4	0.05
	April	8.1	4.5	11	7.0	8.5	6.5	1.5	0.06
200m									
2002	May	4.6	1.5	9.0	3.5	5.7	7.5	1.7	0.08
	June	3.0	0.5	7.0	2.1	4.2	6.5	1.5	0.05
	July	2.9	0.0	7.5	2.1	3.9	7.5	2.0	0.07
	August	4.1	-1.0	13.5	2.2	6.6	14.5	3.0	0.11
	September	2.8	-2.5	18.5	0.0	7.1	21.0	3.5	0.13
	October	5.1	0.0	12.5	2.7	8.1	12.5	2.7	0.10
	November	5.8	-3.5	15.5	2.7	9.4	19.0	3.4	0.13
	December	6.7	-5.0	16.0	2.4	11.1	21.0	3.8	0.14
200m									
2003	January	7.7	-6.0	15.0	4.3	11.3	21.0	3.2	0.12
	February	8.6	2.0	14.0	6.5	10.9	12.0	2.4	0.09
	March	8.0	1.5	14.5	5.8	10.0	13.0	2.5	0.11
	April	-	-	-	-	-	-	-	-
	May	4.7	0.5	19.0	1.9	3.1	18.5	1.8	0.10
	June	3.3	1.0	7.5	2.9	3.9	6.5	1.3	0.05

Appendix 2A cont.

Year	Month	Mean (°C)	Abs. Min	Abs. Max	Mean Min	Mean Max	Range	SD	SE
	July	2.6	1.0	7.0	2.2	3.1	6.0	1.1	0.04
	August	3.0	0.5	6.5	2.4	3.7	6.0	1.5	0.1
	September	3.4	0.5	7.5	2.7	4.1	7.0	1.6	0.06
	October	4.1	0.5	13.0	2.5	6.0	12.5	2.3	0.08
	November	5.1	0.5	12.0	3.0	7.7	11.5	2.2	0.08
	December	6.3	1.0	13.5	4.0	8.6	12.5	2.6	0.10
2004	January	7.5	2.0	16.0	5.2	9.8	14.0	2.8	0.10
	February	8.1	3.0	14.0	5.8	10.0	11.0	2.2	0.08
	March	7.0	1.5	12.5	4.5	9.3	11.0	2.4	0.09
	April	6.8	0.0	16.0	4.5	9.1	16.0	3.4	0.13

Appendix 2B Monthly sea surface temperature (ºC) for Marion Island over a two year period; mean, minimum (min) and maximum (max) temperatures, temperature range, standard deviation (SD) and standard error (SE) are reported.

Year	Month	Mean (ºC)	Min	Max	Range	SD	S.E.
2002	April	7.5	6.8	8.5	1.7	0.45	0.08
	May	7.3	6.2	7.8	1.6	0.45	0.08
	June	5.4	5	7.6	2.6	0.55	0.12
	July	5.2	4.8	5.5	0.7	0.21	0.04
	August	5.1	4.8	5.4	0.6	0.17	0.03
	September	4.4	3.7	5.4	1.7	0.43	0.08
	October	4.7	4	5.4	1.4	0.36	0.07
	November	4.8	4.2	5.1	0.9	0.25	0.05
	December	4.6	4.2	5	0.8	0.18	0.03
2003	January	5.1	4.4	6.4	2	0.51	0.09
	February	6.3	5.7	6.7	1	0.28	0.05
	March	7.2	6.1	8	1.9	0.54	0.10
	April	6.3	5.6	7.3	1.7	0.40	0.07
	May	5.8	5.4	6.2	0.8	0.23	0.04
	June	6.2	5.8	6.5	0.7	0.18	0.03
	July	5.1	4.5	6	1.5	0.39	0.07
	August	4.8	4.1	5.7	1.6	0.34	0.06
	September	4.1	3.7	4.4	0.7	0.19	0.03
	October	4.4	3.8	5	1.2	0.43	0.08
	November	4.2	3.8	4.6	0.8	0.18	0.03
	December	4.9	4	5.7	1.7	0.58	0.10

Appendix 2B cont.

Year	Month	Mean (ºC)	Min	Max	Range	SD	S.E.
2004	January	6.4	5.5	7.4	1.9	0.47	0.08
	February	7.0	6	7.3	1.3	0.25	0.05
	March	7.0	6.5	7.4	0.9	0.24	0.04
	April	6.6	5.3	7.2	1.9	0.47	0.09
	May	5.9	5.3	6.6	1.3	0.40	0.07
	June	6.4	5.8	6.9	1.1	0.28	0.05
	July	6.1	5.6	6.3	0.7	0.19	0.03
	August	5.7	5.4	6.1	0.7	0.17	0.03
	September	5.8	5.2	6.2	1	0.32	0.06