The Effect of Acute and Chronic Elevation of Temperature on Aspects of the Physiology of Antarctic Nototheniid Fishes

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"Temperature is one facet in the mosaic of physical and biotic factors that describes the niche of an animal.
Of the physical factors it is ecologically the most important, for it is a factor that is all-pervasive"

Cossins and Bowler
(Temperature Biology of Animals 1987)

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The notothenioid fishes, which dominate the upper shelf habitats of the Antarctic Continental Shelf, have evolved in a relatively thermally stable environment for at least 10-15 million years. With the upper lethal limits of these fishes around 4-6°C and the lower limits set by the freezing point of seawater, they are described as extreme stenotherms. As a result, it has been hypothesized that these fishes should exhibit marked responses to acute changes in temperature, but that acclimatory effects may be reduced with prolonged exposure to elevated temperatures is not likely to increase their thermal tolerance.

This study was designed to investigate aspects of the physiological response of Antarctic nototheniid fishes to both acute and prolonged increases in temperature. Changes in haematology, metabolic scope for activity, cardiovascular performance, aerobic swimming ability and the capacity to tolerate additional stressors were investigated in the Antarctic nototheniid *Pagothenia borchgrevinki*, with some comparative work carried out on the closely related *Trematomus bernacchii* and the temperate-water notothenioids *Notothenia angustata* and *Bovichtus variegatus*. The responses to an acute change in temperature were found to vary between the closely-related Antarctic nototheniids, most likely as a consequence of their differing ecotypes. Plasma glucose levels were measured in these fish for the first time and exhibited a delayed rise in response to an acute increase in temperature. Cardiac performance was found to be closely linked to prolonged swimming ability through thermal changes in *P. borchgrevinki*, indicating a cardiac limitation of aerobic performance. One factor which became apparent was that the acclimation of Antarctic nototheniids to temperatures only ~2°C above their habitat temperature results in a decrease in thermal sensitivity of a variety of physiological parameters. The most notable finding was that *P. borchgrevinki* possesses sufficient phenotypic plasticity to warm-acclimate prolonged swimming ability, cardiac performance and osmo-regulatory capacity after 4-6 weeks of exposure to 4°C, suggesting that the Antarctic nototheniids may not be as extremely stenothermal as previously assumed and that the consequences of climate change may not be as dire as has been predicted.

Chapter One

General Introduction

ANTARCTICA

Five hundred and ninety million years ago, the Antarctic continent was central to the supercontinent Gondwanaland, along with the present-day continents of Africa, India, Australia, New Zealand and South America. This supercontinent remained intact for some 375 million years before continental drift caused the various continents to separate and drift apart. Isolation of the Antarctic continent was finally achieved about 25 million years ago by formation of the Drake Passage between South America and West Antarctica (Kennett et al. 1983), a separation which established the Antarctic Convergence or Antarctic Polar Front - a well-defined, almost circular, oceanic front located between 50 and 60°S where cold, dense Antarctic surface waters sink beneath the warmer sub-Antarctic waters (Lutjeharms 1990) (Figure 1.1). The north-south flow of water was thereby impeded, and the resulting reduction of heat exchange between Antarctic oceans and warmer northern waters contributed to the gradual cooling of the Antarctic environment (Eastman 1993). The Antarctic Convergence is currently one of the most characteristic hydrographic features of the Southern Ocean, and the dramatic localised 3-4°C change in surface temperature associated with its presence interrupts the gradual rate of increase occurring in a northerly direction (Lutjeharms 1990). The Convergence forms a barrier which affects the distribution of phytoplankton, zooplankton, fish and birds. South of the Antarctic Convergence, the Southern Ocean can be divided into two concentric water masses; close to the continent the currents flow in a counter-clockwise direction, while further north the water masses circulate in a clockwise direction under the influence of the prevailing westerly winds forming the world's most

powerful current (Griffies 2003). Superimposed on these circumpolar movements is a tendency for surface water to drift towards the north.

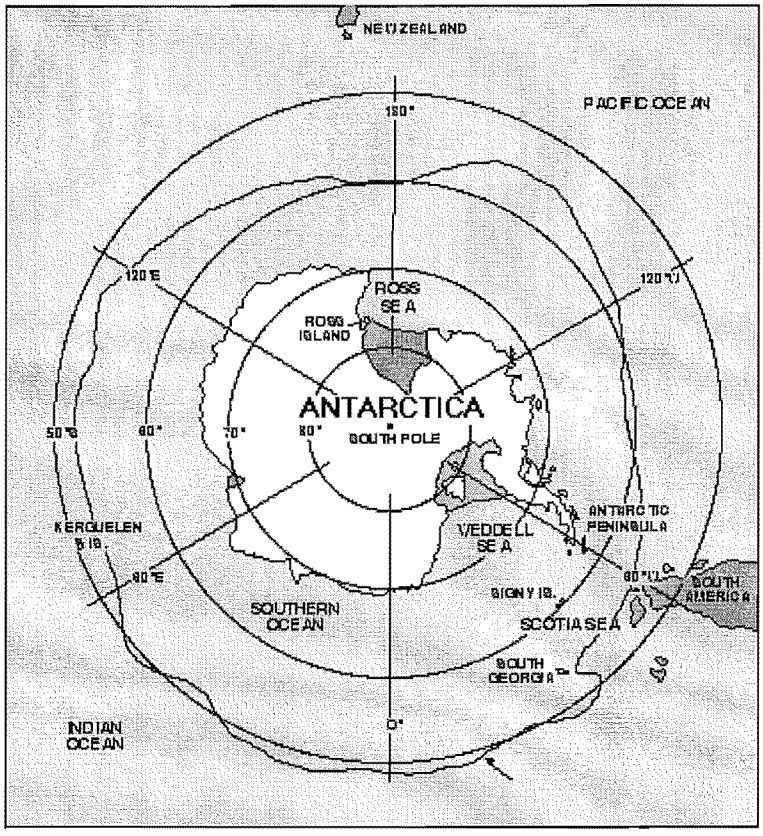


Fig. 1.1. Map of the Antarctic Continent and Southern Ocean. Anonymous World-Wide-Web Antarctic Teaching Resource. Arrowhead marks the location of the Antarctic Convergence.

Following the isolation of Antarctica, the decrease in temperature to the current extremely low levels has been relatively steady, although periods of slight warming and occasional steeper declines have been identified (Kennett 1982, see Clarke and Johnston 1996). Milankovitch cycles (related to variations in the orbit of the earth) have contributed to these fluctuations in the rate of thermal change (Crame 1993). Conditions and climate on the Antarctic Continental Shelf are thought to have become polar with the formation of sea ice some 14-12 million years ago (Kennett 1982). Extensive ice sheets have, however, probably only existed periodically over this time (Kennett 1977; Kennett 1982), and it is thought that as recently as about 3 million years ago relatively warm bodies of water (2-6°C) extended into East Antarctica (Eastman 1991). The current

period of cooling, with its associated ice sheet extension, is thought to have commenced around 2.5 million years ago (Ekau 1991), although even during the past 50 000 years there have been periods when sea ice cover and shelf thickness have been considerably less than at present (Lorius et al. 1985). The Antarctic continent today has the distinction of being the world's coldest (Fortuin and Oerlemans 1990), windiest, highest and most isolated. The continent is covered by 90% of the world's ice at an average thickness of about 2000 metres (Lutjeharms 1990), with the result that most of the sun's heat is reflected back into the atmosphere (Van den Broeke 2000).

The present-day Antarctic Continental Shelf is the product of repeated glacial erosion and is very deep and narrow, compared with shelves of other continents, with a rugged topography (Anderson 1991; Vacchi et al. 1999). Although shelves represent only about 7% of the ocean's surface and 0.2% of the ocean's volume, they are sites of high biodiversity (Eastman and McCune 2000). The Antarctic Continental Shelf is 400 - 600 m deep, some eight times the world average, and has inner shelf depressions over 1200 m in depth (Andriashev 1965; Anderson 1999). This feature has resulted in species diversity among the modern fish fauna being greatest at depths of 300 - 600 m, rather than 100 - 200 m as occurs on temperate continental shelves, a pattern described as glacial submergence (Andriashev 1987). The majority of the Southern Ocean has a depth of 3000 - 5000 m, and with the archipelagos which occur in other environments having been eliminated by glacial action (Vacchi et al. 1999), the Antarctic Continental Shelf is geographically isolated.

McMurdo Sound is an embayment between Ross Island and the Antarctic mainland in the Ross Sea. The entrance to the sound is 72 km wide and about 81 km from the Ross Ice Shelf, and the sound is over 1000 m deep. The waters of the sound are generally covered by 2 to 3 metres of annual sea ice for about 10 months of the year (Littlepage 1965; Jacobs and Giulivi 1999) and are among the coldest and iciest in the world, due to both the extremely high latitude (78°S) and the immediate proximity to the Ross Ice Shelf which is a source of freezing shelf water (Jacobs et al. 1979). The upper 50 m of the water column is ice-laden during winter and spring with several metres of platelet ice adherent to the underside of the sea ice (Littlepage 1965) and the bottom of the sound covered with anchor ice to a depth of 30 metres (Dayton et al. 1969). The temperature of the seawater in McMurdo Sound is therefore maintained at or very close to its freezing point, averaging -1.86°C. Seasonal thermal variation close to the ice shelf was originally thought to be less than 0.2°C (Littlepage 1965), but recently published

results, following two years of continuous high resolution temperature and pressure recording at near-shore shallow-water sites in McMurdo Sound, have revealed substantial warming of the seawater during January and February with temperatures reaching above -0.5°C. The amplitude of change in temperature over the two-year period from 1999 - 2001 was 1.57°C, from -1.92 to -0.35°C (Hunt et al. 2003), which still represents an extremely low degree of annual variation compared with coastal seawater temperatures in other parts of the world. Due to the presence of a large iceberg at the entrance to McMurdo Sound throughout the duration of this study, the reduction in 'annual' sea ice over the two summer periods that fieldwork was carried out was restricted and the study site remained covered with at least three metres of ice for the entire two year period.

In contrast to temperature, both photoperiod and productivity exhibit marked seasonal fluctuation in McMurdo Sound. There are four-month periods of continual darkness in winter and continual daylight in summer, separated by two month transition periods in which there is a rapid daily change in daylight hours (Rivkin and Putt 1987). The light reaching the marine environment is also attenuated by snow, ice, and sea ice microbial communities. At midday during the austral summer, the under-surface of the ~2.5 metre-thick sea ice in McMurdo Sound receives on average 0.1 to 0.16% of the light incident on the ice surface (Littlepage 1965; Schwartz et al. 2003). These Antarctic light cycles impose strong seasonality on primary production and cause a marked seasonal oscillation in the food supply. There is strong evidence that light is the primary driving factor for the growth and production of high latitude macroalgae (Miller and Pearse 1991; Schwartz et al. 2003) and it has therefore been suggested that the presence of ice (Eastman 1993) and seasonality of resources (Clarke and North 1991; Hubold 1991; Vacchi et al. 1999), rather than temperature, may have presented the most significant evolutionary challenges to Antarctic marine organisms. The effect of this seasonality varies with the position of an organism in the food web, with carnivores being slightly more insulated from its influence than herbivores (Clarke 1988). Salinity also fluctuates throughout the year in McMurdo Sound, with a decrease in salinity during summer due to melting sea ice, and an increase in winter as the sea ice forms (Littlepage 1965; Knox 1994). The oxygen tension of the water, however, is continually high as the result of enhanced solubility at sub-zero temperatures. Oxygen tensions even increase above air-saturation during the summer months (Littlepage 1965) due to extensive photosynthesizing phytoplankton blooms (Knox 1994).

The present-day Antarctic continental marine environment is therefore characterised by decreased availability of shelf habitats, the presence of sea ice, large seasonal fluctuations in primary production, isolation both hydrographically and geographically, and temperatures close to the freezing point of seawater. The Antarctic shelf has been described as a continental island (Eastman 2000), and the modern coastal Antarctic fish fauna have evolved in an isolated and unique environment (Kennett 1977).

NOTOTHENIOIDEI

Prior to the fragmentation of the supercontinent Gondwanaland, the Southern Ocean had a relatively temperate climate with water temperatures of 12-15°C and the fish fauna contained representatives of many of the genera found in temperate waters today, such as gadoids and salmonids (Clarke and Johnston 1996). The current fish fauna, however, differs markedly from both the fossil Antarctic ichthyofauna, and from the modern fish fauna of other southern continents (Eastman 1993). The relationship of the notothenioid suborder to other species is obscure, due to the paucity of the fossil record over the past 40 million years (Eastman 1993), although molecular phylogenetics has recently identified the Percidae (perches) as the most likely sister group for the notothenioids (Chen et al. 2003; Dettai and Lecointre 2004), with the larger clade including the Serranidae (sea basses), the genera *Trachinus* (weeverfish), *Chelidonichthys* (gurnard), *Scorpaena* (scorpionfish), and a group comprising the Zoarcoidei (eelpouts), Cottoidei (sculpins), and Gasterosteidae (sticklebacks). In contrast to the populations of other continental shelves, the fauna of the Antarctic continental shelf displays a very high percentage of endemism in different taxonomic groups. Eighty eight percent of the fish species and 76% of the genera are endemic to the Southern Ocean (Andriashev 1987). The current benthic fish fauna of the shelf and upper slope of the Antarctic region includes 213 identified species (Eastman 2000). Ninety six are of the Perciform suborder Notothenioidae, comprising 45% of the fish fauna. In high latitude (71-78°S) shelf areas these notothenioids dominate abundance and biomass at levels of 90-95% (Eastman and Hubold 1999), and some 97% of the species are endemic (Eastman 2000). Notothenioids are often the only fish caught in shallow coastal waters, and therefore the majority of

Antarctic ichthyological research has been carried out on these species (Clarke and Johnston 1996). The range of morpho-ecological changes exhibited by Antarctic notothenioids has enabled the occupation of a variety of different niches which are generally filled by taxonomically unrelated fish in temperate and tropical oceans (Vacchi et al. 1999; Eastman 2000). This degree of variation in body shape and ecology within closely related species is comparable to the radiation of species within the African and Siberian rift lakes (Eastman 1993; Eastman and McCune 2000) and was facilitated by the thermal isolation of Antarctica, the increasing productivity of the Southern Ocean which began some 22 million years ago (Kennett 1982), and the absence of competition from non-notothenioid species (Eastman 2000).

The notothenioid suborder comprises eight families (Table 1.1): Bovichtidae, Pseudaphritidae, Eleginopidae, Nototheniidae, Harpagiferidae, Artedidraconidae, Bathydraconidae, and Channichthyidae (Eastman 2000).

Table 1.1. Species composition of Notothenioid families

Family	Antarctic Species	Non-Antarctic Species	Total
Bovichtidae	1	9	10
Pseudaphritidae	0	1	1
Eleginopidae	0	1	1
Nototheniidae	33	15	48
Harpagiferidae	6	0	6
Artedidraconidae	25	0	25
Bathydraconidae	16	0	16
Channichthyidae	15	0	15
Totals	96	26	122

From Eastman, 2000.

The bovichtids are thought to have diverged very early in the notothenioid evolution, possibly after the separation of New Zealand from Antarctica about 50 million years ago (Bargelloni et al. 1997), although recent results from fossil-calibrated molecular clock analysis suggest that the separation may have occurred some 125 million years ago (Near 2004). The remainder of the notothenioid taxa is thought to have diversified about 10-15 million years ago, when the Antarctic climate was modified by a drop in water temperature, expansion of the ice sheet, and more consistent sea-ice formation (Kennett 1982; Eastman 1993; Eastman and McCune 2000; Poulin et al. 2002). The molecular clock analysis of Near (2004) however, once again indicates an earlier date, some 24 million years ago at the time of the development of the Antarctic Circumpolar Current (Kennett 1982).

Most notothenioids are benthic fishes and are confined to water less than 1000 m deep (Dewitt 1971; Eastman and McCune 2000). They are slow growing and long-lived, generally reaching sexual maturity after about 5 years (Knox 1994). The majority of species live, feed and reproduce on the substrate, which is likely to reflect the ancestral niche of notothenioids as a taxon as these fish are thought to have evolved from a sedentary, benthic ancestor that became isolated from temperate waters following the formation of the Drake Passage (Eastman 1991; Eastman 1993). With life in warm waters likely to represent the original evolutionary situation (Arntz et al. 1994), the Antarctic notothenioids have adapted to their cold and stable environment in a variety of ways. The most notable adaptation is the synthesis of antifreeze glycopeptides (De Vries and Eastman 1981; De Vries 1983; De Vries 1988) which depress the freezing point of fluids by a non-colligative mechanism (De Vries 1988; Fletcher et al. 2001). The antifreeze glycopeptides can be classified into eight size-classes ranging in molecular weight from 2600 to 33 700 Da, characterised by the presence of repeating alanine-alanine-threonine sequences (De Vries 1984). Ice crystals enter Antarctic fish in their natural habitat, but these antifreeze molecules are adsorbed onto the crystals and act to inhibit growth, thereby preventing damage to the fish and allowing survival in contact with ice which would otherwise prove lethal. All antifreeze-producing notothenioids examined so far have aglomerular kidneys (De Vries and Eastman 1981; Eastman 1993) and this evolutionary loss of glomeruli is likely to be an energy-saving mechanism, both in terms of removing the need to reabsorb ions from the filtrate, and in preventing the urinary loss of antifreeze glycopeptides (Dobbs et al. 1974; Eastman 1990). The plasma sodium and chloride concentrations of these fish are almost double those of other marine

teleosts (Dobbs and De Vries 1975; O'Grady and DeVries 1982), which is another factor contributing to lowering of the freezing point and may also serve an energy-conservation purpose in reducing the ionic gradient between seawater and plasma and thus the energy required to maintain this gradient.

The blood of Antarctic notothenioids contains fewer erythrocytes and less haemoglobin than that of temperate-water teleosts, reaching its extreme in the family Channichthyidae (icefishes) with the complete absence of functional erythrocytes (Everson and Ralph 1968; Hureau et al. 1977; Wells et al. 1980). This unique feature is thought to reduce the potentially negative physiological effects of increased blood viscosity at subzero temperatures (Hemmingson and Douglas 1970; Macdonald et al. 1987; Wells et al. 1990; di Prisco et al. 1991), and is made possible by the high oxygen solubility at subzero temperatures which allows considerable quantities of oxygen to be carried in physical solution in the plasma (di Prisco and Giardina 1996). Notothenioids also demonstrate a reduced affinity of haemoglobin for oxygen, although whether this is adaptation or just relaxed selection due to the high ambient levels of dissolved oxygen in the Southern Oceans is unknown (Bargelloni et al. 1994).

Some other adaptations of the Antarctic notothenioids to their sub-zero thermal environment include: increased mitochondrial densities (Dunn 1988; Egginton and Sidell 1989; Londraville and Sidell 1990), polymerisation of tubulins at low temperatures (Williams et al. 1985; Detrich 1991; Detrich 1997), enhancement of nerve conduction rates (Macdonald et al. 1988), increased rates of protein synthesis (Smith and Haschemeyer 1980), greater enzyme catalytic activity (Somero 1991), enhanced lipid metabolism (Crockett and Sidell 1990), cold adaptation of muscle contractile elements (Johnston et al. 1975; Franklin and Johnston 1997), increased membrane fluidity at low temperatures (Macdonald et al. 1987; Hochachka 1988), and heat shock protein expression at 5°C (Maresca et al. 1988). A complete lack of heat shock protein expression has even been reported in at least one species, probably due to the lack of positive selection pressure (Hofmann et al. 2000). Heat shock proteins thus appear not essential for life, although organisms lacking the heat-shock response may have an attenuated range of thermal tolerance, and their survival may therefore be jeopardised by habitat warming (Hofmann et al. 2000).

Survival near thermal limits is generally supported by anaerobic metabolism (Pörtner 2002), and the limited anaerobic capacity for metabolism of the notothenioid fishes (Dunn and Johnston 1986; Davison et al. 1988; Davison et al. 1995) may be

another factor contributing to the extreme stenothermality of these species (Hochachka and Somero 2002). Antarctic notothenioids acclimatised to the environmental conditions of McMurdo Sound die of heat death at temperatures above 4-6°C (Somero and De Vries 1967; Somero 1991; Somero et al. 1998; Hofmann et al. 2000). Several hypotheses have been proposed to explain the occurrence of heat death at such low temperatures including: a temperature-induced phase change in lipids of the nervous system (Somero and De Vries 1967), and the accumulation of large amounts of acetylcholine at synapses due to both the K_m and release of acetylcholine in these fish increasing sharply with temperature (Baldwin 1971; Macdonald and Montgomery 1982; Somero 1991). The dysfunction at high temperatures may also be a failure of integration, since individual elements (e.g. isolated enzymes) can function acutely at >30°C.

The evolution of the Antarctic notothenioids at low and relatively stable temperatures for the past ten to twenty million years is thought to have led to the loss of ability to cope with higher temperatures, and it has been suggested that even prolonged acclimation cannot broaden the thermal tolerance of these extremely stenothermal fishes (Eastman 1993; Knox 1994; Somero et al. 1998; Hofmann et al. 2000).

NOTOTHENIIDAE

Within the notothenioids, the nototheniid family is the most diverse with respect to size, body form and distribution, and they therefore form a useful group for studying the effects of ecological differences on basic physiological processes (Macdonald et al. 1987). The nototheniids are thought to have radiated relatively recently, with divergence of the genus *Trematomus* only about 3.4 million years ago (Ritchie et al. 1996). The status of the genus *Pagothenia* is still being debated and it has recently been suggested that it should be re-evaluated and perhaps placed back within *Trematomus* (Tokita et al. 2002). Within the nototheniids, there is a trend toward diversification into habitats within the water column (Eastman and DeVries 1981; Eastman and DeVries 1985; Eastman 1997) known as pelagization (Klingenberg and Ekau 1996). About 50% of Antarctic nototheniid species are described as pelagic, semipelagic, cryopelagic or epibenthic, rather than benthic, and it is thought that high productivity at the ice-water interface

(Hedgpeth 1977) and the lack of competition for water column habitats may have been the stimuli for this move away from benthic habitats (Eastman 1993). Paedomorphosis, or the retention of larval characteristics, is thought to have been one of the primary mechanisms of pelagization in these fishes (Balushkin 2000; Voskoboinikova 2001).

All nototheniids lack a swimbladder, as the result of their benthic ancestry, and are therefore heavier than water. Those species which have diversified into the water column exhibit a variety of adaptations to increase buoyancy including: a reduction in the extent of bone and scale mineralization, substitution of cartilage for bone, and the deposition of large amounts of lipid (Hochachka and Somero 1973; De Vries and Eastman 1978; Clarke et al. 1984; Eastman and DeVries 1985; Eastman 1993). The tendency to accumulate lipids is correlated with activity level, with pelagic fishes accumulating the greatest amounts (Hubold 1985; Sidell et al. 1995). Most other teleosts which utilise lipid for buoyancy store the lipid in hepatocytes and adipose cells, generally in the from of wax esters (Nevenzel and Menon 1980; Neighbors and Nafpaktitis 1982; Phleger and Grigor 1990). Nototheniids, in contrast, store predominately triacylglycerol (Clarke et al. 1984) in large subcutaneous and intramuscular lipid sacs (De Vries and Eastman 1978; Eastman 1993). This may be due either to the greater fluidity of triacylglycerol at low temperatures (Eastman and DeVries 1981), or due to the fact that triacylglycerol is more accessible for metabolism. The cellular nature of the lipid sac walls indicates that both removal and addition of lipid are likely to be possible (Eastman 1993) and, with lipid substrates constituting the primary fuel for aerobic energy metabolism in oxidative muscles of these fish (Sidell et al. 1995), it is feasible that the stored lipid may also serve a metabolic purpose (Sidell and Hazel 2002). Antarctic fish have low myoglobin levels, with the protein not expressed at all in some icefish (Fitch et al. 1984), and it has been suggested that intracellular lipid may also take on the role usually fulfilled by myoglobin in facilitating the diffusion of oxygen through muscle (Egginton and Sidell 1989; Londraville and Sidell 1990). This may be due to the high affinity of myoglobin at low temperatures making it ineffective for facilitated transport (Egginton, pers. comm.).

The trend toward pelagization within the nototheniids has produced phylogenetically closely-related species which are not necessarily morphologically and ecologically similar (Klingenberg and Ekau 1996). The two Antarctic nototheniids studied in this investigation were two such species: *Pagothenia borchgrevinki* (Figure

1.2) which is adapted for life in the water column, and *Trematomus bernacchii* (Figure 1.3) which is of the ancestral benthic ecotype.

Pagothenia borchgrevinki (Boulenger, 1902), also known as the bald notothen, is a cryopelagic species (Andriashev 1970) which is described as a mobile, semi-pelagic predator (Montgomery and Wells 1993). It is found associated with the underside of the ice and has a circum-Antarctic distribution, having been captured around the shores of the Ross Sea, Davis Sea, Weddell Sea, Antarctic Peninsula, and also the South Orkney and South Shetland Islands (Gon and Heemstra 1990), at depths of 0-550 m (Dewitt et al. 1990). *P. borchgrevinki* is a silvery white fish, with a reflective stratum argentum masking the dark organs and iris and providing good camouflage against the background of platelet ice (Eastman and DeVries 1985). Specimens collected from greater depths, rather than directly beneath the ice, are generally darker in colour (Eastman 1990). Although *P. borchgrevinki* is primarily zooplanktivorous (Eastman and DeVries 1985; Foster et al. 1987; Montgomery et al. 1989; La Mesa et al. 2004), individuals have been observed to feed on dead *Pleuragramma* (Janssen et al. 1992). *P. borchgrevinki* is slightly negatively buoyant (Eastman and De Vries 1982) and would therefore be expected to swim constantly to maintain station in the water column (Montgomery and Macdonald 1984). These fish have, however, been observed to utilise crevices and holes within the platelet ice to rest or avoid predation (Andriashev 1970; De Vries 1970; Eastman and DeVries 1985). They have also been observed clinging to the under-surface of the ice shelf in an almost upside down position (Gutt 2002), although they are reported to lack substrate contact adaptations in the pelvic and anal fins (Eastman and DeVries 1985). *P. borchgrevinki* is regarded as a major food source for Weddell seals, emperor penguins, and south polar skua (Mund and Miller 1995; Cherel and Kooyman 1998; Davis et al. 1999; Ponganis et al. 2000), and as such, represents an important link between the zooplankton and the top predators (La Mesa et al. 2004).

P. borchgrevinki attains a maximum total length of about 28 cm (Dewitt et al. 1990) and is morphologically very different from its close benthic relatives. The fitness ratio, along with indices of trunk shape and flatness of this species, suggest streamlining and drag reduction (Eastman 1993), and these fish also possess a greater volume of subcutaneous and intermuscular adipose tissue than the benthic species (Eastman and De Vries 1982; Clarke et al. 1984). Living a lifestyle which is closely associated with the ice, *P. borchgrevinki* has a greater concentration of antifreeze proteins than those nototheniids which are confined to deeper ice-free water (DeVries and Lin 1977; De

Vries 1988). There is also correlation of other physiological parameters with the increased activity level of this species, including a relatively high haemoglobin concentration, haematocrit (Wells et al. 1980), and rate of resting oxygen consumption (Wells 1987). The oxygen transport system of *P. borchgrevinki* has been identified as one of the most specialised among the notothenioids, with haemoglobin multiplicity (di Prisco and Giardina 1996), and a blood oxygen affinity much lower than that of the sedentary nototheniids (Tetens et al. 1984).

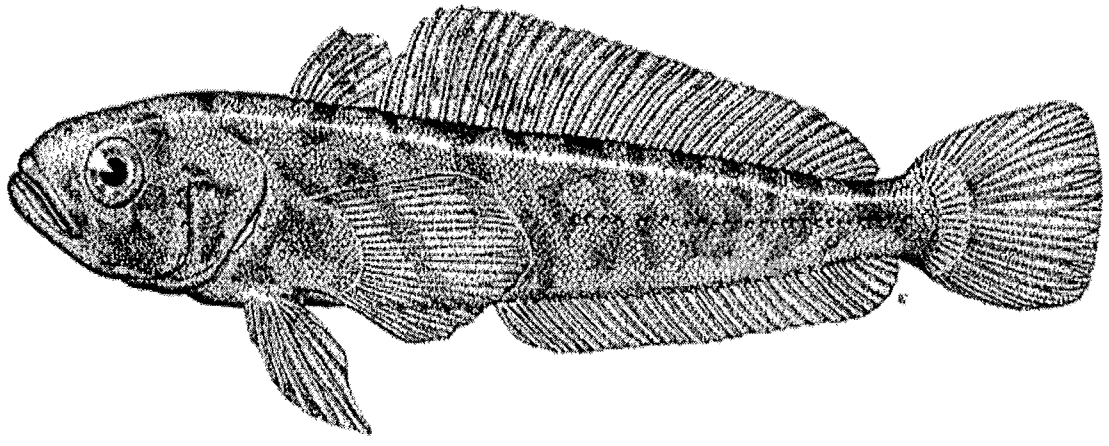


Fig. 1.2. Sketch of the Antarctic nototheniid *Pagothenia borchgrevinki*. Illustration reproduced with permission of the author C. Zimmerman. (www.fishbase.org, 2004)

Trematomus bernacchii (Boulenger, 1902), also known as the emerald rockcod, is a territorial benthic species (Eastman 1990) which has been described as intermittently active (Montgomery and Wells 1993). It inhabits the near-shore and first-slope waters of Antarctica (probably circum-Antarctic), and the nearby islands including Peter I, South Shetland, Elephant and South Orkney Islands (Gon and Heemstra 1990). They have been observed at depths from near the surface to 700 m, although are most common in the upper 200 m (Dewitt et al. 1990). Primarily a benthic ambush feeder (Kiest 1993), but opportunistic where suitable pelagic prey are available (Moreno 1980; Hopkins 1987), *T. bernacchii* is an omnivorous trophic generalist (Eastman and DeVries 1985). The main

food sources are edible benthic organisms such as polychaetes, algae, small fishes, fish eggs and amphipods (Kiest 1993; Vacchi et al. 2000), although in McMurdo Sound *T. bernacchii* will rise into the water column to feed on planktonic molluscs and copepods (Foster and Montgomery 1993; Montgomery et al. 1993). The fish are often seen perched on sponges and therefore have access to a wide variety of prey in the water column (Moreno 1980). The females generally live longer than ten years and grow to around 35 cm total length, while the males live about five years and grow to a length of 28 cm (Wohlschlag 1961). *T. bernacchii* is a patchy mottled brown fish which is well camouflaged against the sea floor and there are two colour morphs, one brown and the other with a white blotch on the head, although the two are not genetically different (Bernardi and Goswami 1997).

Based on morphological observations and measurements, the nototheniid species have been ranked into an ecological series reflecting their mode of life (Ekau 1991). On a scale from 1 to 10, in which 1 represents the most pelagic and 10 the most benthic, *T. bernacchii* has been allocated a rating of 5.67 compared with a score of 3.25 for the cryopelagic *P. borchgrevinki*.

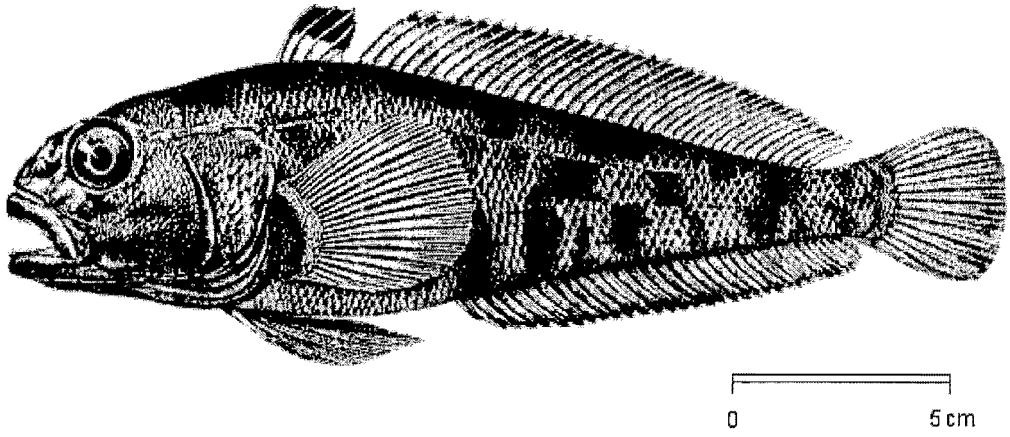


Fig 1.3. Sketch of the Antarctic nototheniid *Trematomus bernacchii*. Illustration reproduced with permission from FAO Species Identification Sheets (Hureau 1985).

Over the past 30 years the nototheniids of McMurdo Sound have been intensively studied by physiologists (see Eastman 1993), and much work has been carried out on the cold adaptations of these stenothermal Antarctic fishes. There is, however, a relative paucity of information relating to their physiological responses to an increase in temperature.

CLIMATE CHANGE

The patterns of climate change and the concept of global warming remain subjects of intense debate, although there is a growing awareness that the earth's temperature regime is changing (Wood and McDonald 1997). The history of the Milankovitch cycles suggests that under natural conditions sea level and climate should remain stable for another few thousand years, after which another ice build-up will begin (Quilty 1990). Over the past 150 years, however, the global mean annual temperature of the earth's lower atmosphere has warmed by 0.6 ± 0.2°C (Intergovernmental Panel on Climate Change, 2001). The twentieth century was the warmest century, and 1990 to 2000 the warmest decade, of the past millennium (IPCC 2001). Alpine glaciers are shrinking rapidly with glaciers in the European Alps having lost more than half their volume since 1850 (Hardy 2003), polar sea ice in the Northern Hemisphere decreased about 15% during the last half of the 20th century (IPCC 2001), and sea ice cover in the Bellingshausen Sea decreased in the late 1980s and early 1990s (Jacobs and Comiso 1993). These changes have been accompanied by an average increase in global precipitation, and a faster rate of warming on land than in the ocean. As a result of the increase in snowfall, some glaciers such as the Franz-Josef Glacier in New Zealand have actually been advancing over the past 20 years (IPCC 2001). Ocean surface temperature has increased on average 0.15°C during the 1950-93 period and global ocean heat content in the upper 300 m has increased by 0.04°C per decade since the late 1950s (IPCC 2001).

Increases in seawater temperature of as little as 0.5°C have been demonstrated to have marked effects on phytoplankton production and zooplankton composition (Southward 1980), and warming has already been associated with changes in the distribution (Beukema 1992; Southward et al. 1995) and production of fish stocks over the past few decades (O'Brien et al. 2000; McGinn 2002), including a decline in the

Southern Ocean icefish population (Kock and Everson 2003). Any increase in mean global temperature is paralleled by a rise in the frequency and magnitude of thermal fluctuations (Mohnen and Wang 1992), and therefore animals must not only adapt to changing temperature ranges, but also cope with extreme events (Sommer et al. 1997). From an ecological point of view, the annual maximum temperature may be of greater importance than the mean temperature value. There are also other factors likely to be altered by climate warming that may influence the realised thermal niche of an organism, including changes in the availability of dissolved oxygen, alteration of primary productivity, and changes in water level and the duration of ice cover (Magnuson and Destasio 1997; Magnuson 2002).

The most rapid warming over the past 50 years has taken place in parts of the Arctic, the Tibetan Plateau (Liu and Chen 2000), and on the Antarctic Peninsula (Domack et al. 2003). Surface air temperatures over the Antarctic Peninsula have risen by up to 2.5°C over the past 50 years (Jacobs and Comiso 1997; King and Harangozo 1998; Marshall and King 1998; King et al. 2003) and a series of very warm summers in the 1990s was followed by retreat and/or disintegration of the Larsen A, Prince Gustav, Wordie, Larsen B, Wilkins, George VI and Larsen C ice shelves (Gates 1993; Vaughan and Doake 1996; Malakoff 2002; Scambos et al. 2003; Shepherd et al. 2003), probably as a result of an increase in melt-water ponds and associated ice shelf weakening (Scambos et al. 2000). This is in contrast to a cooling between 1966 and 2000 in the Dry Valleys of Victoria Land (Doran et al. 2002), but the relevance of this isolated trend for the remainder of the Antarctic Continent has been debated (Turner et al. 2002; Walsh et al. 2002). Meteorological observations only began in earnest in 1957 in the Antarctic and therefore the records are short and influenced by decadal changes (Van den Broeke 2000). Antarctic ice cores, however, are beginning to shed light on the longer-term thermal fluctuations (EPICA 2004).

The geography and latitude of the Antarctic Peninsula differ from most of continental Antarctica and hence it is not surprising that its weather and climate exhibit special characteristics (Domack et al. 2003). The supposedly anthropogenic Antarctic ozone hole may be resulting in a change in the Antarctic Oscillation, and the associated shifts in winds and air rising over the continent could account for the majority of the summertime cooling over eastern Antarctica and warming over the Antarctic Peninsula (Kerr 2002). When corrected for changes in the oscillation, an Antarctic background

warming of 1.30 ± 0.38°C per century has been derived from data from 1957-1995 (Van den Broeke 2000).

Mid-depth Southern Ocean temperatures rose by 0.17°C between the 1950s and the 1980s (Gille 2002) which is as fast as the global rate of ocean change. The sub-surface Southern Ocean has warmed during the past 50 years and as the Southern Ocean plays a critical role in global climate, transmitting climatic signals between the Pacific, Atlantic and Indian Oceans, this may have broader implications as water from the Antarctic spreads around the globe. In addition, because warm water can hold less dissolved gas than cold water, the carbon dioxide storage capacity of the Southern Ocean has declined over the past 50 years which is also likely to have an impact on global climate (Gille 2002).

The current models of climate change predict an increase in mean global temperature of 1.4 to 5.8°C for the period from 1990 to 2100 (IPCC 2001; Reilly et al. 2001; Wigley and Raper 2001). Warming is predicted to be greatest at high latitudes in winter (King 1994; Hardy 2003) and the IPCC projections for the period 1990 to 2100 include forecasts for increased precipitation in high latitudes, including Antarctica, which may actually result in an enlargement of the Antarctic ice sheet (Comín and Rodríguez-Arias 2003). Although the substantial mass of ice and water constituting Antarctica has the ability to act as a huge heat sink, there is a very real possibility that if current trends continue the water temperature of the Southern Ocean will rise. Concern about the effects of temperature changes of only a few degrees Celsius on organismal distribution, species distribution limits, and community structure has thus become more than an issue of purely academic concern (Wood and McDonald 1997; Pörtner 2002), and the IPCC has mandated that a clearer understanding be achieved of how temperature changes in the range of 1.5-4.5°C affect the basic physiological and biochemical functions of organisms, especially when these changes occur at or near the upper limits of the thermal optima of these species. Projected extinctions due to changes in species distribution limits as the result of global warming are as high as 15-37% (Thomas et al. 2004). For eurythermal species living in the middle of their temperature range, the impacts of global warming are likely to be considerably less than for stenothermal species living toward the upper extreme of their thermal tolerance range, such as the Antarctic nototheniids. The general response of organisms to a slow climatic shift is a parallel change in the geographic range (Clarke and Johnston 1996), but this is not an option available to the Antarctic fishes, living at the southernmost extreme of the marine habitat.

The most rapid periods of warming and cooling in the past are 10-100 times slower than predicted by global warming models for the next century (IPCC 2001). An increase in temperature of 1°C per 100 000 years has been reported as within the adaptive capabilities of organisms (Schopf 1980), but greater rates could result in generation time becoming the limiting factor for adaptation. Assuming that the phylogenetic trees (Fago et al. 1992; Bargelloni et al. 1994; Cheng et al. 2003) are correct in identifying an Antarctic evolutionary history for *N. angustata*, the occurrence of this species in temperate New Zealand waters may be taken as evidence that evolutionary adaptation to thermal change is possible in the Antarctic notothenioids but, with Antarctic fishes being slow to achieve sexual maturity (Knox 1994), the scope for adaptations in the genotype in response to the rapid change predicted by global warming may be limited and the capacity for alteration of the phenotype may ultimately determine survival. Phenotypic plasticity is the term used to refer to the malleability of an organism's phenotype (physiology, morphology, behaviour) in response to the environmental conditions experienced by that organism (Scheiner 1993). Physiological systems have evolved to take into account probable operating conditions, based on past experiences of the species, and such information is stored in the genotype and expressed as the phenotype. Phenotypic variability is generally greater in animals which operate over a wide thermal range (Randall and Brauner 1991) and it is thought that specialisation to a narrow range of environmental conditions may restrict the ability to respond to environmental change (Huey and Hertz 1984; Huey and Kingsolver 1989; Angiletta et al. 2003). The adaptation of Antarctic nototheniids to their stable thermal environment over the past 10-15 million years may therefore have obviated the need to retain the functional plasticity necessary for survival in more variable ecosystems (Eastman 1993; Knox 1994; Somero et al. 1998; Hofmann et al. 2000).

A review of the thermal physiology research carried out on fish to date has identified several trends (see Wood and McDonald 1997). Firstly, it has been traditional among physiologists to study how organisms adjust to cope with the rate-depressing effect of low temperature, rather than regarding a temperature-mediated increase in rate as a physiological problem. Although both increases and decreases in temperature represent a physiological challenge in that homeostasis is disturbed, the nature of the challenge is different, depending on the direction of temperature change (Clarke 1991; Clarke 1998). In general, reductions in temperature from the upper end of the physiological range down to the lowest temperatures that a species tolerates are less

perturbing of enzymes than increases in temperature above the physiological temperature range (Somero et al. 1996). Secondly, eurythermal species have been studied much more than stenothermal species. The responses of cold stenothermal species to temperature change differ dramatically from those of the well-researched temperate eurytherms and therefore results cannot be extrapolated from one to the other. And finally, the majority of temperature acclimation studies have involved large changes in temperature, rather than the few degrees Celsius likely to be the result of global warming. The most pertinent questions were recently posed by Hochachka and Somero (2002) – "Do some organisms now live so close to the upper limits of their thermal tolerance ranges that rising temperatures will lead to their disappearance from current habitats? How much phenotypic plasticity is available to allow acclimatisation to temperature change, thus enabling species to stay put despite increases in habitat temperature?" The stenothermal Antarctic nototheniids form an ideal group to provide some insight into the answers.

PHYSIOLOGICAL EFFECTS OF TEMPERATURE

Temperature has been coined the "ecological master factor" for fish (Brett 1971), and the strong and frequently dominant role that it plays in governing the distribution patterns of organisms provides good reason to analyse its effects on living systems. Temperature exerts effects on virtually all levels of biochemical and physiological organisation, from the rates of molecular diffusion and biochemical reactions, through cellular, tissue and organ function, to the function of the whole organism (Johnston and Bennett 1996; Guderley and St Pierre 2002; Hochachka and Somero 2002). Temperature is an especially critical determinant of physiological performance in ectotherms such as fish, where body temperature is dependent on and fluctuates in response to the organism's thermal environment (Brill et al. 1994) and the thermal extremes of the spectrum are often good starting points in the study of temperature as the physical constraints operating on organisms are greater at these minimum and maximum temperatures, with the organisms effectively living at the critical limits of life (Guderley and St Pierre 2002).

The physiological effects of changes in temperature can be described both qualitatively and quantitatively by reference to various aspects of the evoked stress response. Stress, in biological terms, is any condition in which the dynamic equilibrium of organisms known as homeostasis is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli or stressors (Wendelaar Bonga 1997) and temperature has been described as the most pervasive environmental stressor (Crawshaw 1979; Wedemeyer et al. 1990). The effects of stressors are twofold: they produce effects which threaten or disturb the homeostatic equilibrium, and then elicit a coordinated set of compensatory behavioural and physiological responses, enabling the animal to overcome the threat (Wendelaar Bonga 1997). In cases where the level of stress is sufficiently severe, however, the response may lose its adaptive value and become dysfunctional (Selye 1974).

Theoretically, if an ectotherm is rapidly moved to a new temperature, various biological rates adjust up or down depending on the direction of the temperature change (Precht et al. 1973). The acute temperature dependence of biological rate processes of an individual organism may, however, undergo modification during acclimation or acclimatisation over a period of weeks or months to a new temperature. The definitions of the terms acute, acclimation, acclimatisation and adaptation adopted in this study are those of Clarke (1991); acute responses are those which involve the adjustment of an organism to an immediate change in temperature; acclimation responses occur in an organism following longer-term manipulation of a single variable (such as temperature) in the laboratory setting; acclimatisation refers to the adjustment of an organism to changes occurring in the environment where a variety of factors, such as temperature, photoperiod and food availability, will generally vary in tandem; and adaptation is the evolutionary adjustment occurring over successive generations.

The acute stress response is characterized by a cascade of physiological and metabolic changes (Pickering 1992; Wendelaar Bonga 1997) which can be divided into primary, secondary and tertiary responses according to the level of biological organisation involved (Mazeaud et al. 1977; Donaldson 1981; Mazeaud and Mazeaud 1981; Wedemeyer and McLeay 1981; Randall and Perry 1992). Primary responses refer to the activation of the endocrine system and the release of stress hormones, such as catecholamines and cortisol, into the blood stream. It is a general response of fish to release catecholamines (adrenaline and noradrenaline) from chromaffin tissue in the head kidneys and associated with the walls of the posterior cardinal veins into the bloodstream

in severe stress, and this catecholamine release is primarily mediated via sympathetic nerves (Randall and Perry 1992). Catecholamines exert effects on respiratory and cardiovascular parameters, as well as on metabolite mobilisation and blood oxygen transport capacity. The interaction of catecholamines with erythrocytes results in enhancement of blood oxygen transport (Nikinmaa and Tufts 1989; Randall and Perry 1992; Thomas and Perry 1992), while the interaction of catecholamines with hepatocytes enhances the metabolic potential of an animal as a result of the mobilisation of glucose (Mommsen et al. 1988; Sheridan 1988). Antarctic notothenioids, however, appear to have an attenuated catecholamine release response during exposure to moderate stressors (Egginton 1994; Davison et al. 1995). Cortisol is the predominant corticosteroid hormone in fish (Donaldson 1981) and is released from interrenal cells located around the posterior cardinal veins and in the head kidneys, in response to adrenocorticotropic hormone and other hormones secreted by the pituitary gland, following corticotrophin-releasing hormone secretion from the hypothalamus. Cortisol has important functions in the regulation of both hydromineral balance and energy metabolism of fish (Wendelaar Bonga 1997) and a delayed release of cortisol has been reported in response to stress in the Antarctic nototheniid *P. borchgrevinki*.

The effects of these hormones at blood and tissue levels are defined as the secondary stress responses and include changes in haematology, oxygen uptake rate, cardiac output, mobilisation of energy substrates, and disturbances of ionic and osmotic balance. Tertiary stress responses extend to the level of the organism and population and include inhibition of growth, reproduction and the immune response, impairment of locomotory ability, and a reduction in the capability to tolerate additional stressors. An increase in temperature has been shown to elicit a range of responses at both secondary and tertiary levels in fish, including alterations of protein synthesis and structure, membrane structure, muscle function, osmoregulation, metabolism, cardiovascular performance, aerobic swimming ability, growth and development, reproduction and behaviour (see Wood and McDonald 1997 for a review).

In Antarctic notothenioids, the documented secondary stress responses to an acute elevation of temperature include haematological changes (Franklin et al. 1991; Davison et al. 1994; Ryan 1995; Egginton 1997; Lowe and Wells 1997; Forster et al. 1998), and increases in the rates of oxygen consumption (Johnston et al. 1991; Wilson et al. 2002), mitochondrial respiration (Johnston et al. 1994), muscle contraction (Johnston and Johnston 1991), and in the rate of the atrial pacemaker (Macdonald 1997). At the tertiary

level, the anaerobic burst swimming performance of *P. borchgrevinki* has been shown to be independent of both an acute increase in temperature and warm-acclimation (Wilson et al. 2001), while the predominantly aerobic, prolonged swimming speed of this species declined rapidly with an acute increase in temperature (Wilson et al. 2002).

OVERALL OBJECTIVE

The overall objective of this study was to fill in some of the gaps in the understanding of the physiological responses of stenothermal Antarctic nototheniid fishes to elevated temperature. A variety of physiological parameters were investigated in order to build up a physiological and performance capacity profile for both short and longer-term increases in temperature. The majority of investigations were carried out on the relatively active, cryopelagic Antarctic nototheniid *Pagothenia borchgrevinki.* Haematological and metabolic responses to increased temperature were also studied in the benthic Antarctic nototheniid *Trematomus bernacchii* to explore the effect of lifestyle, and metabolic rates of the temperate-water notothenioids *Paranotothenia angustata* and *Bovichtus variegatus* were determined at various temperatures to identify the effect of adaptation temperature.

GENERAL METHODS

The Antarctic nototheniid fishes *Pagothenia borchgrevinki* and *Trematomus bernacchii* were caught from late October until early December in 2002 and 2003. The capture site for *P. borchgrevinki* was in McMurdo Sound (77° 53.573' S, 166° 45.425' E), some 6 km south-west of Scott Base in close proximity to the Ross Ice Shelf. A 1.2 m diameter hole was drilled in the ~3 metre thick sea ice and a heated fishing hut placed over the top to keep the hole from completely freezing over between fishing trips. The depth of the Sound where the hole was located was around 400-600 metres, and there was an abundance of platelet ice which floated up through the hole with the slightest disturbance

under the ice and had to be cleared daily. The fish were caught just below the ice surface by jigging 3.5 m hand-lines through the hole, using barb-less hooks baited with fish. Catch patterns throughout the study support the idea that *P. borchgrevinki* are schooling fish and that schools are size-specific. Poor catches were taken on days with strong currents indicating that the fish had either moved deeper or had taken refuge within the platelet ice. After capture, the fish were held briefly in insulated bins before being transported to the Wet Laboratory of Scott Base (New Zealand Science Support Base, 77° 49'S, 166°40'E) where they were kept in 45-60 L opaque blue tanks in a flow through aquarium system (seawater temperature -1.0 ± 0.3°C). The oxygen tension of the seawater within the system was close to air-saturation. Any fish that had gills affected by X-cell disease (Franklin and Davison 1987; Davison 1998) were excluded from this study.

T. bernacchii were caught through similiarly-sized holes drilled by diving teams in an area of 20-40 metre deep water 200-300 metres off Cape Armitage, some 2 km west of Scott Base. The majority of fish were caught just above the ocean floor using baited, barb-less hand-lines. A few larger specimens were netted by the divers working out of the holes. Fishing holes were rapidly "fished out" which relates well to the territorial benthic nature of this species. The territories were re-colonised within a matter of days, but generally by smaller fish. After capture, *T. bernacchii* were also held in insulated bins and returned to the Wet Laboratory at Scott Base where they were kept in tanks in the flow-through aquarium.

Chapter Two

The effect of an acute increase in temperature and warm-acclimation on the haematology of two Antarctic nototheniids

INTRODUCTION

The physiological systems of fishes can be "stressed" by a variety of biological, chemical and physical factors, of which temperature is one of the most significant (Wedemeyer et al. 1990). The original definition of Selye (1950) described stress as "the sum of all the physiological responses by which an organism tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force", and Pickering (1981) introduced the term "stressor" to describe the force or challenge responsible for eliciting this compensatory physiological response.

The stress response of fish is characterized by stimulation of the hypothalamus, resulting in activation of the neuro-endocrine system and a subsequent cascade of physiological and metabolic changes that enable the animal to cope with the changing environment (Pickering 1992; Wendelaar Bonga 1997). The response can be classified according to the speed of recruitment and the level of biological organisation involved (Mazeaud et al. 1977; Donaldson 1981; Mazeaud and Mazeaud 1981; Wedemeyer and McLeay 1981; Randall and Perry 1992). Primary responses refer to responses of the endocrine system and the release of stress hormones, such as cortisol and catecholamines, into the blood stream. The secondary responses occur as a direct result of these hormones and include alteration of both blood and tissue chemistry. These responses are initially adaptive, in that they attempt to restore and maintain homeostasis,

but may become maladaptive in cases of chronic or very severe acute stress (Schreck 1981). Acclimation to a change in environmental conditions is possible if the compensatory stress response can re-establish a satisfactory relationship between the new conditions and the organism (Wedemeyer et al. 1990).

The stress response was originally reported to be non-specific and characterised by a "general adaptation syndrome" of reactive processes (Selye 1936). Aquaculture has, however, been the primary motivation for studies of the stress response in fish and the variety of species investigated in the past has therefore been limited. More recent indications are that the responses may be as diverse as the adaptations of these organisms to their remarkably wide range of aquatic environments (Wendelaar Bonga 1997).

The haematological response of Antarctic nototheniids to severe acute thermal stress (exposure to a water temperature of 10°C) has previously been investigated in both *Pagothenia borchgrevinki* and *Trematomus bernacchii* (Franklin et al. 1991; Davison et al. 1994; Forster et al. 1998). A marked increase in haematocrit and haemoglobin concentration was observed in the former (Franklin et al. 1991; Forster et al. 1998), while a qualitatively similar response of smaller magnitude (Davison et al. 1994) and no change at all (Forster et al. 1998) have both been reported from the latter. Exposure to 5 and 8°C also results in the elevation of haematocrit, haemoglobin concentration, and plasma cortisol levels of *P. borchgrevinki* (Ryan 1995). Plasma chloride concentrations have been shown to increase in both of the Antarctic nototheniids during acute 10°C exposure (Franklin et al. 1991; Davison et al. 1994), while longer-term exposure of *T. bernacchii* to an elevated temperature (4°C for 4-5 weeks) results in a hypo-osmotic change in the plasma (Gonzalez-Cabrera et al. 1995; Guynn et al. 2002).

This study was designed to expand upon the previous studies of haematological variables during acute and chronic exposure to increased temperature and had two major objectives. The first was to quantify and compare several aspects of the secondary stress response to acute thermal change in two closely-related, but eco-physiologically different Antarctic nototheniid fishes. Both species inhabit McMurdo Sound but while *P. borchgrevinki* has been described as a mobile semi-pelagic predator, *T. bernacchii* is benthic and only intermittently active (Montgomery and Wells 1993). Associated with this objective, it was intended to measure plasma glucose levels to determine their effectiveness as an indicator of the stress response in these fishes. Hyperglycaemia has been reported as a feature of the stress response in a variety of fishes (McLeay 1977; McLeay and Gordon 1980; Barton and Schreck 1987; Barton and Iwama 1991; Braley

and Anderson 1992; Vijayan and Moon 1992; Carragher and Rees 1994; Franklin et al. 1996; Elofsson et al. 2000; Begg and Pankhurst 2004) but there are no records of plasma glucose levels in nototheniid fishes subjected to stress. In vertebrates, the elevation of blood sugar is typically due to the action of catecholamines and functions to provide caloric energy for the "fight-or-flight" reaction (Pottinger et al. 2000). Catecholamines, such as adrenaline, rapidly direct the phosphorylation of the inactive form of glycogen phosphorylase resulting in an increase in glycogenolysis (Vijayan and Moon 1992), with the primary source of glycogen being the liver and muscle (Wedemeyer et al. 1990). Gluconeogenesis may, however, gain greater importance when the glycogen stores of the liver have been depleted (Janssens and Waterman 1988; Mommsen et al. 1988). The increase in circulating adrenaline levels is rapid and transient (Wells and Weber 1990), and the hyperglycaemia almost immediate. The corticosteroid hormone cortisol has also been demonstrated to cause hyperglycaemia in fish (Leach and Taylor 1980; van der Boon et al. 1991; Pickering and Pottinger 1995; Vijayan et al. 1997; Mommsen et al. 1999; Begg and Pankhurst 2004) following activation by the hypothalamic-pituitary-interrenal (HPI) axis (Pickering 1981; Sumpter 1997), probably as the result of gluconeogenesis (Vijayan et al. 1991). The release of cortisol, however, is slow, compared with the release of catecholamines, and its effects are more prolonged (Gamperl et al. 1994; Waring et al. 1996). Three and 6°C were selected as water temperatures for the investigation, being either side of the 4°C upper viable temperature identified for these fish (Guynn et al. 2002).

The second objective was to determine the effect of acclimation to 4°C on the haematology of *P. borchgrevinki*, in particular to ascertain whether this species exhibits the hypo-osmoregulation typical of other Antarctic nototheniids.

MATERIALS AND METHODS

SERIES 2.1. EFFECT OF AN ACUTE INCREASE IN TEMPERATURE

Pagothenia borchgrevinki (mass 73.2 ± 22.3 g (mean ± SD), range 29.4 – 144.2 g, total length 204 ± 18 mm (mean ± SD), range 159 – 260 mm) and *Trematomus bernacchii* (mass 44.9 ± 20.6 g (mean ± SD), range 13.8 – 149 g, total length 151 ± 19 mm (mean ±

SD), range 110 – 225 mm) were captured near Scott Base, Antarctica during November 2002 and 2003, as described in Chapter One. The fish were held in a flow-through aquarium system (seawater temperature -1.0 ± 0.3°C) in the Wet Laboratory of Scott Base for at least three days prior to use, and were not fed. Following the recovery period, eight randomly-selected fish of each species were sampled to provide baseline haematological data. The remainder of the fish were placed (in groups of up to 10 of the same species) into 65 L tanks isolated from the flow-through system. The seawater temperature in the tanks was maintained at either 3.0 or 6.0°C (± 0.3°C) using a heat exchanger linked to an adjacent freshwater tank containing a thermostatically controlled heater. A portion of the seawater in the static tanks was able to be replaced daily without altering the temperature more than 0.3°C. The air temperature in the laboratory was consistently between 3 and 6°C, which assisted in the maintenance of stable seawater temperatures.

Individual fish were randomly dip-netted from the tanks at pre-determined time intervals (5, 10, 20, 30 minutes and 1, 2, 4, 8, 12, and 24 hours) as quickly and carefully as possible in order to minimize stress. A total of eight fish were sampled at each time period. The fish were killed immediately by a sharp blow to the head and blood samples of approximately 0.5 mL drawn into pre-heparinized (ammonium heparinate, Sigma Ltd.) hypodermic syringes with 25-gauge needles. The blood was obtained by acute cardiac puncture, and the entire sampling procedure was completed within 30 seconds of netting the fish. The fish were then blotted to remove any excess water, weighed and measured (from nose to tip of tail).

In order to determine percentage haematocrit an aliquot of whole blood was drawn into a capillary tube. The tube was sealed at one end with haematocrit clay and centrifuged at 20 000g for 90 seconds. The tubes were measured immediately upon removal from the centrifuge. A 5 µL sample of whole blood was frozen in liquid nitrogen in an Eppendorf tube for the determination of haemoglobin concentration in Christchurch. The remaining blood was centrifuged (3000g for 2 minutes) and the plasma drawn off by pipette. The plasma (frozen in liquid nitrogen) was transported to Christchurch for assessment of osmolarity and glucose concentration. All blood samples were kept in a liquid nitrogen dewar for up to two weeks at Scott Base before being transferred into a chilly bin of dry ice and transported by LC130 Hercules (8-hour flight) to New Zealand. At Canterbury University the samples were stored for up to 1 month at -80°C prior to analysis.

SERIES 2.2. EFFECT OF WARM-ACCLIMATION

Pagothenia borchgrevinki (mass 67.7 ± 16.7 g (mean ± SD), range 47.1 – 112 g, total length 209 ± 2 mm (mean ± SD), range 189 – 239 mm, n = 20) were caught and transported to the Wet Laboratory of Scott Base in the same manner as described in Series 2.1. The fish were held for three days in the flow-through aquarium system, at a water temperature of -1.0 ± 0.3°C, to allow recovery from the stress of capture. Ten fish were then kept in a 65 L tank in the flow-through aquarium system for a four-week period at a temperature of -1°C, while the remaining 10 were transferred to a 100 L tank, isolated from the flow-through system with a water temperature of 4°C. The water temperature of the isolated tank was maintained using a heat exchange system and daily water replacement, as described in Series 2.1. The fish were not fed and a natural (24-hour daylight) photoperiod was maintained. At the end of the acclimation period, both groups of fish participated in the acclimated swimming performance investigation (see Chapter 6), and were therefore maintained at their respective acclimation temperatures for 5-6 weeks in total. Twenty-four hours after the final swimming trial the fish were quickly killed by a sharp blow to the head and blood samples were obtained. The only difference between blood sampling protocols of this experiment and Series 2.1 is that blood was obtained from the caudal vein, rather than the heart, in order to preserve the heart tissue for biochemical analyses. The blood samples were stored and analysed in the same manner as those of Series 2.1.

DETAIL OF ANALYSIS METHODS

Haemoglobin Concentration

Blood haemoglobin concentration was determined using the cyanmethaemoglobin method (Sigma diagnostics kit 525). The Sigma procedure is based on the oxidation of haemoglobin and its derivatives to methaemoglobin, in the presence of alkaline potassium ferricyanide. Methaemoglobin reacts with potassium cyanide to form cyanmethaemoglobin which has maximal absorption at 540 nm, and the colour intensity at 540 nm is proportional to the total haemoglobin concentration. Absorbance of the samples was measured using a Unicam 8625 UV/VIS spectrometer, and total haemoglobin concentration was determined from the absorbance values using a

calibration curve drawn from various dilutions of a cyanmethaemoglobin standard solution.

Mean Corpuscular Haemoglobin Content

Mean corpuscular haemoglobin content (MCHC) was calculated from:

Haemoglobin concentration / Fractional haematocrit.

Plasma Osmolarity

The osmolarity of 8 μL plasma aliquots was determined using a Wescor 5100C vapour pressure osmometer. The osmometer was calibrated at the beginning and end of each experimental run using 100, 290 and 1000 mOsm L^{-1} standard solutions.

Plasma Glucose Concentration

Determination of the glucose concentration of plasma samples was made using the glucose oxidase / peroxidase enzymatic method (Sigma diagnostics kit 510). The Sigma procedure is based on the following coupled enzymatic reactions:

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{o-Dianisidine} \xrightarrow{\text{Peroxidase}} \underset{\text{(colourless)}}{\text{Oxidized o-Dianisidine}}_{\text{(brown)}}$$

The intensity of the brown colour measured at 450 nm is proportional to the original glucose concentration. The absorbance of samples was measured using a Unicam 8625 UV/VIS spectrometer and glucose levels were determined by comparison with a 5.56 mmol L^{-1} glucose standard solution and blank reference tube:

$$\text{Plasma glucose (mmol L}^{-1}) = \frac{A(\text{Test}) / A(\text{Standard}) \times 100}{18}$$

where: A = absorbance at 450 nm.

Condition Factor

Fulton's condition factor for each fish was calculated from length and weight measurements using the following formula:

$$\text{Condition Factor} = \frac{100 \times (\text{body mass, g})}{(\text{body length, cm})^3}$$

STATISTICAL ANALYSIS

Data were compared by one-way analysis of variance (ANOVA). Where a treatment effect was present in Series 2.1, Dunnett's post-hoc test was employed to compare treatment groups with the control. Where a treatment effect was indicated in Series 2.2, inter-group differences were determined using the Tukey-Kramer post-hoc test. The data were log transformed where indicated necessary by Bartlett's test, to improve homogeneity of variance. A level of difference of $p<0.05$ was regarded as statistically significant. Statistical analyses were all carried out using GraphPad Prism version 4.00 software for Windows, and values are presented as mean ± SEM, unless otherwise stated.

RESULTS

BASELINE HAEMATOLOGY

The baseline haematological values (Table 2.1) were obtained from fish kept in the flow-through aquarium system (water temperature -1.0 ± 0.3°C) for 72 hours post-capture.

Table 2.1. Resting haematological parameters of control fish 72-hours post-capture at -1°C.

Species	*Pagothenia borchgrevinki*	*Trematomus bernacchii*
Haematocrit (%)	16.3 ± 1.0	13.4 ± 0.9*
Haemoglobin (g dL^{-1})	3.7 ± 0.2	2.5 ± 0.4*
MCHC (g L^{-1})	228.6 ± 9.8	192.1 ± 32.7
Osmolarity (mOsm L^{-1})	574.0 ± 8.9	561.4 ± 6.4
Glucose (mmol L^{-1})	4.6 ± 0.7	2.3 ± 0.7*

Values are mean ± SEM. N = 8 for both species. * Significantly lower than *P. borchgrevinki*.

SERIES 2.1. EFFECT OF AN ACUTE INCREASE IN TEMPERATURE

Haematocrit

The haematocrit of *P. borchgrevinki* was elevated above the control value after 5 minutes at 3°C and remained elevated for the majority of the 24-hour period, although no significant difference was detected between the two values after 2, 8, and 12 hours (Fig. 2.1). At 6°C, the haematocrit was significantly higher than the control values after 10 minutes, and remained elevated for the entire 24-hour period. The maximum haematocrit at 3°C (26.8 ± 1.7%) was reached after 24 hours, while the maximum haematocrit at 6°C (28.1 ± 2.5%) was attained after 2 hours.

Exposure of *T. bernacchii* to either 3 or 6°C for 24 hours elicited no change in haematocrit (Fig. 2.1).

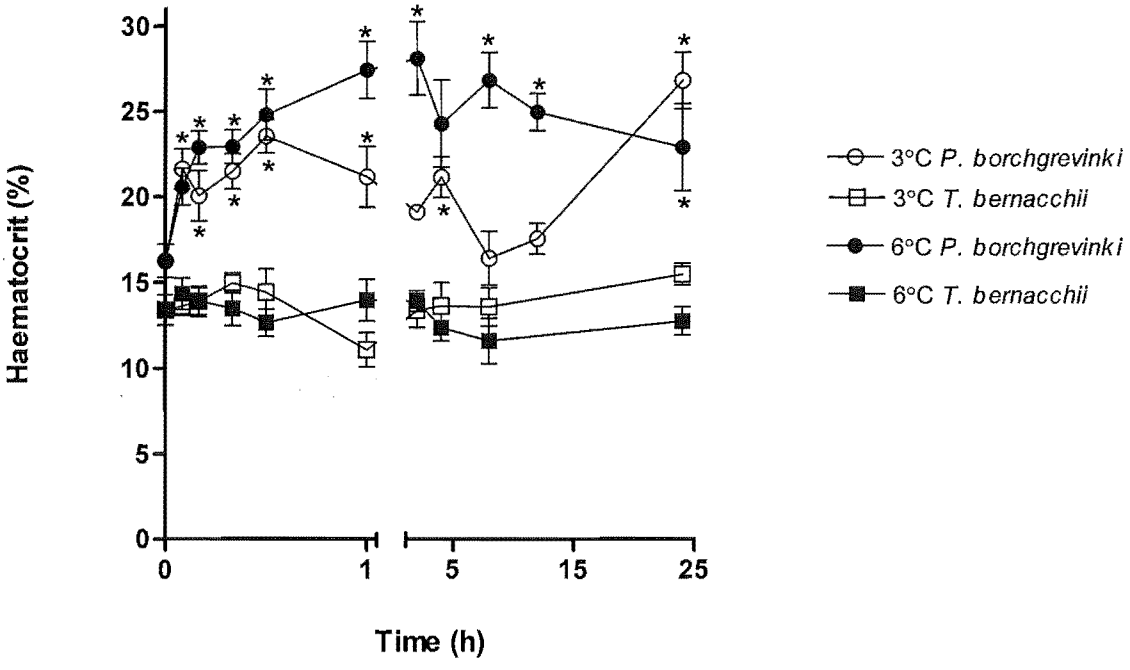


Fig. 2.1. Haematocrit of two Antarctic notothenioids during acute exposure to two different temperatures. The circles represent data from *P. borchgrevinki*, and the squares represent values from *T. bernacchii*. * Significantly different from T= 0 control value.

Haemoglobin Concentration

The changes in haemoglobin concentration of *P. borchgrevinki* at both 3 and 6°C (Fig. 2.2a) mirror the changes in haematocrit (Fig. 2.1), although none of the haemoglobin concentrations were significantly different from the baseline value at -1°C.

There was no significant difference between the blood haemoglobin concentrations of *T. bernacchii* at 3 or 6°C and the baseline value at -1°C (Fig. 2.2b).

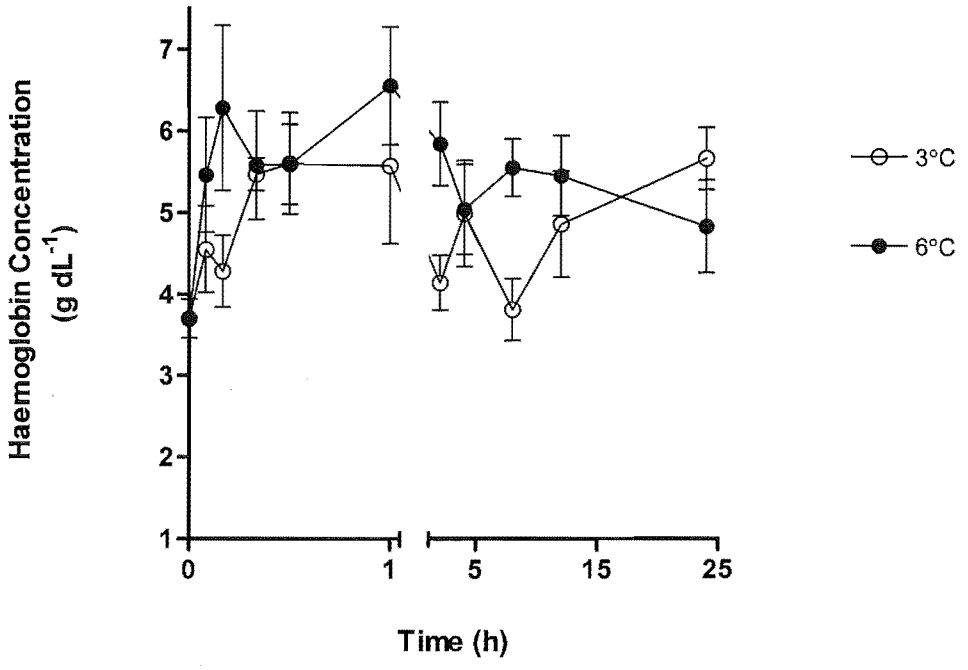


Fig. 2.2a. Blood haemoglobin concentration of *P. borchgrevinki* during acute exposure to an increase in water temperature. None of the values are significantly different from the T= 0 control value.

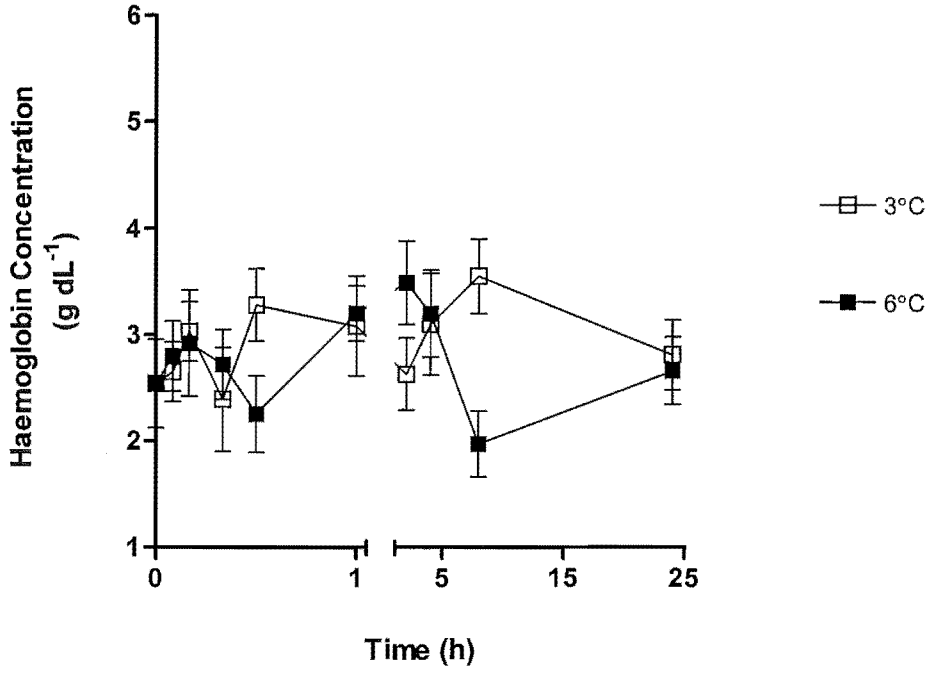


Fig. 2.2b. Blood haemoglobin concentration of *T. bernacchii* during acute exposure to an increase in water temperature. None of the values are significantly different from the T= 0 control value.

Mean Corpuscular Haemoglobin Content

No change in mean corpuscular haemoglobin content (MCHC) was measured in either *P. borchgrevinki* or *T. bernacchii*, at 3 or 6°C.

Plasma Osmolarity

The plasma osmolarity of *P. borchgrevinki* was unchanged from the -1°C baseline level throughout a 24-hour period of exposure to 3°C, but during exposure to 6°C there was a 9.7% increase in osmolarity (to 629.7 ± 9.8 mOsm L^{-1}) after 12 hours (Fig. 2.3a).

The plasma osmolarity of *T. bernacchii* was also unaffected by exposure to 3°C, but increased to 608.4 ± 10.5 mOsm L^{-1} (an 8.4% increase) after 8 hours at 6°C (Fig. 2.3b). In both species, the plasma osmolarities had returned to baseline levels after 24 hours at 6°C.

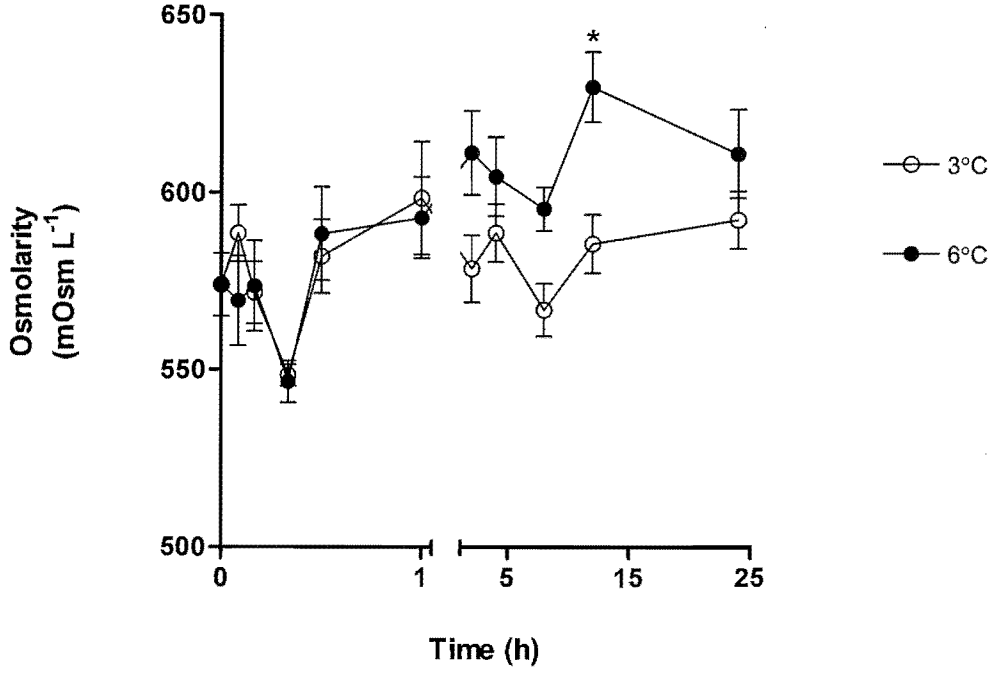


Fig. 2.3a. Plasma osmolarity of *P. borchgrevinki* during acute exposure to an increase in water temperature. * Significantly different from T= 0 control value.

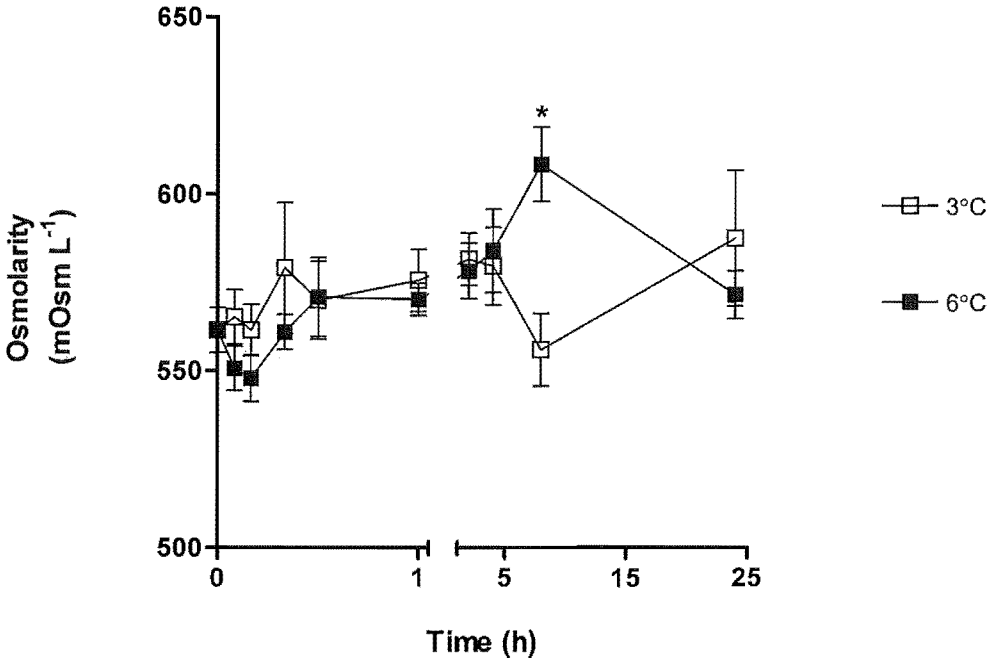


Fig. 2.3b. Plasma osmolarity of *T. bernacchii* during acute exposure to an increase in water temperature. * Significantly different from T= 0 control value.

Plasma Glucose Concentration

The plasma glucose concentration of *P. borchgrevinki* increased slowly during exposure to an elevated temperature and was significantly higher than the -1°C baseline level after 24 hours at both 3 and 6°C. The maximum glucose concentrations were attained after 24 hours and were 10.0 ± 1.6 mmol L^{-1}, and 9.5 ± 1.1 mmol L^{-1}, at 3 and 6°C respectively (Fig. 2.4a).

The plasma glucose concentrations of *T. bernacchii* were significantly higher than the -1°C control value after both 1 and 24 hours at 3°C, reaching a maximum of 7.7 ± 1.7 mmol L^{-1} after 24 hours. At 6°C, the glucose concentrations were elevated after 2, 4, 8 and 24 hours, attaining a maximum of 6.4 ± 1.0 mmol L^{-1} after 24 hours (Fig. 2.4b).

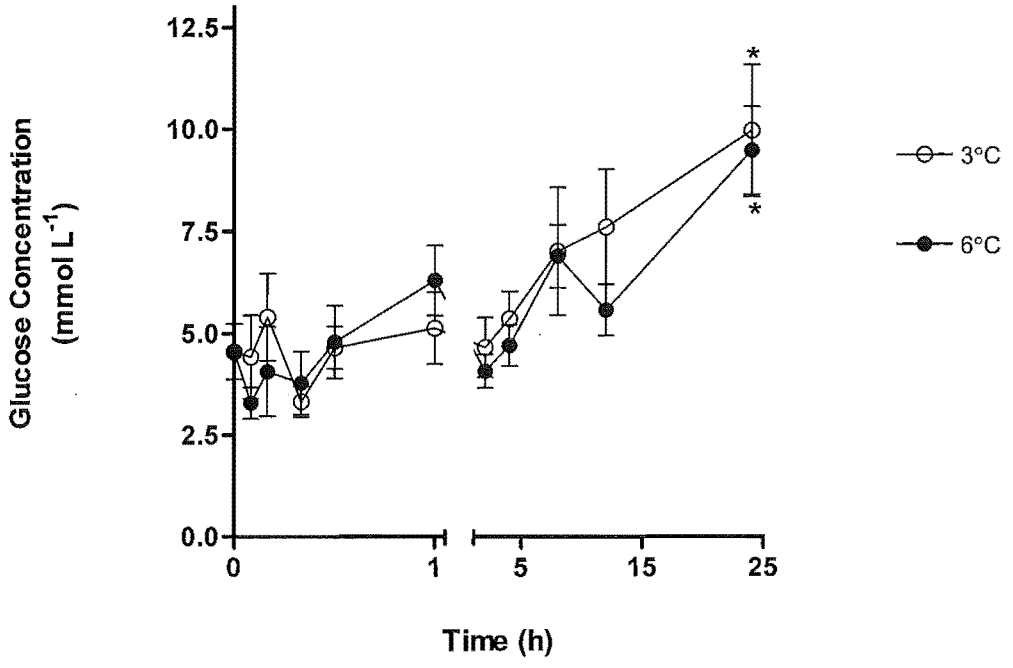


Fig. 2.4a. Plasma glucose concentration of *P. borchgrevinki* during acute exposure to an increase in water temperature.* Significantly different from T= 0 control value.

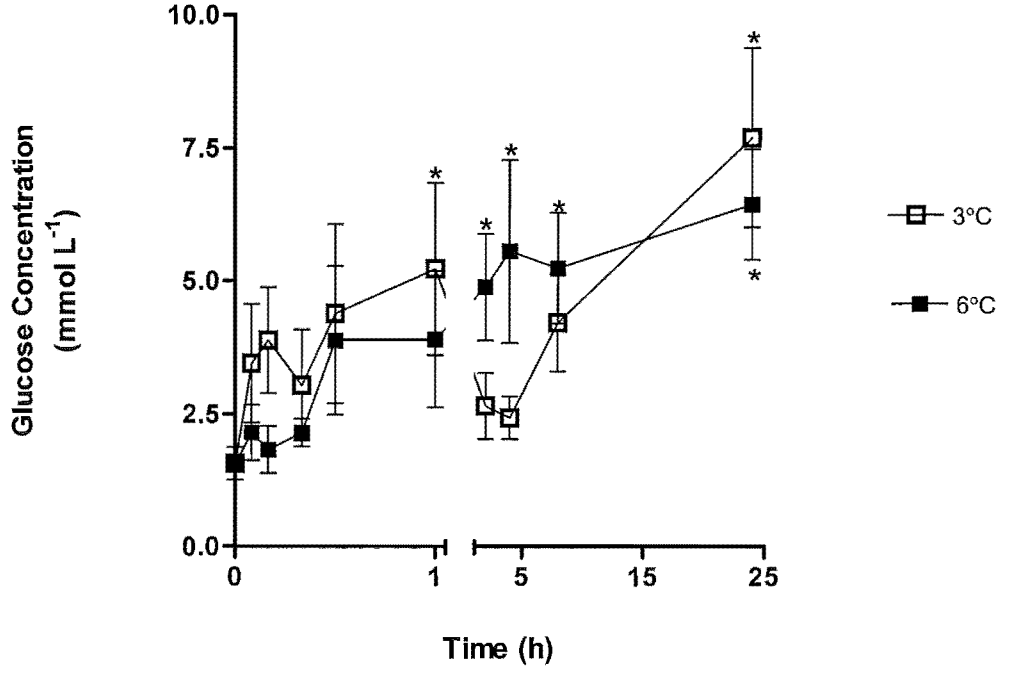


Fig. 2.4b. Plasma glucose concentration of *T. bernacchii* during acute exposure to an increase in water temperature. * Significantly different from T= 0 control value.

Condition Factor

The mean condition factor of *P. borchgrevinki* was 0.84 ± 0.09, with a range from 0.62 to 1.06. The condition factor of *T. bernacchii* was significantly higher than that of *P. borchgrevinki*, with a value of 1.22 ± 0.13 (range 0.86 – 1.54). Within each species there was no significant difference in weight, length or condition factor between the control fish and any of the treatment groups.

SERIES 2.2. EFFECT OF WARM-ACCLIMATION

Haematology

There were no significant differences in any of the haematological parameters between fish which had been kept in the flow-through aquarium at Scott Base (water temperature -1.0 ± 0.3°C) for 3 days after capture, and fish which had been kept in the same system for 5-6 weeks.

There were no differences in haematocrit (Fig. 2.5), haemoglobin concentration, mean corpuscular haemoglobin concentration, or plasma glucose concentration (Fig. 2.6) between -1°C and 4°C-acclimated *P. borchgrevinki*, but there was a significant reduction in plasma osmolarity of the 4°C-acclimated fish (Fig. 2.7). Data from two of the 4°C-acclimated fish was excluded from the results due to contamination of the blood samples with a clear fluid, possibly spinal fluid.

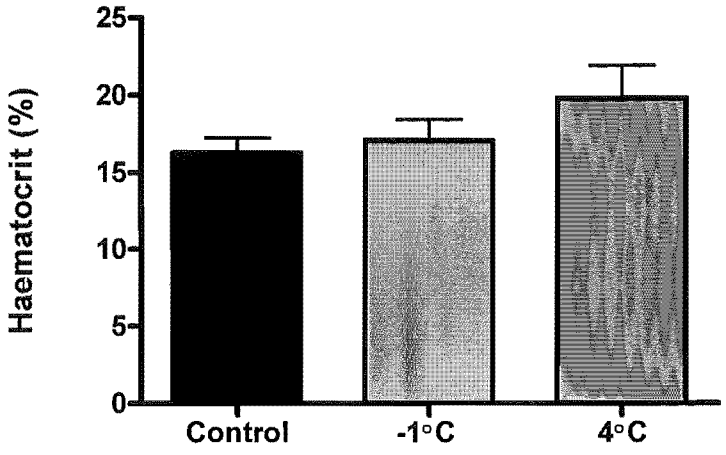


Fig. 2.5. Haematocrit of *P. borchgrevinki.* Values from fish acclimated to two different temperatures for five weeks and control fish sampled at -1°C, 72-hours post-capture. There are no significant differences between the values.

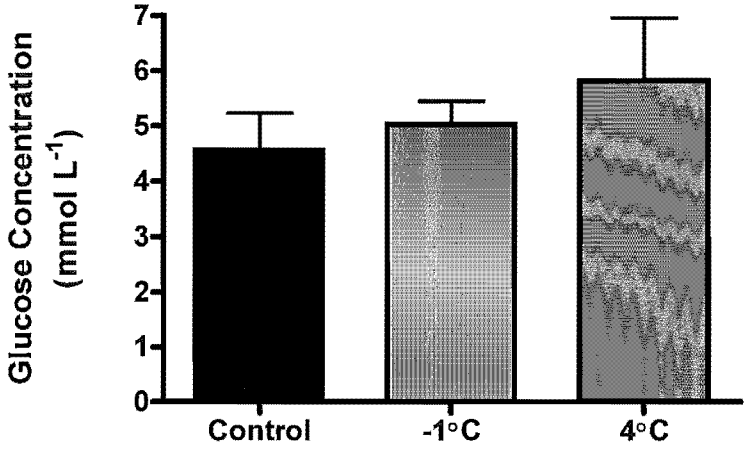


Fig. 2.6. Plasma glucose concentrations of *P. borchgrevinki.* Values from fish acclimated to two different temperatures for five weeks and control fish sampled at -1°C, 72 hours post-capture. There are no significant differences between the values.

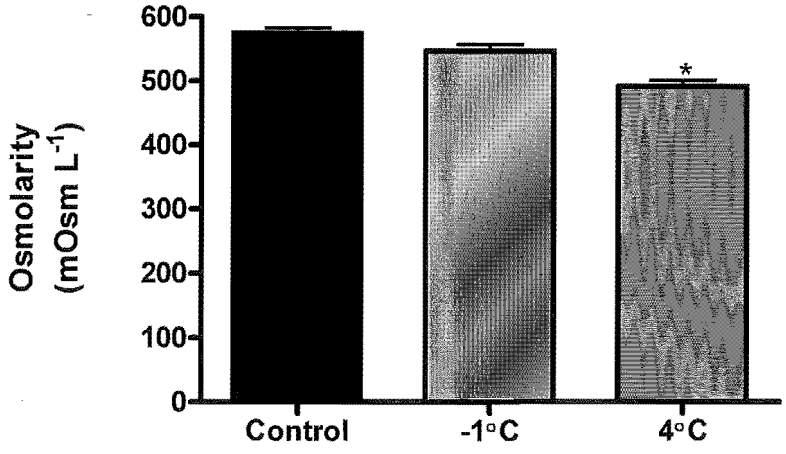


Fig. 2.7. Plasma osmolarity of *P. borchgrevinki*. Values from fish acclimated to two different temperatures for five weeks and control fish sampled at -1°C, 72 hours post-capture. * Significantly different from values of both control and -1°C-acclimated fish.

Table 2.2. Haematological and physical parameters of *P. borchgrevinki* acclimated to two different temperatures.

Treatment	Control	-1°C-acclimated	4°C-acclimated
Weight (g)	67.2 ± 5.7	60.7 ± 3.8	74.7 ± 5.8
Length (mm)	197.3 ± 4.8	200.9 ± 3.8	217.5 ± 4.5$^{\#}$
Condition Factor	0.86 ± 0.03	0.74 ± 0.02*	0.71 ± 0.02*
Haematocrit (%)	16.3 ± 1.0	17.1 ± 1.4	19.8 ± 2.1
Haemoglobin Conc. (g dL^{-1})	3.7 ± 0.2	4.5 ± 0.4	3.8 ± 0.5
MCHC (g L^{-1})	228.6 ± 9.8	268.5 ± 15.7	262.9 ± 14.9
Osmolarity (mOsm L^{-1})	574.0 ± 8.9	546.8 ± 10.1	491.4 ± 8.8$^{\#}$
Glucose (mmol L^{-1})	4.6 ± 0.7	5.0 ± 0.4	5.8 ± 1.1

Control fish were kept at -1°C and sampled 72 hours post-capture. Acclimated fish were maintained for 5-6 weeks at their respective acclimation temperatures. Values are all mean ± SEM. N = 8 for control and 4°C-acclimated fish, N = 10 for -1°C-acclimated fish. * Significantly different from control value. $^{\#}$ Significantly different from both control, and -1°C-acclimated values.

Condition Factor

The mean condition factors of both groups of acclimated fish were significantly lower than that of the control fish (Fig. 2.8). The mean body length of the 4°C-acclimated fish was also significantly greater than those of both control and -1°C-acclimated fish (Table 2.2).

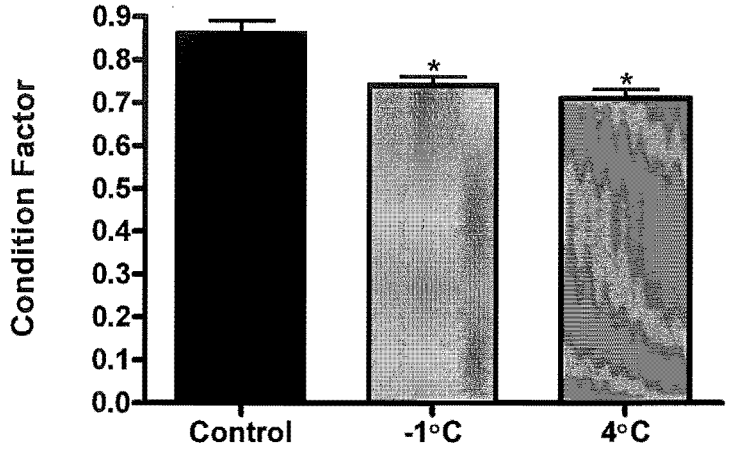


Fig. 2.8. Condition factor of *P. borchgrevinki*. Values from fish acclimated to two different temperatures and control fish sampled at -1°C, 72 hours post-capture. * Significantly different from control group.

DISCUSSION

A marked difference was observed between the relatively active, cryopelagic *Pagothenia borchgrevinki* and the more sedentary, benthic *Trematomus bernacchii* in terms of the effect of an acute increase in temperature on haematocrit. The 64% and 72% increases in haematocrit of *P. borchgrevinki*, at 3 and 6°C respectively, contrasted with the lack of change in haematocrit of *T. bernacchii* at either temperature. This lack of response of *T. bernacchii* compares well with a previous report of no change in response to acute 10°C exposure (Forster et al. 1998), although an earlier study reported a 64% increase in haematocrit due to the same stressor (Davison et al. 1994). The 64% increase is, however, considerably less than the 100% increase in haematocrit of *P. borchgrevinki*

acutely exposed to 10°C (Franklin et al. 1991; Forster et al. 1998). The increase in haematocrit of *T. bernacchii* in response to other stressors, such as hypoxia and exhaustive exercise, is also reported to be less that of *P. borchgrevinki* (Davison et al. 1988; Davison et al. 1994; Lowe and Wells 1997). Being members of the same taxonomic family, it is likely that the differing responses are due primarily to the effect of ecotype. Another sluggish Antarctic sit-and-wait predator, the notothenid *Notothenia neglecta,* does not alter haematocrit during moderate exercise (Egginton et al. 1991), and modest, if any, changes in haematocrit have been detected in response to stress in other benthic and demersal species (Qvist et al. 1977; Tetens et al. 1984; Wells et al. 1984; Egginton et al. 1991), in support of an effect of lifestyle. Antarctic notothenioids maintain lower resting haematocrits than fish from warmer latitudes, presumably to reduce blood viscosity and thereby reduce the amount of energy required to pump blood around the body (Farrell 1984; Macdonald and Wells 1991; Axelsson et al. 1992; Egginton 1996). Sedentary species with low levels of metabolic activity, such as *T. bernacchii* and *N. neglecta*, may maintain these low haematocrits during exposure to low to moderate stressors as an energy conservation measure. Active predators in the water column, such as *P. borchgrevinki*, are likely to have a greater requirement for the additional oxygen-carrying capacity that an increase in haematocrit would contribute in times of stress. It has been proposed, however, that the insensitivity of haematocrit observed in some Antarctic fishes may not reflect the lifestyle (benthic or pelagic) of the species concerned, but rather the development of a cholinergic-dominated system of cardiovascular control (Egginton 1996). The inhibitory cholinergic tone on the heart of *T. bernacchii* is approximately twice the inhibitory tonus on the heart of *P. borchgrevinki* (Axelsson et al. 1992; Axelsson et al. 1994; Franklin et al. 2001), indicating that cholinergic control mechanisms may assume greater importance in the benthic species. The stress-related release of catecholamines is attenuated in *T. bernacchii* (Davison et al. 1995), and may be combined with poor adrenoceptor responses, as hypothesized for other sedentary notothenioids (Egginton 1994). The reserves of erythrocytes in the spleen are also reported to be five times lower in benthic than in pelagic temperate-water fishes (Soldatov 1996), although this has not been investigated in the Antarctic species. With increases in haematocrit of 64-75% measured in *T. bernacchii* in response to enforced continuous swimming and acute 10°C exposure in the absence of marked erythrocyte swelling (Macdonald and Wells 1991; Davison et al. 1994) it appears that significant erythrocyte reserves are present.

The elevation of haematocrit of *P. borchgrevinki* in response to acute thermal stress was achieved within 5 – 10 minutes at both temperatures. Such an immediate increase in haematocrit could be the result of recruitment of erythrocytes from the spleen (Yamamoto 1987; Yamamoto 1988; Yamamoto and Itazawa 1989; Wells and Weber 1990; Franklin et al. 1993), and/or from erythrocyte swelling (Nikinmaa 1983; Wells and Weber 1990) and haemoconcentration due to efflux of water from the plasma. Polycythaemia, resulting from the release of erythrocytes from the spleen, is reported to be the major cause of stress-induced elevation of haematocrit in Antarctic nototheniids (Wells et al. 1989; Franklin et al. 1993) and the stimulus for splenic contraction in *P. borchgrevinki* appears to be neural and cholinergic, rather than the result of circulating catecholamines as in the majority of teleosts (Kita and Itazawa 1990; Nilsson et al. 1996). It is unlikely that haemoconcentration contributed significantly to the increase in haematocrit in this study with only a 10% increase in serum osmolarity of *P. borchgrevinki*, and no change in the haematocrit of *T. bernacchii* despite an 8% increase in serum osmolarity. Fish erythrocytes generally increase in volume in response to increased levels of adrenaline in the circulation (De Vries and Ellory 1981; Nikinmaa 1982; Ling and Wells 1985; Fuchs and Albers 1988; Salama and Nikinmaa 1988) but, as previously mentioned, nototheniids are unusual in that they do not release significant amounts of catecholamines into the blood stream in response to moderate levels of stress (Egginton 1994; Davison et al. 1995). With a large increase in haematocrit occurring over a short time frame, and no alteration of mean corpuscular haemoglobin content (MCHC: an index of erythrocyte volume), it seems likely that the largest contributing factor was the recruitment of sequestered erythrocytes from the spleen. Ryan (1995) also detected no change in MCHC of *P. borchgrevinki* acutely exposed to 5°C, indicating that polycythaemia was the primary contributor to the increase in haematocrit at this temperature. At 8°C, however, a reduction in MCHC was detected in the same study which relates well to the increase in plasma catecholamine concentrations measured in *T. bernacchii* and *P. borchgrevinki* during acute exposure to 10°C (Forster et al. 1998). Treatment of temperature-stressed *P. borchgrevinki* erythrocytes with the β-adrenergic antagonist sotalol inhibits swelling, indicating that the Na^+/H^+ antiporter does respond to endogenous catecholamines when they are released in times of severe stress (Forster et al. 1998).

The 64%, 72%, (Chapter Two) and ~100% (Franklin et al. 1991; Forster et al. 1998) increases in haematocrit of *P. borchgrevinki* during exposure to 3, 6, and 10°C

respectively, indicates a positive relationship between the magnitude of the thermal stressor and the level of response. A positive correlation between stress level and degree of increase in haematocrit has previously been reported following measurement of the smaller responses of *T. bernacchii* (Davison 2001). Whether the greater increase in haematocrit of *P. borchgrevinki* during exposure to relatively high temperatures (10°C) is a direct response to the greater demand for oxygen, or whether it is the result of an enhanced primary stress response at higher stress levels is unknown. Large increases in haematocrit have been measured in *P. borchgrevinki* in response to hypoxia (Wells et al. 1989), exercise (Davison et al. 1988; Wells et al. 1989; Franklin et al. 1993), and handling (Wells et al. 1984), indicating that substantial increases in haematocrit are a general feature of the stress response of this species.

The haemoglobin concentration of *T. bernacchii* was unaltered by acute exposure to 3 or 6°C, relating well to the lack of change in haematocrit. There were also no significant changes in the haemoglobin concentration of *P. borchgrevinki*. If the marked increase in haematocrit of this species was due primarily to splenic contraction, as hypothesized, the haemoglobin concentration of this species should have increased. In comparing Figures 2.1 and 2.2a, it is apparent that there are increases and decreases in haemoglobin concentration of *P. borchgrevinki* during the 24-hour period which mirror the changes in haematocrit. The lack of statistical significance is likely to be the result of high levels of inter-individual variation combined with low subject numbers. Previous studies have reported both increased and unchanged blood haemoglobin concentrations accompanying the elevation of haematocrit of Antarctic nototheniids during the stress response (Egginton et al. 1991; Franklin et al. 1993; Davison et al. 1994; Egginton 1994; Davison et al. 1995; Ryan 1995; Lowe and Wells 1997). It has been suggested that the primary role of haemoglobin is to protect against environmental hypoxia (Holeton 1974), a stressor not likely to be encountered in the sub-zero Antarctic waters, which would explain the reduction in haemoglobin levels of Antarctic nototheniids relative to marine teleosts from lower latitudes (Everson and Ralph 1968; Wells et al. 1990). The reduced resting haemoglobin concentrations of these species may, however, necessitate an increase in order to meet the elevated oxygen demands of stressful situations.

The baseline haematocrits, blood haemoglobin concentrations, mean corpuscular haemoglobin contents (MCHC), serum osmolarities and plasma glucose concentrations of *P. borchgrevinki* and *T. bernacchii* compare well with the majority of previously published values (Tetens et al. 1984; Macdonald and Wells 1991; Franklin et al. 1993;

Davison et al. 1994; Davison et al. 1995; Ryan 1995; Lowe and Wells 1997; Forster et al. 1998), although the haematocrits are higher and the MCHC values lower than those obtained by chronic cannulation (Wells et al. 1990). It has been suggested that the use of chronically implanted cannulae is the only reliable method of sampling blood from resting fish (Wells et al. 1990; Macdonald and Wells 1991) and that much of the data on fish haematocrits is in error due to the stress of acute sampling, with an acutely sampled haematocrit of 15% probably representing a true level of 11-12% (Macdonald and Wells 1991). The surgical cannulation procedure itself, however, may contribute to the alteration of haematology via blood loss and chronic stress. The majority of fish used in the current study were too small for the surgical implantation of cannulae, and acute sampling was therefore employed. During blood sampling, the amount of air exposure and handling were kept to an absolute minimum, with the sampling process completed within 30 seconds of first netting the fish.

Both Antarctic nototheniids were almost completely inactive while held in the tanks, tending to rest quietly on the floor of the tank or perch on submerged pumps. *P. borchgrevinki* kept at 3°C remained silvery-white in colour, while fish exposed to 6°C darkened almost immediately on immersion in the warmer water. Melanocytes are usually under both hormonal (pituitary release of melanocyte stimulating hormone) and neural control (antagonistic sympathetic and parasympathetic innervation), although the relative importance of these mechanisms in Antarctic fishes is not known (Egginton and Davison 1998). The only mortality during this experiment was a single *P. borchgrevinki* which died after between 12 and 24 hours of exposure to 6°C. An attempt was made to keep *P. borchgrevinki* at 6°C for a longer period but 50% of a group of eight fish died within four days of increasing the water temperature abruptly from -1 to 6°C. The survivors were returned to -1°C tanks in the flow-through aquarium system but all remained very dark in colour and died within the next four days. *P. borchgrevinki* acclimated to close to their environmental temperature have been demonstrated to tolerate and acclimate to a sudden elevation of temperature to 4°C in this study, but an abrupt increase from -1°C to 6°C appears to be a greater change than they can successfully adjust to. The next step will be to carry out a progressive increase and determine whether fish acclimated to 4°C can restore and maintain homeostasis at 6°C, and therefore survive.

The baseline haematocrit and haemoglobin concentration of *T. bernacchii* were significantly lower than those of the relatively active *P. borchgrevinki*. A relationship

between the haematocrit and activity level of fish has previously been reported in some (Farrell 1991; Fange 1992), but not all studies (Wells and Baldwin 1990). With higher levels of both haematocrit and haemoglobin concentration in *P. borchgrevinki*, it appears as though haematocrit is the major determinant of haemoglobin concentration, as hypothesized by Gallaugher and Farrell (1998).

The baseline plasma glucose concentration of *T. bernacchii* was also lower than that of *P. borchgrevinki*. Resting plasma glucose concentrations of various temperate and tropical teleosts range from 1.0 – 4.5 mmol L^{-1} (Schwalme and Mackay 1985; Flos et al. 1988; Braley and Anderson 1992; Vijayan and Moon 1994; Iwama et al. 1995; Vijayan et al. 1997; Pottinger 1998; Wells and Pankhurst 1999; Grutter and Pankhurst 2000; Pottinger et al. 2000; Trenzado et al. 2003; Begg and Pankhurst 2004; Bracewell et al. 2004), indicating that *P. borchgrevinki* either has routinely high blood glucose levels, or that the levels were elevated by the stress of capture and handling. The Antarctic nototheniid *N. neglecta* has a plasma glucose concentration similar to that of *T. bernacchii* and within the range of most other fishes (1.67 ± .93 mmol L^{-1}), while the plasma glucose concentration of cannulated, routinely active *N. rossii* has been measured as 14.43 ± 5.6 mmol L^{-1} (Egginton et al. 1991). *N. neglecta* is a sedentary benthic species, while *N. rossii* is described as a demerso-pelagic burst and glide fish (Morris and North 1984). Also taking into account the extremely low plasma glucose concentration of the very sedentary Antarctic eelpout (0.20 ± 0.05 mmol L^{-1}) (van Dijk et al. 1999), and the difference in baseline values between *T. bernacchii* and *P. borchgrevinki*, there appears to be an effect of lifestyle on the resting plasma glucose concentrations of these fishes.

In contrast to the lack of change in haematocrit, *T. bernacchii* exhibited a more rapid and proportionally greater increase in plasma glucose levels (~200%) than *P. borchgrevinki* (~100%). With lower resting glucose concentrations in the benthic species, it is possible that a larger relative increase may be required to meet the additional demands associated with stressful situations, but there also may be a difference in the substrates preferentially metabolised by the two species in times of stress. Fishes commonly become hyperglycaemic in response to a variety of stressors (Barton and Schreck 1987; Braley and Anderson 1992; Vijayan and Moon 1992; Carragher and Rees 1994; Elofsson et al. 2000; Begg and Pankhurst 2004; Bracewell et al. 2004) including thermal stress (Kindle and Whitmore 1986; Staurnes et al. 1994; van Dijk et al. 1999), with a response typically occurring within 15-30 minutes (Begg and Pankhurst 2004;

Bracewell et al. 2004). Plasma glucose concentrations reach 10 - 18 mmol L^{-1} in the majority of stressed relatively active temperate-water teleosts (Schwalme and Mackay 1985; Braley and Anderson 1992; Vijayan and Moon 1994; Wells and Pankhurst 1999), although levels in the less active sea raven and a tropical labrid only increase to about 4 mmol L^{-1} during stress (Vijayan and Moon 1994; Grutter and Pankhurst 2000). In this study, the plasma glucose concentrations of both *P. borchgrevinki* and *T. bernacchii* were still increasing after 24 hours and may not have attained their peak, but the values after 24 hours fall somewhere between those of inactive and relatively active temperate-water species.

In vertebrates, the elevation of blood sugar as the stress response begins is generally due to the action of catecholamines and functions to provide caloric energy for the "fight-or-flight" reaction (Pottinger et al. 2000). Catecholamines, such as adrenaline, rapidly direct the phosphorylation of the inactive form of glycogen phosphorylase, resulting in an increase in glycogenolysis (Vijayan and Moon 1992). The primary source of elevated blood sugar is therefore glycogen from liver and muscle (Wedemeyer et al. 1990), although gluconeogenesis may become more important when the glycogen stores of the liver have been depleted (Janssens and Waterman 1988; Mommsen et al. 1988). In the majority of fish, the increase in circulating adrenaline is rapid and transient (Wells and Weber 1990), resulting in an almost immediate hyperglycaemia. Slower changes in plasma glucose levels, such as that measured after 24 hours during the recovery of tropical damselfish from stress, are generally attributed to the effects of cortisol (Begg and Pankhurst 2004). Secretion of the corticosteroid hormone cortisol by the interrenal tissue is a characteristic reaction of teleost fish to almost all forms of environmental stress (Donaldson 1981) and this hormone has been demonstrated to cause hyperglycaemia in a variety of fishes (Leach and Taylor 1980; van der Boon et al. 1991; Pickering and Pottinger 1995; Vijayan et al. 1997; Mommsen et al. 1999), probably as the result of gluconeogenesis (Vijayan et al. 1991). The elevation of corticosteroids is generated by activity of the hypothalamic-pituitary-interrenal (HPI) axis (Pickering 1981; Sumpter 1997) and most fish demonstrate a rapid increase in cortisol with significant elevations occurring as early as five minutes after the first disturbance (Strange 1980; Sumpter et al. 1986; Robertson et al. 1988; Pankhurst et al. 1992; Grutter and Pankhurst 2000; Pörtner 2002), although the increase is generally more sustained than that of the catecholamines. In *P. borchgrevinki*, the release of cortisol is slow compared with the majority of temperate-water fishes (Sumpter et al. 1986; Robertson et al. 1988; Pankhurst

et al. 1992; Grutter and Pankhurst 2000; Pörtner 2002). During acute exposure to 5°C the first detectable increase in plasma cortisol concentration occurs after 30 minutes, and the level reaches a peak after 3 hours (Ryan 1995). There is evidence for an effect of environmental temperature on the rate at which plasma cortisol levels are elevated in stressed fish, with slower responses at low temperatures (Davis et al. 1984; Barton and Schreck 1987; Pankhurst et al. 1992), but the response of *P. borchgrevinki* is even slower than that of temperate fishes exposed to cooler temperatures (Pankhurst et al. 1992) indicating that it is more likely to be due to a less sensitive HPI axis than to the rate-depressing effects of temperature (Ryan 1995). The sea raven, a sluggish sit-and-wait predator living on rocky bottoms, also exhibits a cortisol response during stress that is slower than most salmonids (Vijayan and Moon 1994), raising the possibility of an effect of lifestyle on the sensitivity of the HPI axis. The relative importance of catecholamines and corticoid hormones in the response to stress is known to vary between species depending on lifestyle and habitat (Mazeaud et al. 1977; Leach and Taylor 1980; Wendelaar Bonga 1997) and, with an attenuated stress-related release of catecholamines in *T. bernacchii* and other sedentary notothenioids (Davison et al. 1995; Egginton 1997), cortisol may have the dominant controlling influence over substrate mobilisation. There are, however, other hormones, such as thyroxine and glucagon, which are also implicated in the breakdown of glycogen (Janssens and Waterman 1988; Soengas et al. 1992).

With hyperglycaemia detected in *T. bernacchii* in the absence of a change in haematocrit, and an increase in plasma glucose levels of both Antarctic nototheniids at a temperature below their upper viable limit, plasma glucose levels are a sensitive indicator of stress in these species. The considerable delay prior to the elevation of glucose levels, the lack of any relationship between the magnitude of the hyperglycaemia and that of the stressor, and the high level of inter-individual variability, however, result in plasma glucose levels being less-than-ideal as a parameter by which to describe the stress response. Plasma glucose concentrations are easily determined but variability is the major disadvantage associated with their use as the levels are affected by a variety of extrinsic factors including diet, life stage, time since last feeding, and season of the year. All of these factors act to influence liver glycogen stores and therefore affect the magnitude of the hyperglycaemic response elicited by a given stress factor (Nakano and Tomlinson 1967; McLeay 1977; Gordon and McLeay 1978). Although none of the fish in this study had been fed post-capture, their pre-capture feeding history could well explain some of the variation observed. The specific dynamic action (increase in

metabolic rate following feeding) of Antarctic fish has been estimated at 10 - 16 days (Johnston and Battram 1993; Boyce and Clarke 1997) and therefore those fish which had fed immediately prior to capture would still have been within the absorptive period.

The temperature-induced change in plasma osmolarity of the two Antarctic nototheniids was of similar magnitude and time-frame. The proportional increases in plasma osmolarity of *P. borchgrevinki* and *T. bernacchii* at 6°C (9.7% and 8.4%, respectively) were slightly higher than the 7.5% increase reported from *P. borchgrevinki* following a 10 minute exposure to 10°C (Franklin et al. 1991), while no change was detected in either species at 3°C. The amplitude of temperature change has previously been reported to have an influence on the level of osmotic disruption (Pickering 1981), but in the Antarctic species it appears that the magnitude of hyper-osmotic change does not vary with the severity of stressor above the threshold for osmotic dysfunction. Water-breathers typically exhibit disruptions of osmotic and ionic balance following moderate or large temperature shifts (Crawshaw 1979), and the effects tend to be more pronounced when temperatures near the upper or lower survival limits are encountered (Pickering 1981). In the Antarctic nototheniids, it appears as though osmotic changes only occur at temperatures close to or exceeding the upper lethal limits, and that even these changes are transient, in support of the theory that these fishes with their high resting plasma osmolarities and the resulting reduction in osmotic gradient between blood and seawater should exhibit less of an osmotic dysfunction than temperate-water fishes (Franklin et al. 1991). The direction of the osmotic change measured in this study compares well with the increase in plasma osmolarity and ionic concentrations demonstrated by a variety of marine species in stressful situations due to the efflux of water and/or influx of ions (Stanley and Colby 1971; Maetz and Evans 1972; Bourne 1986). There are a variety of factors which may be contributing to the transient increase in osmolarity including: osmotic efflux of water from the plasma (haemoconcentration), increased diffusion at higher temperatures with osmoregulatory mechanisms unable to excrete the greater number of ions, and permeability changes resulting from activation of the primary stress response (release of corticosteroids and/or catecholamines into the blood stream). As discussed above, catecholamine release is generally rapid and transient (Wendelaar Bonga 1997) and attenuated in *T. bernacchii* (Davison et al. 1995), and therefore unlikely to be responsible for the osmotic change. The corticoid stress response may have an influence as both adrenal and pituitary hormones produce alterations in ion permeability and ion transport (Evans 1975; Evans 1978) and cortisol, specifically, is known to exert

an effect on the regulation of hydromineral balance (Wendelaar Bonga 1997). The 8-12 hour latency prior to detection of osmotic change in *P. borchgrevinki* at 6°C relates well to the delayed rise in cortisol levels reported in this species by Ryan (1995). The primary cause of the transient hyper-osmolarity is more likely to be a temporary inability of osmoregulatory control mechanisms to keep pace with the increased influx of ions at the higher temperature.

The oxygen affinity of human haemoglobin (Colombo et al. 1992) and of carp (Kwiatkowski and Noble 1993, cited in Jensen et al. 1998) decreases with increasing blood osmotic pressure, and although this effect has not been investigated in Antarctic fish, it could possibly represent an additional means by which to compensate for the increase in oxygen demands at higher temperatures.

The baseline plasma osmolarities measured in this study (574.0 ± 8.9 mOsm L^{-1} in *P. borchgrevinki*, and 561.4 ± 6.5 mOsm L^{-1} in *T. bernacchii*) are consistent with previous observations that Antarctic fishes have higher plasma osmolarities than temperate marine teleosts. The plasma sodium and chloride levels of *P. borchgrevinki* are approximately 50% higher than the majority of other marine teleosts (Dobbs and De Vries 1975; O'Grady and DeVries 1982), and as a consequence of these ions and the presence of antifreeze, the plasma osmolarity is approximately 2-fold greater in these fish. The Antarctic nototheniid *N. neglecta*, however, does not to conform to this general trend and has a plasma osmolarity similar to that of temperate-water teleosts (Romao et al. 2001). In contrast to the hyper-osmotic response elicited by acute elevation of water temperature, acclimation of *P. borchgrevinki* to 4°C for 4-5 weeks resulted in a hypo-osmoregulatory shift. The 14% decrease in serum osmolarity of *P. borchgrevinki*, compares well with the results of previous studies on Antarctic nototheniid fishes. Gonzalez-Cabrera et al. (1995) acclimated both *T. bernacchii* and *T. newnesi* to 4°C for five weeks and measured reductions in osmolarity of 24% and 20%, respectively, while a more recent study by Guynn et al. (2002) reported an 18% decrease in serum osmolality following a five week acclimation of *T. bernacchii* to the same temperature. Both studies detected an increase in the activities of gill Na^+/K^+-ATPases following warm acclimation which would increase the excretion rate of serum Na^+ and Cl^-, and contribute to the observed decrease in osmolarity. Other species exhibiting enhanced hypoosmoregulation at higher temperatures include: *Myoxocephalus scorpius* and *M. quadricornis* in which serum osmolarity was higher at -0.1°C than at 2°C (Oikari 1975, cited in Guynn et al. 2002), *Salvelinus alpinus* (Arctic char) which had a higher serum osmolarity when

acclimated to 5°C than to 10°C (Staurnes 1993), the Arctic teleost *Microgadus tomcod* which increased serum osmolarity in response to winter water temperatures of -1.7°C (Gonzalez-Garcia et al. 1987), and the killifish *Fundulus heteroclitus* which had a higher osmolarity at 1.5°C than at 4°C (Umminger 1969). Acclimation of the eurythermal non-Antarctic nototheniid, *N. angustata* to its upper viable temperature (14°C), however, resulted in no change in osmolarity (Guynn et al. 2002) in agreement with the observation that the trend for an inverse relationship between environmental temperature and serum osmolarity appears to be restricted to organisms with environmental temperatures approaching 0°C (Burton 1986). The high osmolarities observed in cold waters could be the result of reduced overall osmoregulatory capacity due to incomplete cold-adaptation (Hochachka 1988), although the role of hyper-osmolarity of Antarctic teleosts in energy conservation has been questioned as Antarctic fish are able to actively regulate plasma osmolarity during changes in ambient salinity from 50 to 200% (O'Grady and DeVries 1982). The high plasma osmolarity therefore appears to be actively regulated by a shift of the set point of pumps involved, rather than the result of insufficient capacity of ion regulation (O'Grady and DeVries 1982; Gonzalez-Cabrera et al. 1995).

Another factor which could contribute to the decrease in serum osmolality following warm acclimation is a reduction in the concentration of antifreeze molecules in the blood. Gonzalez-Cabrera et al. (1995) determined, however, that the Na^+ and Cl^- concentrations paralleled the changes in osmolarity, providing evidence that the majority of the observed decline in osmolarity was the result of electrolyte losses. They also ruled out the possibility that the reduction in osmolarity following warm acclimation was a sign of osmoregulatory failure, as both *T. bernacchii* and *T. newnesi* maintained the new Na^+ and Cl^- levels as long as they were alive. The increased Na/K-ATPase activity of *T. bernacchii* was not accompanied by an increase in the number of Na/K-ATPase sites or a measurable change in the enzyme's affinity for ouabain. Therefore, as pump activity is a function of pump density and pump turnover, the increased activity observed after warm acclimation is likely to be the result of a change in turnover of the enzyme (Guynn et al. 2002). Weighing the fish at the start and end of their 35-day warm-acclimation trial also revealed no difference, indicating that the decline in serum osmolarity was not due to an enhanced hydration state (Gonzalez-Cabrera et al. 1995).

In summary, there appears to be a two-fold response of osmoregulatory capacity to increased temperature in the Antarctic nototheniids: an initial and transient

hyperosmotic change, due probably to an initial inability to keep up with the greater influx of ions at the higher temperature, and a longer-term hypo-osmotic shift due to an increase in the activity of Na^+/K^+ ATP-ases.

Acclimation to a higher temperature could also be expected to result in an increase in resting haematocrit if it is assumed that haematocrits are maintained at reduced levels at low temperatures as an adaptation to reduce viscosity-related cardiac work (Farrell 1984; Macdonald and Wells 1991; Axelsson et al. 1992). Fewer erythrocytes could be stored in the spleen and a greater number released into circulation without increasing the cardiac workload, due to the decrease in blood viscosity at higher temperatures. Non-Antarctic notothenioid species, such as New Zealand's *Notothenia angustata,* have slightly higher haematocrits than their Antarctic relatives (Tetens et al. 1984; Macdonald et al. 1987), and a significant increase in both haematocrit and haemoglobin concentration, with no indication of erythrocyte swelling, has previously been reported in *P. borchgrevinki* acclimated to 4.5°C for 8 – 13 days (Tetens et al. 1984). A relationship between acclimation temperature and haematocrit has also been demonstrated in goldfish (Houston and Murad 1992), winter flounder (Cech et al. 1976), and rainbow trout (Jones 1971; Martinez et al. 1994; Perry and Reid 1994), although not in all studies (Wood 1979; Barron et al. 1987; Taylor et al. 1993), and the suggestion has been made that the relationship in temperate and tropical fishes acts primarily to increase carrying capacity to offset the decreasing solubility (Taylor et al. 1997). Although the haematocrits of 4°C-acclimated *P. borchgrevinki* were slightly higher than those of -1°C-acclimated fish in this study, the differences were not significant and the haemoglobin concentrations were slightly lower. Lane et al. (1981) demonstrated that both haematocrit and haemoglobin concentrations of trout were reduced during prolonged starvation and therefore the 5-6 week fast of *P. borchgrevinki* may have neutralised any increase resulting from the warm-acclimation. The condition factors of both -1°C and 4°C-acclimated *P. borchgrevinki* were significantly lower than condition factors of freshly caught fish caught this season and condition factors of healthy *P. borchgrevinki* caught during previous seasons (0.82 ± .006 and 1.01 ± .012) (Davison 1998). A critical value of 0.7 has previously been identified in temperate-water fish as the threshold between "normal" and malnourished fish (Reimers 1963), although values are species-specific as indicated by the significant difference in values between the laterally-compressed benthic *T. bernacchii* and the more streamlined *P. borchgrevinki*. Warm-acclimation of regularly fed fish will be required to determine whether haematocrit does in fact increase.

With regard to identifying the haematological parameter which provides the best representation of the thermal stress response in Antarctic nototheniids, it appears that as reported from studies on other species (Buckley et al. 1985; Barton 1988; Pottinger and Carrick 1999), haematological profiles enable a better picture of the stress response to be obtained than single tests. While haematocrit alone is a good indicator of the magnitude of thermal stress in *P. borchgrevinki*, it is not a good indicator in *T. bernacchii*; osmotic changes are transient, restricted to high temperatures, and complicated by the reverse direction of acclimatory change; plasma glucose increases are slow and do not reflect the magnitude of the stressor; and MCHC is sensitive only to extreme thermal changes. Plasma lactate levels are not a good indicator in these fishes as Antarctic notothenioids do not generally exhibit the elevation of plasma lactate levels typical of other teleosts in response to stress (Qvist et al. 1977; Davison et al. 1988; Egginton et al. 1991; Davison et al. 1995; Lowe and Wells 1997), probably as the result of a low glycolytic capacity (Dunn and Johnston 1986; Davison et al. 1988; Egginton et al. 1991). Plasma free fatty acid levels is an area for future research, although the response may be slow if an increase in cortisol levels is the primary stimulant.

In conclusion, ecotype appears to have a significant influence on the stress response, at least with regard to acute increases in temperature. Marked differences were observed between the secondary stress responses (most apparent in haematocrit) of the two nototheniids and there is scope for further research to determine whether these are directly related to differences in the primary stress responses. With regard to which haematological parameter gives the best idea of the stress response, there appears to be no single parameter which reflects the magnitude of the thermal stressor in the Antarctic nototheniids, although both cortisol and plasma fatty acid levels (see Chapter Nine) warrant further investigation. Plasma glucose concentrations are elevated by acute thermal stress, but the response is slow and unrelated to the magnitude of the stressor. The combination of haematological information with other physiological parameters (metabolic, ventilatory, cardiovascular, and whole-body performance measures) is therefore likely to provide a clearer picture of the response.

P. borchgrevinki does exhibit the hypo-osmotic shift characteristic of Antarctic nototheniids during warm-acclimation, adding support to the theory that this response is typical of fishes with environmental temperatures close to 0°C.

Chapter Three

The effect of an acute increase in temperature on heart rate and blood pressure

INTRODUCTION

Temperature exerts a major influence over the physiology of poikilothermic organisms such as fishes in that it alters biochemical reaction rates by a factor of two to three for every change of 10°C, and also shifts the equilibria between the formation and disruption of non-covalent interactions which stabilise biological structures. The heart, as a chemo-mechanical converter of energy, is particularly sensitive to thermal change, with temperature defined as the single most important environmental determinant of heart rate in fish (Driedzic and Gesser 1994; Farrell 1997). The effects of change in temperature on the physiology of animals are generally described as Q_{10} effects, where Q_{10} is the increase in a rate caused by an increase in temperature of 10°C. If a rate doubles over a 10°C interval, Q_{10} is 2 and if it triples, Q_{10} is 3 (Videler 1993). During acute changes in temperature within a species' thermal range, heart rate usually changes with a Q_{10} of near 2, varying from 1.3 to 3 depending upon the species, temperature range, and acclimation temperature (Farrell 1984; Farrell and Jones 1992). Eurythermal species, in general, show a remarkable ability to compensate cardiovascular performance for the direct consequences of seasonal changes in environmental temperature, while stenothermal species have developed cardio-circulatory adaptations for functioning within a very restricted thermal regime (Hazel and Prosser 1974; Cossins and Bowler 1987). The Antarctic nototheniids, with their remarkably low upper lethal temperatures (Somero and De Vries 1967), are among the most stenothermal of fishes. They possess a variety of

cardiovascular adaptations to the cold, including increased cardiac mass (Johnston et al. 1983; Montgomery and Wells 1993), enlargement of ventricular myocytes (Zummo et al. 1995) and greater volume densities of mitochondria (Johnston et al. 1983; Tota et al. 1991; Zummo et al. 1995), with these adaptations attaining their peak in the haemoglobin-less icefish.

Heart rate is set by the intrinsic rhythm of the pacemaker in the sinus venosus or in the sino-atrial ring, and is generally related to lifestyle increasing from benthic to more active pelagic species (Farrell 1991). Resting heart rate in fish, however, is rarely the exact pacemaker rate with at least four effector mechanisms modulating the pacemaker rhythm including: stretch of the pacemaker cells, adrenergic nerve fibres, cholinergic nerve fibres, and hormones (Randall 1970; Santer 1985). All teleost fish species studied so far have a cholinergic inhibitory innervation of the heart, and most teleosts also possess an adrenergic excitatory innervation (Axelsson et al. 1998), which together constitute the major influences on the teleost heart (Farrell 1991). There is also, however, a controlling input from a number of neuropeptides, amines (5-hydroxytryptamine), purine derivatives (adenosine and its nucleotides), and the renin-angiotensin system (Nilsson and Holmgren 1992; Olson 1992). By sequentially injecting a cholinoceptor antagonist (e.g. atropine) and a β-adrenoceptor antagonist (e.g. sotalol) *in vivo*, it is possible to determine the cholinergic and adrenergic influences on the heart and to express the percentage change in heart rate as cholinergic and adrenergic tone (see Altimiras et al. 1997). In contrast to the majority of temperate and tropical teleosts, cholinergic control appears to be the dominant mechanism in many physiological systems of Antarctic fish, including the spleen (Nilsson et al. 1996), gills (Axelsson et al. 1994) and heart (Axelsson et al. 1992; Axelsson et al. 1994; Davison et al. 1997; Franklin et al. 2001). Adrenergic control, however, is still potentially important, as some blood vessels are more responsive to adrenaline than to acetylcholine (Nilsson et al. 1996). Antarctic nototheniids also tend to have relatively greater cholinergic than adrenergic tonus on the heart at rest, unlike the majority of teleosts (Axelsson et al. 1987) and have some of the highest levels of inhibitory cholinergic tone on the heart recorded from any fishes, with an 80% inhibitory cholinergic tone on the heart of the sedentary benthic *Trematomus bernacchii* at 0°C (Axelsson et al. 1992), and even higher values recorded from the large mesopelagic *Dissostichus mawsoni* (Egginton and Campbell unpublished). The cholinergic tone on the heart of the relatively active, cryopelagic *Pagothenia borchgrevinki* is lower, at 44.6 – 54.5% (Axelsson et al. 1992; Axelsson et

al. 1994; Franklin et al. 2001), although still high compared with most teleosts. The relative tonic influence of the cholinergic and adrenergic systems varies widely between species (Axelsson et al. 1987; Axelsson et al. 1998), but it has been proposed that there is a trend for a greater cholinergic component of control at lower temperatures (Priede 1974; Wood et al. 1979). This has recently been brought into question, however, by reports of a cholinergic tone of 30% on the heart of the Antarctic dragonfish *Gymnodraco acuticeps* (Axelsson et al. 2000), and 18% on the heart of the Antarctic notothenid *T. newnesi* (Egginton and Campbell unpublished). Following a study of cardio-respiratory control in seven Antarctic notothenids, Egginton et al. (unpubl. data) suggested that there may an effect of lifestyle, with more sedentary species having a higher level of cholinergic tone.

Previous research into the effect of an increase in water temperature on the cardiovascular systems of Antarctic notothenid fish has demonstrated that the heart rates of these fishes are remarkably thermally independent (Axelsson et al. 1992; Franklin et al. 2001). This thermal independence has been reported as high as 10°C in *P. borchgrevinki* (Forster et al. 1998), although the heart rate of *T. bernacchii* appears to be temperature-dependent above about 3°C (Axelsson et al. 1992; Forster et al. 1998). The thermal independence of heart rate of *P. borchgrevinki* from -1.2 to 3°C has been attributed to an increase in the level of inhibitory cholinergic tonus (Franklin et al. 2001). Ventral aortic pressure has also been reported as thermally sensitive in the majority of studies (Axelsson et al. 1992; Forster et al. 1998), although Franklin et al. (2001) observed a 20% decrease in ventral aortic pressure of *P. borchgrevinki* during an increase in water temperature from -1.2 to 3°C.

This study was designed to investigate further this thermal insensitivity of heart rate and blood pressure. The Antarctic notothenid *P. borchgrevinki* was acutely exposed to water temperatures of 3, 6, and 10°C and heart rates and ventral aortic pressures were monitored during both exposure and recovery. The relative levels of cholinergic and adrenergic influence on the heart were determined at -1 and 6°C in order to compare the values with those previously determined at -1 and 3°C by Franklin et al. (2001) and gain greater insight into the mechanisms controlling heart rate at different temperatures.

MATERIALS AND METHODS

Pagothenia borchgrevinki were caught during October and November 2003, as described in Chapter One, and transported to Scott Base where they were kept in the flow-through aquarium system (water temperature -1.0 ± 0.3°C) in the Wet Laboratory at Scott Base, Antarctica. The fish held for a minimum of three days before experiments commenced. During this time they were not fed and a natural photoperiod (24-hour daylight) was maintained. Any fish with gills affected by X-cell disease (Franklin and Davison 1987; Davison 1998) were excluded from the study as this has been shown to produce chronic hypertension (Davison and Franklin 2003).

In order to cannulate the ventral aorta, the fish were individually anaesthetised in a 0.1 g L^{-1} solution of unbuffered MS222 (ethyl m-aminobenzoate methanesulphonate) dissolved in -1°C seawater, taking 10 - 15 minutes to achieve mild anaesthesia. Each fish was then placed on its right side in a surgical sling, allowing access to the left operculum, which was retracted back with a loose clamp exposing the gill arches beneath. Whilst on the operating table, the gills were continuously irrigated with an aerated 0.05 g L^{-1} MS222 solution. A small hole was made in the afferent vessel of the second gill arch using a 25-gauge needle, and the vessel cannulated with polyethylene tubing (PE10 connected to PE50). The tubing was tied, using silk suture thread, around the gill arch, and also held in place by two sutures placed on the skin dorsal to the operculum. Heparinsed (ammonium heparinate, Sigma Ltd.) physiological saline was used to flush the cannula. The physiological saline was based on analysis of *P. borchgrevinki* serum (Dobbs and De Vries 1975), as described by Macdonald (1997) (Appendix 1). Following surgery, the fish were kept in smaller tanks within the continuously-flowing aquarium system for 24 hours to recover from the effects of anaesthesia and surgery. Opaque sheeting was employed to screen the fish from nearby human activities. Following the recovery period, the fish were randomly assigned to the experiments of Series 3.1 and Series 3.2.

SERIES 3.1. EFFECT OF AN ACUTE INCREASE IN TEMPERATURE

P. borchgrevinki (mass 117.7 ± 29.6 g (mean ± SD), range 78.4 – 135 g, total length 233.9 ± 18.3 mm (mean ± SD), range 208 – 263 mm, n = 8) were used for this experiment. The ventral aortic cannula of an individual fish was connected to an ADInstruments disposable pressure transducer which fed into an ADI Powerlab data acquisition system, sampling at 20 Hz. The Powerlab was connected to a portable laptop running Chart for Windows Version 4 recording software. Great care was taken to ensure that all air-bubbles were removed from the lines to avoid interference with the response. Heart rate and blood pressure were monitored for an hour at -1°C to obtain baseline data, following which the effect of handling was determined by briefly lifting the fish from the water and monitoring recovery for an hour. The fish was then lifted from the -1°C tank and placed into an adjacent, identical tank maintained at 3.0 ± 0.3°C. Recording continued for an hour with the fish held at 3°C, and recovery was then monitored for an hour following return of the fish to the -1°C tank. The procedure was repeated at a temperature of 6°C. After an hour of recovery from exposure to 6°C, four of the fish were subjected to a 15 minute immersion in 10°C water. On completion of the experiment, mass and total length (from nose to tip of tail) of each fish was measured.

As a control, a second group of *P. borchgrevinki* (mass 95.4 ± 14.0 g (mean ± SD), range 78.2 – 113.2 g, total length 224.3 ± 9.2 mm (mean ± SD), range 212 – 236 mm, n = 6) was exposed to 6°C without prior exposure to 3°C in order to assess whether there had been a cumulative response.

SERIES 3.2. CHOLINERGIC AND ADRENERGIC TONE

Pagothenia borchgrevinki (total length 219 ± 16 mm (mean ± SD), range 179 – 240 mm, n = 20) were randomly divided into three treatment groups. The set-up used to record heart rate and blood pressure was identical to that described in Series 3.1. The first group of fish (n = 8) were injected with the cholinoceptor antagonist atropine sulphate (1.5 mg kg^{-1} body weight) via the afferent branchial cannula following a 30 minute period of recording baseline levels at -1°C. The fish were left for 30 minutes for the drug to take effect, as indicated by the stabilisation of heart rate and blood pressure to new levels, before being rapidly transferred into an identical, adjacent tank with a water temperature

of 6.0 ± 0.3°C. After 20 minutes of recording, the cholinoceptor agonist carbachol (0.1 mg kg^{-1}) was injected via the cannula and heart rate was monitored for a further 15 minutes. The second group of fish (n = 6) was injected with atropine and then the β-adrenoceptor antagonist sotalol (3 mg kg^{-1} body weight) following the 30 minute baseline recording. These fish were also transferred to 6°C after 30 minutes. After recording for 20 minutes, the adrenergic agonist adrenaline (10 μg kg^{-1}) was injected into the cannula and heart rate was monitored for 15 minutes. The third group of control fish (n = 6) were injected with physiological saline following baseline recording. They were given 30 minutes to recover before being transferred to 6°C and heart rate was then recorded for 20 minutes. The injection volumes were 0.1 mL for group one, 0.2 mL in total for group two, and 0.2 mL of saline for the third group.

DATA ANALYSIS AND STATISTICS

Mean heart rate and ventral aortic pressure were averaged over one minute blocks. In order to determine the cholinergic and adrenergic tone on the heart, the heart rate was averaged over the final 5-minute period at both -1 and 6°C. The tone was then calculated using the following formulae, modified from Cameron (1979) (see Axelsson 1988):

$$\text{Cholinergic Tone} = \frac{\text{Heart Rate (ATROPINE)} - \text{Heart Rate (CONTROL)}}{\text{Heart Rate (INTRINSIC)}}$$

$$\text{Adrenergic Tone} = \frac{\text{Heart Rate (ATROPINE)} - \text{Heart Rate (INTRINSIC)}}{\text{Heart Rate (INTRINSIC)}}$$

The control heart rate was determined 30 minutes following the injection of 0.2 mL saline, while the intrinsic heart rate was the rate measured after treatment with both atropine and sotalol.

Q_{10}

Q_{10} values for both series were calculated using the Van't Hoff equation (Hoar 1975):

$$Q_{10} = (R_2 / R_1)^{10/(T_2-T_1)}$$

where: R_1 and R_2 are the rates at temperatures T_1 and T_2, respectively.

Statistical Analysis

Evaluation of statistically significant differences was made by one-way analysis of variance (ANOVA) using the repeated-measures method, except in comparisons with the 10°C data where standard ANOVA was employed due to the absence of matched data pairs. Where a treatment effect was indicated, post-hoc comparisons were performed. Dunnett's test was used to compare handling responses with the control values, and Tukey-Kramer tests were employed to compare the heart rates at different temperatures. The data were log transformed where indicated necessary by Bartlett's test, to improve homogeneity of variance. Analyses were carried out using GraphPad Prism version 4.00 software for Windows, with statistical significance taken at the level of $p<0.05$. Data are represented as mean ± SEM, unless otherwise stated.

RESULTS

The resting heart rate and ventral aortic pressure of *P. borchgrevinki* at -1°C were 16.0 ± 0.5 beats min^{-1}, and 3.2 ± 0.1 kPa, respectively.

HANDLING STRESS

Heart rate was unaffected by lifting the fish from the water (Fig. 3.1a), although there was a transient increase in ventral aortic pressure between 2 and 4 minutes post-handling (Fig. 3.1b).

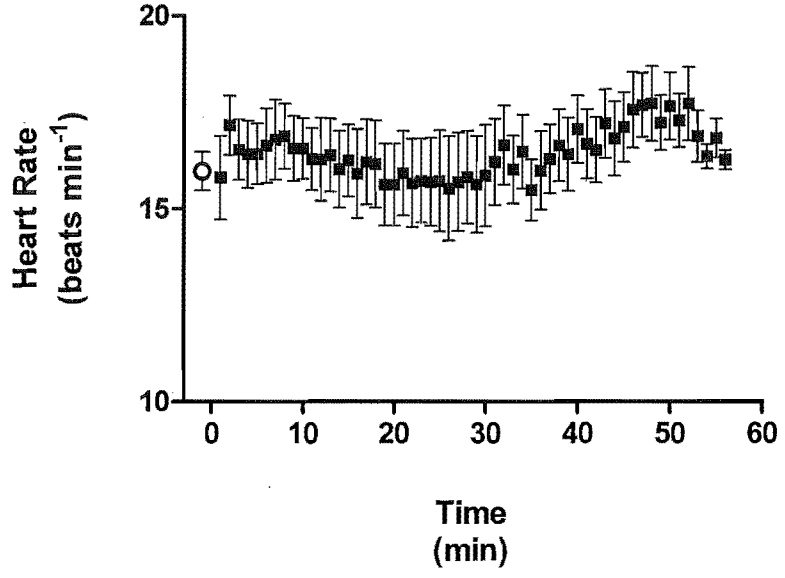


Fig. 3.1a. Heart rate following handling at -1°C. Handling stress occurred at T= 0. The open circle represents resting heart rate. N = 8.

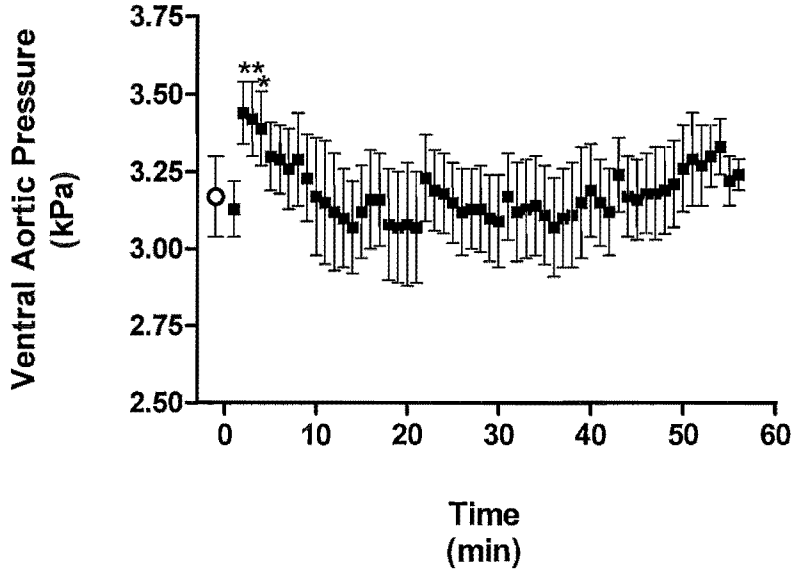


Fig. 3.1b. Ventral aortic pressure following handling at -1°C. Handling stress occurred at T= 0. The open circle represents resting ventral aortic pressure. * Significantly different from resting value. N = 8.

SERIES 3.1. EFFECT OF AN ACUTE INCREASE IN TEMPERATURE

Exposure to an Increase in Temperature

There was no significant difference in heart rate or ventral aortic pressure between the fish exposed to 3 and then 6°C and those exposed to 6°C alone. The data presented in the results are from the group of fish exposed to the two temperatures consecutively.

Transfer of the fish into 3°C water elicited an immediate but transient bradycardia (Fig. 3.2a) and accompanying drop in ventral aortic pressure (Fig. 3.3a). Heart rate then rapidly increased to be consistently above the -1°C rate after 15 minutes. The mean heart rate after 45 minutes at 3°C was 25.3 ± 2.6 beats min^{-1}. Ventral aortic pressure also increased, to a maximum value of 3.9 ± 0.2 kPa after 5 minutes, but then rapidly decreased back to the baseline level.

At 6°C, the initial bradycardia was less marked and the increase in heart rate more rapid than at 3°C (Fig. 3.2b). Heart rate was elevated above both -1°C and 3°C levels after 2 minutes, and remained elevated for the entire 6°C exposure. Mean heart rate after 45 minutes at 6°C (33.7 ± 2.6 beats min^{-1}) was significantly higher than at 3°C. Ventral aortic pressure during acute exposure to 6°C exhibited a similar pattern to that at 3°C, with a drop during the first minute, followed by a transient increase before levels rapidly returned to baseline (Fig. 3.3b).

At 10°C, the bradycardia during the first minute was less apparent than at 3 and 6°C (Fig. 3.2c), although there was a similar drop in ventral aortic pressure (Fig. 3.3c). Heart rate rapidly increased and was consistently higher than rates at 3 and 6°C for the entire 15-minutes period, although the differences were not always significant due to the higher level of inter-individual variation at 10°C. The mean heart rate after 10 minutes at 10°C was 38.5 ± 7.8 beats min^{-1}. The ventral aortic pressure had not returned to the -1°C baseline value after 1 hour of recovery from 6°C exposure and was thus still significantly elevated at the time of exposure to 10°C. The ventral aortic pressure during 10°C exposure attained a higher peak value than at 3 and 6°C, reaching 4.0 ± 0.2 kPa after 3 minutes, although the effect was still transient with a rapid return to baseline levels.

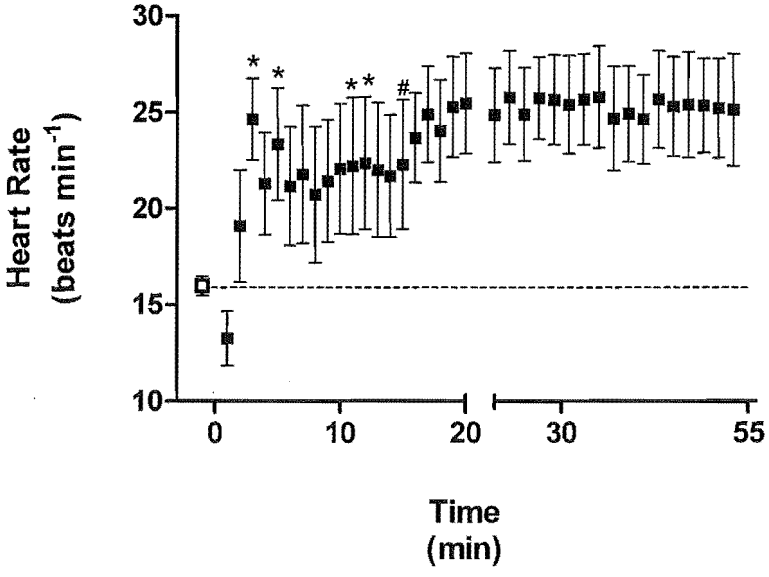


Fig. 3.2a. Heart rate of *P. borchgrevinki* during acute exposure to a water temperature of 3 °C. The dotted line and open symbol represent resting heart rate at -1°C immediately prior to the increase in temperature at T = 0. N = 8. * Significantly different from -1°C baseline heart rate . # Significantly different from -1°C baseline rate from this point on.

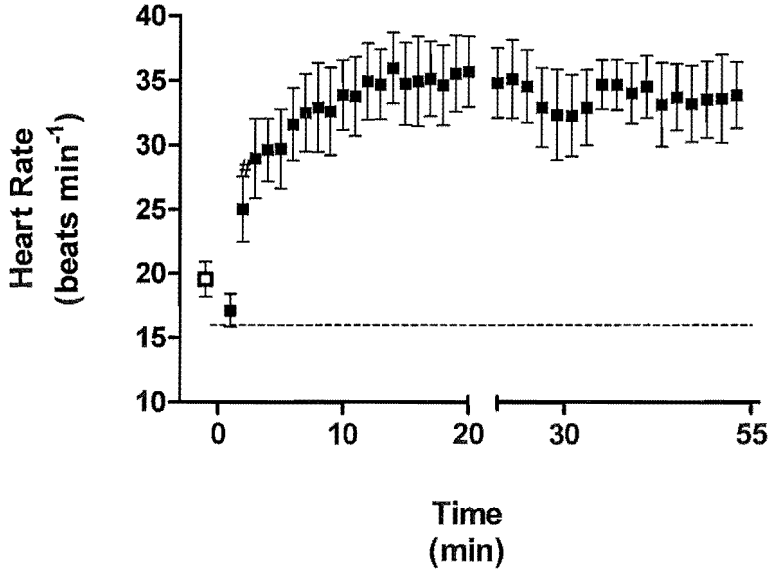


Fig. 3.2b. Heart rate of *P. borchgrevinki* during acute exposure to a water temperature of 6°C. The open symbol represents heart rate at -1°C immediately prior to the increase in temperature at T= 0, and the dotted line represents baseline heart rate at -1°C. #Significantly different from rates at -1 and 3°C from this point on. N = 8.

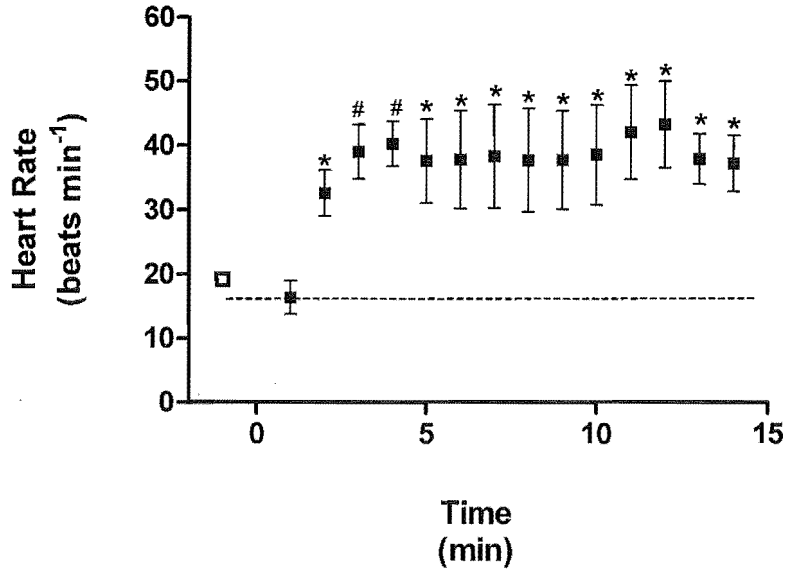


Fig. 3.2c. Heart rate of *P. borchgrevinki* during acute exposure to a water temperature of 10°C. The open symbol represents resting heart rate at -1°C immediately prior to the increase in temperature at T= 0, and the dotted line represents baseline heart rate at -1°C .* Significantly different from rates at -1 and 3°C. # Significantly different from rates at -1, 3, and 6°C.

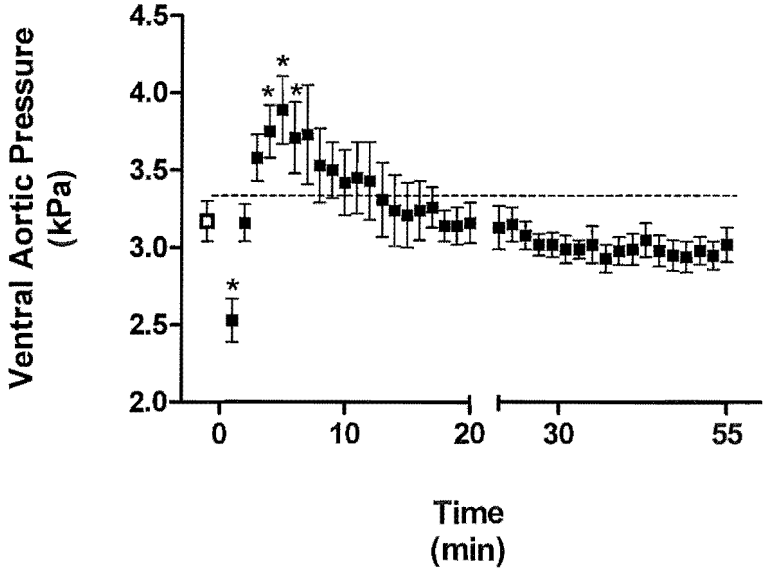


Fig 3.3a. Ventral aortic pressure of *P. borchgrevinki* during acute exposure to 3°C. The open symbol represents ventral aortic pressure at -1°C immediately prior to the increase in temperature at T= 0, and the dotted line represents baseline ventral aortic pressure at -1°C. N = 8. * Significantly different from -1°C baseline ventral aortic pressure.

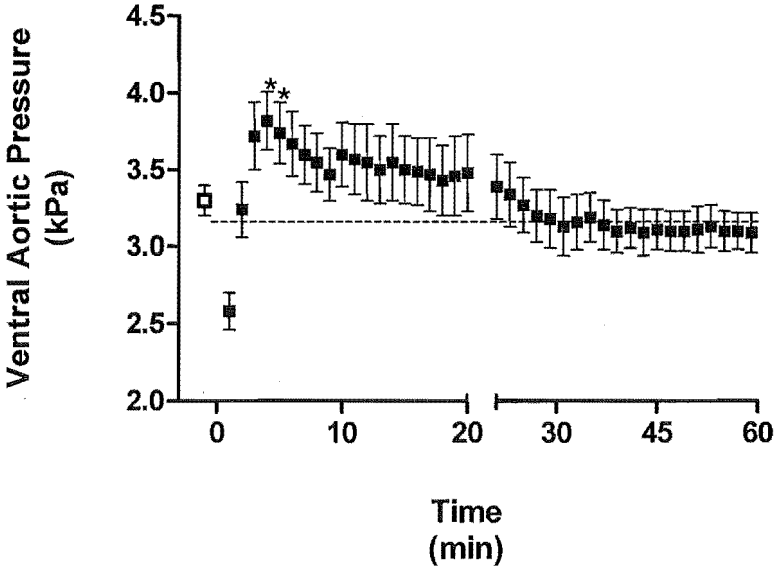


Fig 3.3b. Ventral aortic pressure of *P. borchgrevinki* during acute exposure to 6°C. The open symbol represents ventral aortic pressure at -1°C immediately prior to the increase in temperature at T= 0, and the dotted line represents baseline ventral aortic pressure at -1°C. N = 8. * Significantly different from -1°C baseline ventral aortic pressure.

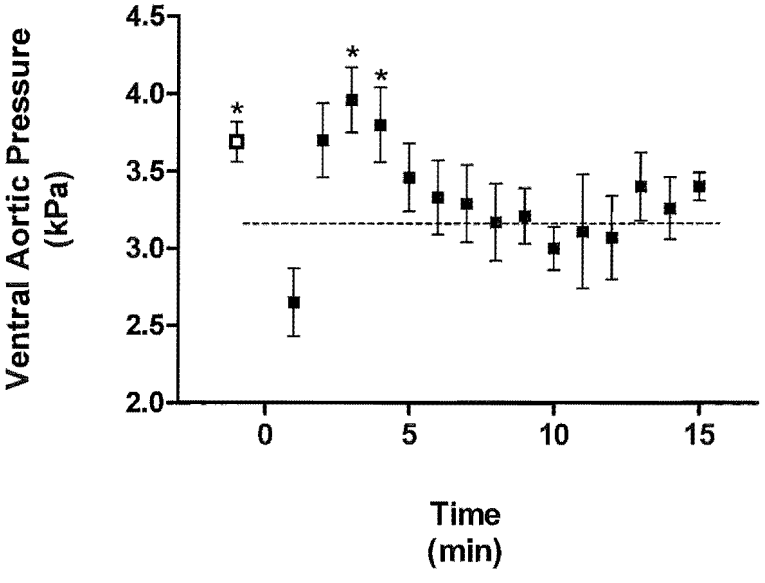


Fig 3.3c. Ventral aortic pressure of *P. borchgrevinki* during acute exposure to 10°C. The open symbol represents ventral aortic pressure at -1°C immediately prior to the increase in temperature at T= 0, and the dotted line represents baseline ventral aortic pressure at -1°C. N = 4. * Significantly different from -1°C baseline ventral aortic pressure.

The rate of increase in heart rate was greatest from -1 to 3°C (Q_{10} = 3.3), with a Q_{10} value of 2.5 from 3 to 6°C, and relative thermal insensitivity (Q_{10} = 1.2) from 6 to 10°C (Fig. 3.4, Table 3.1). At 3°C, the heart beats were regular and even, while at 6°C and 10°C the majority of fish exhibited phasic increases and decreases in heart rate as illustrated in Fig. 3.5.

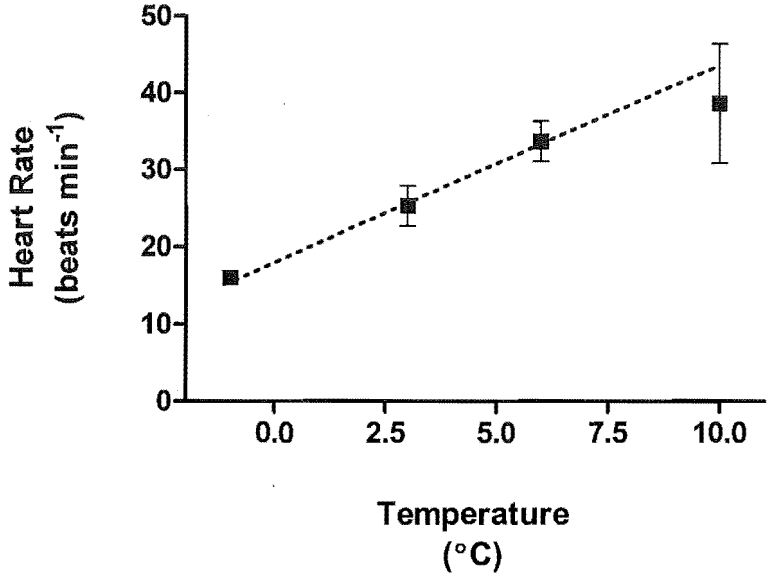


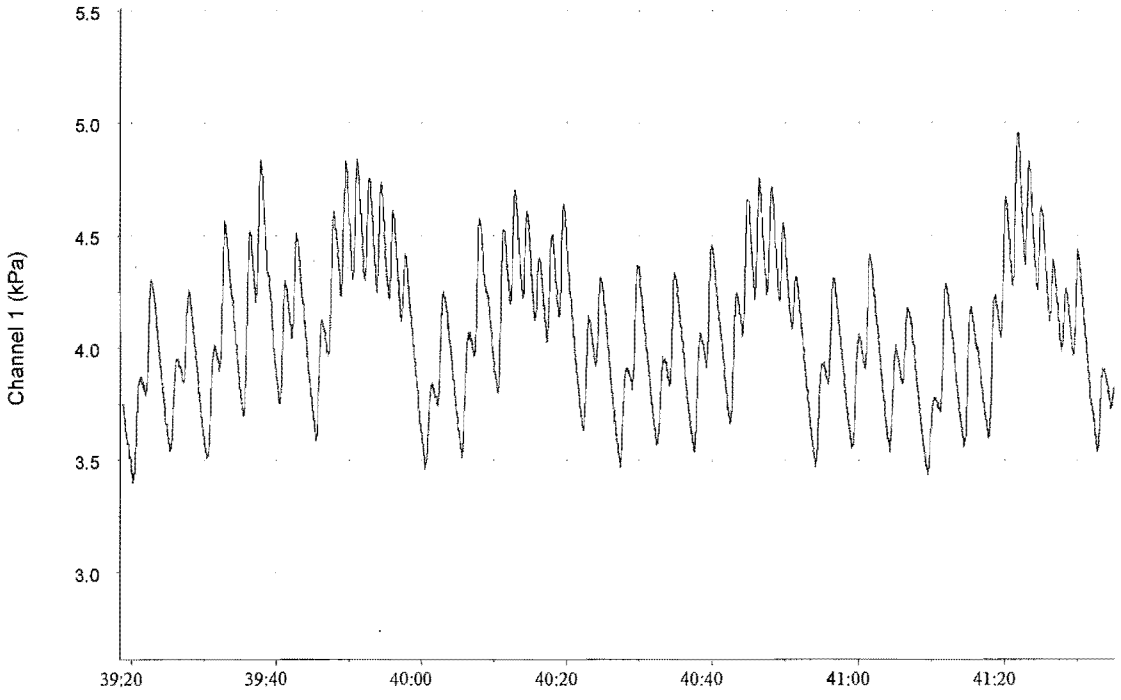
Fig. 3.4. Effect of an acute increase in temperature on heart rate of *P. borchgrevinki*. Heart rate data obtained after 45 minutes at 3 and 6°C, and after 10 minutes at 10°C. The slope of the regression line is 2.52 ± 0.13 (r^2 = 0.997).

Table 3.1. The effect of an acute increase in temperature on heart rate and ventral aortic pressure.

Temperature (°C)	Heart Rate (beats min^{-1})	Ventral Aortic Pressure (kPa)
-1	16.0 ± 0.5	3.2 ± 0.1
3	25.3 ± 2.6*	3.0 ± 0.1
6	33.7 ± 2.6#	3.1 ± 0.1
10	38.6 ± 7.8*	3.0 ± 0.1

Heart rates obtained after 45 minutes at 3 and 6°C, and after 10 minutes at 10°C. N = 8 for -1, 3 and 6°C groups, N = 4 for 10°C group. * Significantly different from rate at -1°C. # Significantly different from rates at -1 and 3°C.

Fig. 3.5. Raw Powerlab ventral aortic pressure trace from a fish in 6°C water.



Recovery from Exposure to an Increase in Temperature

Following return of the fish to -1°C water, heart rate steadily declined to reach baseline levels within an hour after exposure to both 3 and 6°C (Fig. 3.6a and 3.6b). In contrast to the brief and transient hypertension of the fish on exposure to an increase in temperature, the return to -1°C elicited a prolonged elevation of ventral aortic pressure (Fig. 3.7a and 3.7b). Upon return to -1°C following 3°C exposure the pressure was elevated after 2 minutes, attained a maximum value of 3.98 ± 0.16 kPa after 6 minutes, and had returned to baseline levels after 20 minutes. Return of the fish to -1°C following 6°C exposure resulted in a significant elevation of ventral aortic pressure within 1 minute and a maximum value of 3.83 ± 0.20 kPa after 11 minutes. The ventral aortic pressure was still elevated above the -1°C baseline level 60 minutes after the return from 6 to -1°C.

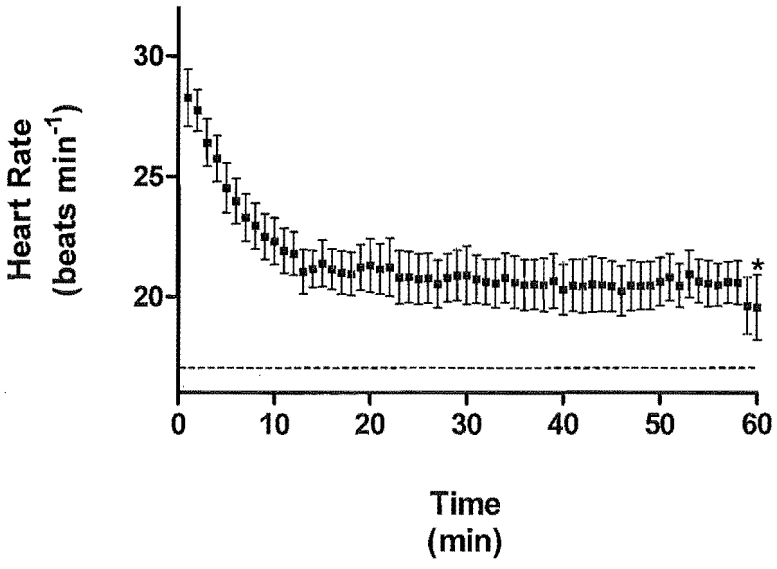


Fig. 3.6a. Heart rate of *P. borchgrevinki* returned to -1°C at T= 0 after 1 hour of exposure to 3 °C. The dotted line represents baseline heart rate at -1°C. N = 8. * **Not** significantly different from baseline heart rate.

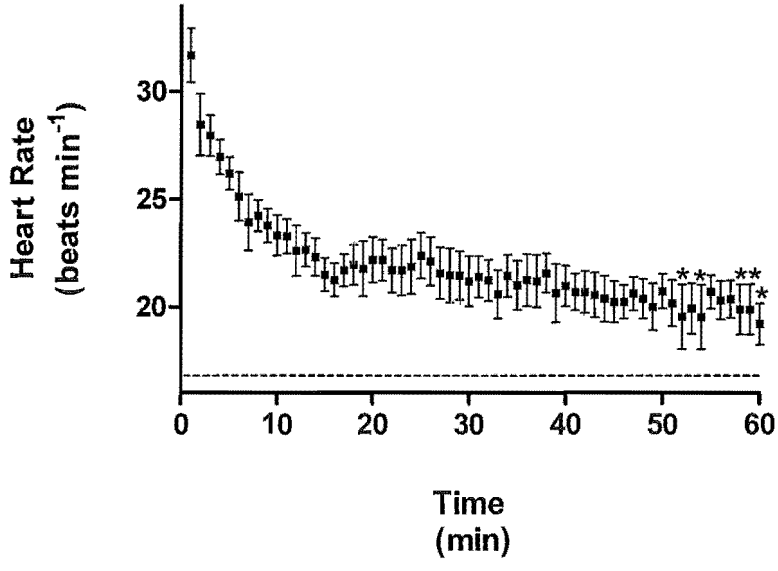


Fig. 3.6b. Heart rate of *P. borchgrevinki* returned to -1°C at T= 0 after 1 hour of exposure to 6°C. The dotted line represents baseline heart rate at -1°C. N = 8. * **Not** significantly different from baseline heart rate.

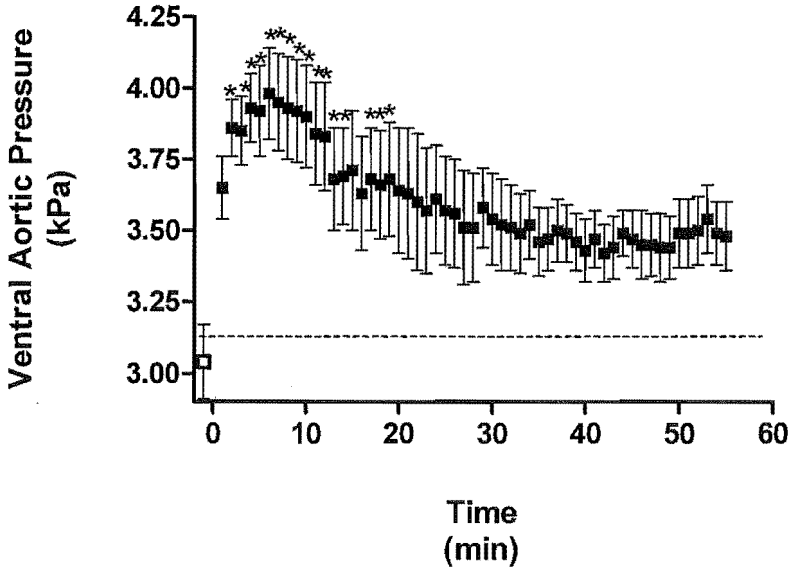


Fig. 3.7a. Ventral aortic pressure of *P. borchgrevinki* returned to -1°C at T= 0 after 1 hour of exposure to 3°C. The dotted line represents baseline ventral aortic pressure at -1°C. N = 8. * Significantly different from -1°C baseline ventral aortic pressure.

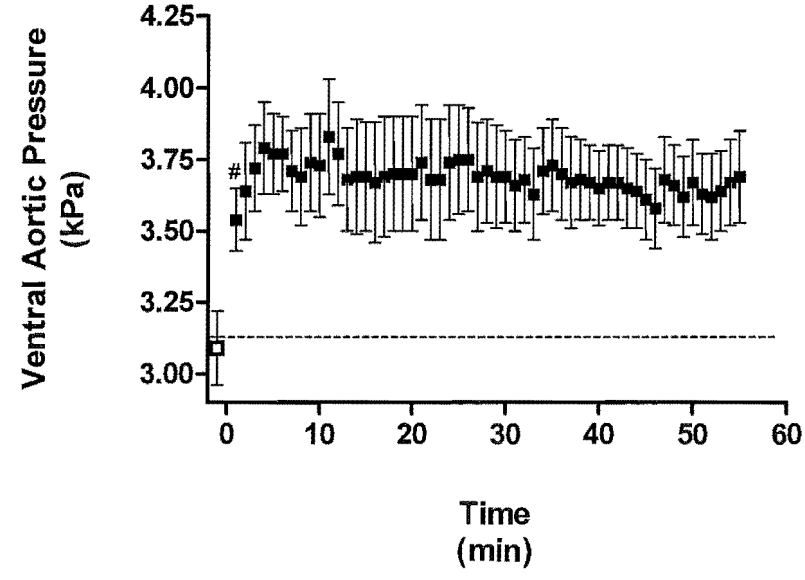


Fig. 3.7b. Ventral aortic pressure of *P. borchgrevinki* returned to -1°C at T= 0 after 1 hour of exposure to 6 °C. The dotted line represents baseline ventral aortic pressure at -1°C. N = 8. # Significantly different from -1°C baseline ventral aortic pressure from this point on.

SERIES 3.2. CHOLINERGIC AND ADRENERGIC TONE

The heart rate (16.0 ± 1.6 beats min^{-1}) of *P. borchgrevinki* measured 30 minutes after injection of 0.2 mL saline via the ventral aortic cannula at -1°C was not significantly different from the mean value of all 20 fish at -1°C prior to treatment (15.8 ± 0.7 beats min^{-1}). The resting heart rate of saline-injected fish at 6°C (26.4 ± 3.0 beats min^{-1}) was significantly higher than the rate at -1°C, although lower than the 33.7 ± 2.6 beats min^{-1} recorded in Series 3.1.

Following the treatment with atropine, heart rate increased at both -1 and 6°C, with rates of 24.3 ± 0.5 and 45.7 ± 1.0 beats min^{-1} at the two temperatures, respectively. Combined atropine/sotalol treatment produced a less marked increase in heart rate at both -1 and 6°C, resulting in intrinsic heart rates of 18.9 ± 0.7 and 32.3 ± 1.4 beats min^{-1}, respectively (Fig. 3.8). Both intrinsic and actual heart rates increased with similar temperature coefficients (Q_{10} values) from -1 to 6°C (Table 3.2). Treatment with carbachol and adrenaline had no significant effect on the heart rates following the atropine and atropine/sotalol treatments respectively, indicating that complete blockage of both cholinergic and adrenergic control mechanisms had been achieved.

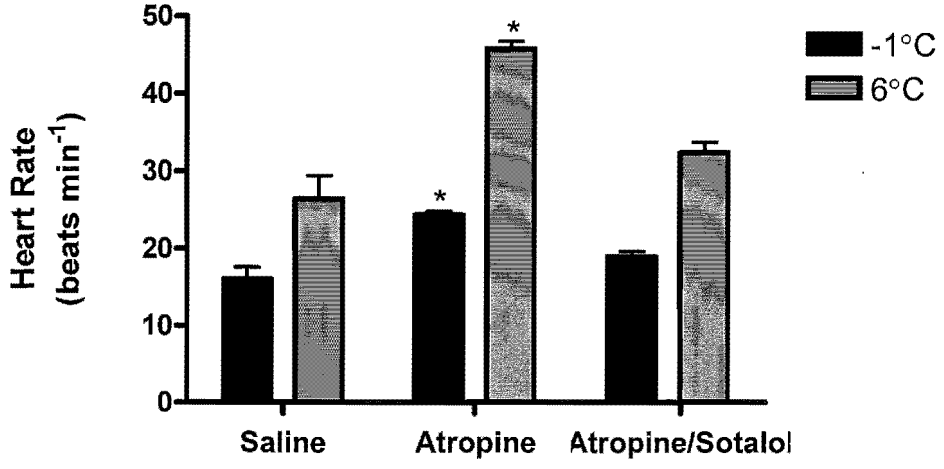


Fig. 3.8. Heart rate of *P. borchgrevinki* after treatment with saline, atropine and sotalol at two different temperatures. The -1°C values were recorded 30 minutes after injection of the drug/saline through a ventral aortic cannula, and the 6°C values recorded 20 minutes after subsequent transfer to 6°C water. * Significantly different from rates after both saline and atropine/sotalol treament.

The resting heart rate of *P. borchgrevinki* at -1°C was the result of an inhibitory cholinergic tone of 43.8% and an excitatory adrenergic tone of 28.7%, while the resting heart rate at 6°C was due to 59.9% cholinergic tone and 41.6% cholinergic tone (Table 3.2). This represents a 37% increase in the level of cholinergic tone and a 45% increase in adrenergic tone from -1 to 6°C.

Table 3.2. Heart rate and cholinergic and adrenergic tonus on the heart of *P. borchgrevinki* at -1°C and 6°C.

Temperature (°C)	Actual Heart Rate (beats min^{-1})	Intrinsic Heart Rate (beats min^{-1})	Cholinergic Tone (%)	Adrenergic Tone (%)
-1	16.0 ± 1.6	18.9 ± 0.7	43.8	28.7
6	26.4 ± 3.0	32.3 ± 1.4	59.9	41.6
Q_{10} (-1 to 6°C)	2.0	2.2	1.6	1.7

The actual heart rates were obtained following saline treatment. Intrinsic heart rates were determined following combined atropine/sotalol treatment. N = 6 for saline and atropine /sotalol groups, N = 8 for atropine group.

There was no change in ventral aortic pressure following the administration of 0.2 mL saline via the ventral aortic cannula, and no difference between the ventral aortic pressures at -1 and 6°C in any of the treatment groups.

DISCUSSION

The heart rate of *Pagothenia borchgrevinki* was found to be thermally sensitive from -1 to 6°C, in contrast to previous findings (Forster et al. 1998; Franklin et al. 2001). The gradual (0.1°C $minute^{-1}$) elevation of temperature by Franklin et al. (2001) may have contributed to the different response, as might the ~15 minute delay prior to elevation of heart rate at 3°C observed in this study. Forster et al. (1998), however, employed a sudden temperature change similar to that of the current study.

In another Antarctic notothenid, *Trematomus bernacchii*, a slow rate of temperature increase resulted in minimal change of heart rate up to 2.5°C due to an increase in vagal tonus, although as temperature increased above 2.5°C there was a rapid increase as the vagal tone was released (Axelsson et al 1992). In the current study, the 65% increase in heart rate of *P. borchgrevinki* from -1 to 6°C was associated with a 45% increase in the excitatory adrenergic tone on the heart (from 28.7 to 41.6%), masking the 37% increase in inhibitory cholinergic tone (from 43.8 to 59.9%). This is in contrast to a greater increase (57%) in cholinergic tone masking a smaller increase in adrenergic tone (21%) reported by Franklin et al. (2001) in explanation of their observation of thermal insensitivity of heart rate from -1 to 3°C. Given the significant increase in heart rate from -1 to 3°C in the current study, it is possible that sudden temperature changes may reduce the ability of these fish to maintain control of heart rate through increases in cholinergic tone. Warm-adapted eels display a lower heart rate than cold adapted eels at a similar temperature, although the difference is able to be removed by vagal blockade, indicating an increase in vagal tone with increasing temperature during thermal acclimation (Seibert 1979). At both 6 and 10°C, periods of phasic decrease in the heart rate of *P. borchgrevinki* were observed (Fig. 3.5). These have previously been described as a form of cholinergic bradycardia, followed by periods of escape from the vagal tone which result in increased heart rate (Axelsson et al. 1992), indicating the inability of the cardiac system to maintain cholinergic control at higher temperatures. These phasic decreases were more frequent at 10°C, which contributed to a greater level of variance within the small data set at this temperature and complicated the assessment of heart rate. The phasic decreases were disregarded during the quantitative determination of heart rate in this study.

Oxygen consumption (Forster et al. 1987), mitochondrial respiration rates (Johnston et al. 1994), muscle contractile rates (Johnson and Johnston 1991), and atrial pacemaker rates (Macdonald 1997) all increase with an acute increase in the body temperature of Antarctic notothenioid fishes, and it is therefore not surprising that heart rate should follow a similar pattern. In the majority of teleosts, the effect of increased temperature on heart rate and stroke volume involves positive chronotropic and inotropic responses and it has been suggested that these responses represent a compensatory mechanism by which cardiac output is intrinsically increased to meet the increased metabolic needs of the tissues due to temperature (Farrell 1984). Although an increase in heart rate was originally thought to represent a mechanism by which cardiac output was

increased to meet the increased metabolic needs of the tissues (Farrell 1984), it has more recently been realised that an increase in heart rate is not a good predictor of cardiac function at high temperatures (Farrell et al. 1996; Farrell 1997) due to the general trend for decreasing stroke volume at higher heart rates (Graham and Farrell 1989; Keen and Farrell 1994). The majority of fishes, in contrast to mammals, birds, reptiles and amphibians, primarily modulate stroke volume rather than heart rate to effect changes in cardiac output (Farrell 1991). Tunas have been identified as an exception to the generality (Brill and Bushnell 1991) and, from the data presented in this study, it appears as though Antarctic notothenioid fishes could be another exception. With the large inhibitory cholinergic tone on the heart of Antarctic notothenioid fishes at rest, there is scope for large increases in heart rate and, as a result, there appears to be a greater reliance on chronotropic rather than inotropic changes to alter cardiac output (Axelsson et al. 1992). The thermal insensitivity of heart rate observed by Franklin et al. (2001), combined with the marked increase in haematocrit of *P. borchgrevinki* in response to acute elevation of temperature (Franklin et al. 1991), led the authors to suggest that the increased metabolic needs resulting from elevated temperature may be met primarily by an increase in haematocrit, rather than a change in cardiac output. Acute exposure of *T. bernacchii* to 5°C increases heart rate and decreases stroke volume, resulting in an increase in cardiac output of only 10% (Axelsson et al. 1992), although the increase in haematocrit of this species is not as marked as that of *P. borchgrevinki* (Davison et al. 1988; Davison et al. 1994; Lowe and Wells 1997). The increase in heart rate of *P. borchgrevinki* in the current study does not necessarily represent an increase in cardiac output and therefore the hypothesis of Franklin et al. (2001) may still apply.

The vagal tone on the heart of *P. borchgrevinki* is thought to be due to vagal innervation (Axelsson et al. 1992; Axelsson et al. 1994; Nilsson et al. 1996). The cholinergic nerve fibres reach the heart via the cardiac branch of the vagus, which may also contain adrenergic fibres that enter the vagus trunk from the sympathetic chains (Morris and Nilsson 1994). It is unknown, however, whether the adrenergic tone is the result of humoral or neural effects. Adrenergic control of the circulatory system in vertebrates may originate from three systems: endocrine cells which release their stored catecholamines into the blood stream, paracrine cells which modulate cardiac activity by the local release of catecholamines, and an adrenergic innervation of the heart, the branchial vasculature and the systemic vasculature (Axelsson et al. 1998). Antarctic notothenioids do not tend to increase levels of circulating catecholamines in response to

moderate levels of stress (Egginton 1994; Davison et al. 1995; Egginton 1997; Lowe and Wells 1997; Egginton and Davison 1998), although both *P. borchgrevinki* and *T. bernacchii* can release significant quantities of adrenaline and noradrenaline into the bloodstream in response to the severe stressor of acute 10°C exposure (Forster et al. 1998). This increase in circulating catecholamine levels has been demonstrated to have little effect on heart rate of these species (Forster et al. 1998), or of other Antarctic notothenioids (Egginton 1997), indicating that the increase in adrenergic tone on the heart of *P. borchgrevinki* at higher temperatures is likely to be predominantly neurally mediated.

The study of cardiac autonomic regulation using reductionistic methods, such as the pharmacological isolation of autonomic effectors in this study, has provided a consistent body of knowledge but is limited by the need to disrupt the system under study by addition of drugs (Altimiras 1999). The relative cholinergic and adrenergic tones calculated on the heart of *P. borchgrevinki* in the current study are very similar to those obtained from bilaterally vagotomised fish (Egginton et al. unpubl. results), indicating that the effect of any physiological side-effects of the drugs on the results was minimal. It is possible that there may be a chronic effect of stress on fish following anaesthesia and surgery but, due to the problems associated with keeping cannulated animals for long periods of time, 24 hours was longest practical recovery period. MS222 is reported to be cleared from the blood of anaesthetised trout after 3 – 6 hours (Houston and Woods 1972), and as these fish were only lightly anaesthetised it is unlikely that there any residual effects of the anaesthetic after 24 hours, even at -1°C. Considering the possibility that cannulated fish at warmer temperatures have a greater propensity for blood loss, the hole in the vessel was made as small as possible. Egginton et al. (unpubl. data) have indicated that heart rate of Antarctic notothenиids may require at least 48 hours to return to resting levels following anaesthetisation and surgery, although the values from the current study are not significantly different from the values they measured 96-hours post-surgery, suggesting that the fish in this study were well-recovered.

The increases in heart rate of *P. borchgrevinki* from -1 to 6°C in both sections of this study (Q_{10} values of 2.9 and 2.0 in Series 3.1 and 3.2, respectively) represent a typical thermal response (Farrell and Jones 1992), with resting heart rates of most temperate fishes increasing in response to acute changes in temperature with an average Q_{10} of about 2.0 (Priede 1974; Butler and Taylor 1975; Cech et al. 1976; Graham and Farrell 1985; Blank et al. 2002; Tiitu and Vornanen 2002). The temperature coefficient

(Q_{10}) describing the increase in heart rate of *P. borchgrevinki* during an acute increase in temperature decreased as the temperature was increased, with Q_{10} values of 3.3 from -1 to 3°C, and 2.5 from 3 to 6°C. From 6 to 10°C, however, thermal sensitivity of heart rate was reduced with a Q_{10} value of 1.2 over this temperature range. The rate of increase in heart rate generally declines at temperatures near the upper lethal limit (Morita and Tsukuda 1994), and the thermal insensitivity of *P. borchgrevinki* above 6°C agrees well with the previously reported upper thermal limit of this species (Somero and De Vries 1967). With a plateau in heart rate from 6 to 10°C and a probable decrease in stroke volume, cardiac output is likely to have declined, as has been observed in this species at 8°C (Chapter 7). *P. borchgrevinki*, acclimated to -1°C, die within one to two hours at 10°C (Macdonald et al. 1988) and it has been suggested that a decline in cardiac performance may be the primary limitation at high temperatures (Farrell 1997).

The resting heart rates at 0°C (16.0 ± 0.5 and 15.8 ± 0.7 beats min^{-1} in the two groups of fish) compare well with those of previous studies, with the resting heart rate of *P. borchgrevinki* previously reported as 11.3 ± 2.9 beats min^{-1} at 0°C (Axelsson et al. 1992), 20.6 ± 0.9 beats min^{-1} at -0.5°C (Axelsson et al. 1994), 15.9 ± 0.8 beats min^{-1} at -1°C (Forster et al. 1998), and 17.7 ± 0.6 beats min^{-1} at -1.2°C (Franklin et al. 2001). The resting heart rate at -1°C was the result of an inhibitory cholinergic tone of 43.8% and an excitatory adrenergic tone of 28.7%. This level of cholinergic tonus is similar to the 44.6% previously measured in this species kept for 3-4 days at -1.2°C in the aquarium system at Scott Base (Franklin et al. 2001). Higher cholinergic tones (50.0-54.5%) have been recorded from *P. borchgrevinki* transported to New Zealand and held in captivity at -0.5 to 0°C for two months prior to the experiments (Axelsson et al. 1992; Axelsson et al. 1994). The increase in cholinergic tone on the heart of fish held captive for long periods may be due to the difference in temperature, with Q_{10} values varying from 1.56 (-1 to 6°C, this study) to 2.93 (-1 to 3°C, Franklin et al. 2001), or to a strengthening of cholinergic control as the metabolism of an animal slows down over long periods in captivity (Axelsson et al. 1992). The excitatory adrenergic tonus on the heart measured in the current study was also closer to the 35.5% value from fish held for 3-4 days at -1.2°C (Franklin et al. 2001), than to the 3.2% excitatory input recorded from fish held for 2 months at 0°C (Axelsson et al. 1992). Egginton et al (unpubl. data) recently determined relative levels of cholinergic and adrenergic tonus on the heart of seven Antarctic nototheniids and concluded that ecotype, rather than genotype, was the dominant influence, with sedentary species having a higher cholinergic tone. Being one of the more

active Antarctic nototheniids and with a lower level of cholinergic tone on the heart than the more sedentary species, *P. borchgrevinki* may possess a greater scope for increasing cholinergic tone on the heart and therefore a greater capacity to maintain control of heart rate during increases in temperature than its benthic relatives.

In contrast to the majority of teleosts (Axelsson et al. 1987), the cholinergic tone on the heart of *P. borchgrevinki* was higher than the adrenergic tone at both -1 and 6°C, resulting in intrinsic heart rates which were higher than the relatively low resting rates. Abolishing both cholinergic and adrenergic tone on the heart of *P. borchgrevinki* at -1°C resulted in an intrinsic heart rate of 18.9 ± 0.7 beats min^{-1}, which falls at the low end of the previously range from 19.5 to 24.3 beats min^{-1} (Axelsson et al. 1992; Macdonald 1997; Franklin et al. 2001). The thermal sensitivities of resting heart rate and intrinsic heart rate were similar from -1 to 6°C (Q_{10} values of 2.0 and 2.2, respectively) and the intrinsic heart rate at 6°C (32.3 ± 1.4 beats min^{-1}) compares well with the 31.5 beat min^{-1} intrinsic heart rate of *T. bernacchii* at 5°C (Axelsson et al. 1992). This value is also similar to the 36 beat min^{-1} intrinsic heart rate of rainbow trout at 5°C (Graham and Farrell 1989), indicating a high level of compensation in heart rate and suggesting that the intrinsic pacemaker frequency of Antarctic fish is not very different from that of temperate teleosts.

Following treatment with atropine alone, heart rates at both -1 and 6°C were higher than both resting and intrinsic values. Infusion of atropine has been shown to result in sequestering of erythrocytes in the spleen (Nilsson et al. 1996) and it is possible that with fewer erythrocytes in circulation, and therefore lower oxygen transport capacity, a large increase in heart rate may be required following atropine treatment to increase oxygen transport to the tissues (Franklin et al. 2001).

The resting ventral aortic pressure measured in the current study (3.2 ± 0.1 kPa at -1°C) compares well with values from earlier studies, which vary from 2.72 ± 0.11 kPa to 3.60 ± 0.20 kPa at temperatures from -1.2 to 0°C (Axelsson et al. 1992; Axelsson et al. 1994; Forster et al. 1998; Franklin et al. 2001). An immediate bradycardia and ventral aortic hypotension, as observed in this study, have been reported as an immediate response to a variety of external stimuli in other teleosts (Nilsson 1983) and have been attributed to air exposure in largemouth bass (Cooke et al. 2003). Whether the response of *P. borchgrevinki* was the result of the thermal change or due to the air exposure remains to be determined but the response was abolished by atropine, as reported in other species (Randall 1968; Stevens et al. 1972; Priede 1974; Wahlqvist and Nilsson 1980;

Axelsson et al. 1987), indicating that it is likely to be a vagally-mediated. The subsequent transient hypertension was observed following both handling and the acute elevation of temperature and it is therefore likely to be representative of the generalized immediate stress reaction of this species, rather than a temperature-specific response. Whether this brief hypertension is the result of an increase in cardiac output or due to an increase in the total vascular resistance is unknown. The rapid return to baseline pressures and the regulation of ventral aortic pressure during continued exposure to the elevated temperatures is in line with the majority of previous studies (Axelsson et al. 1992; Forster et al. 1998), although a 20% decrease was reported during a more gradual increase in temperature from -1.2 to 3°C (Franklin et al. 2001). The cholinergic and cholinergic/adrenergic blockade did not alter the insensitivity of ventral aortic pressure to temperature, which is in contrast to the previously reported decline in pressure of fish treated with atropine at 5°C (Axelsson et al. 1992). The regulation of ventral aortic pressure has been reported in the presence of significant increases in the concentrations of circulating catecholamines (Forster et al. 1998) indicating that these substances only have very transient effects on ventral aortic pressure.

Previous studies have reported differing results with regard to the effect of an increase in temperature on blood pressure, with thermal independence of arterial blood pressure following acute temperature transitions in some fishes (Holeton and Randall 1967; Cech et al. 1976; Axelsson et al. 1992), but not in others (Bailey and Driedzic 1989). The thermal insensitivity of ventral aortic pressure of *T. bernacchii* during exposure to a water temperature of 5°C was associated with no alteration of systemic vascular resistance (Axelsson et al. 1992), indicating that cardiac output remained constant. In contrast, winter flounder increase cardiac output but decrease vascular resistance at higher temperatures to result in thermal insensitivity of blood pressure (Cech et al. 1976). The cardiac output of *P. borchgrevinki* has been shown to double with an acute change in temperature from -1 to 6°C (Chapter Seven), although the effect was not statistically significant. The increase in cardiac output suggests that the regulation of ventral aortic pressure may be through a decrease in branchial and/or systemic resistance, although further investigation is required.

Following return of the fish to -1°C, heart rate decreased rapidly to reach control levels within an hour. The fact that there was no cumulative effect of temperature on the fish exposed to 3°C prior to 6°C, confirms that recovery had occurred within the hour. These fish have been reported not to build up an oxygen debt (Forster et al. 1987) and

exhibit limited synthesis of ATP by glycolytic pathways (Davison et al. 1988) and the rapid decrease in heart rate relates well to the absence of any significant oxygen debt requiring repayment. The ventral aortic pressure of *P. borchgrevinki*, in contrast, increased on return of the fish to -1°C and this hypertension was maintained for about 20 minutes following 3°C exposure and for over an hour after exposure to 6°C. A hypertensive effect such as this has not previously been observed in *P. borchgrevinki* during recovery from other stressors such as forced swimming or hypoxia (Axelsson et al. 1992). The vasculature of teleosts, unlike that of elasmobranches, is usually well innervated by adrenergic nerves and vascular resistance in the systemic circulation is generally modulated by neural not humoral adrenergic tone with circulating catecholamines having an effect only under very adverse conditions (Wood and Shelton 1975; Smith et al. 1985; Axelsson 1988). Antarctic notothenioids are reported to rely more on neural than hormonal control of cardiovascular function (Axelsson et al. 1992; Axelsson et al. 1994; Nilsson et al. 1996), although catecholamine infusion has been demonstrated to elicit a brief increase in ventral aortic pressure of *P. borchgrevinki*, as has Angiotensin II, due to a major increase in systemic vascular resistance (Axelsson et al. 1994). An increase in vascular resistance may therefore be responsible for the initial transient increases in the ventral aortic pressure of this species. Prolonged responses have, however, been attributed to an increase in cardiac output (Axelsson et al. 1992; Axelsson et al. 1994) and it is thought that the Antarctic notothenioids regulate blood pressure predominantly through modulation of cardiac performance rather than through changes in vascular resistance (Macdonald and Wells 1991; Axelsson et al. 1994), possibly as a consequence of the low level of total vascular resistance (Axelsson et al. 1992). With the rapid drop in heart rate of this species during recovery, there would need to be a considerable increase in stroke volume in order to increase ventral aortic pressure without an increase in vascular resistance. Further research is clearly required to determine which mechanism/s are responsible for the ventral aortic hypertension of *P. borchgrevinki* during recovery.

In conclusion, the results of this study indicate that the heart rate of -1°C-acclimated *P. borchgrevinki* is thermally sensitive to sudden changes in temperature, following a typical Q_{10} relationship to about 6°C. The level of inhibitory cholinergic tone on the heart increases as temperature is increased, but from -1 to 6°C this increase in inhibitory tonus is masked by the combination of direct temperature effects and an increase in excitatory adrenergic tone. Above 6°C, the heart rate is relatively insensitive

to further increases in temperature, which relates well to the ~6°C upper lethal temperature (Somero and De Vries 1967) of this species. As previously reported, ventral aortic blood pressure was regulated from -1 to 10°C, but an unexpected finding was the prolonged hypertension associated with the recovery from exposure to increased temperature. The mechanisms behind this increase have yet to be identified. In comparing the results of this study with those of previous investigations, it is apparent that differences in acclimation temperature of only 1-2°C, and variation in the speed of thermal change can have marked effects on the thermal sensitivity of the cardiovascular system of *P. borchgrevinki*.

Chapter Four

The effect of an acute increase in temperature on oxygen consumption rate and aerobic scope

INTRODUCTION

Lavoisier (1743-1794) is generally credited with the demonstration that respiration is a chemical phenomenon in which oxygen is used and carbon dioxide is produced. Respiration by fish includes the uptake of oxygen from the environment at sites of gas exchange (typically the gills), the use of oxygen at mitochondria within individual cells, and the excretion of waste gases to the environment (Cech 1990). Within the mitochondria, oxygen is involved in aerobic conversion of the energy contained in food to high-energy chemical bonds, such as those formed when adenosine diphosphate (ADP) is phosphorylated to form adenosine triphosphate (ATP) and the energy stored in these bonds can then be used for maintenance and to allow fish to move, digest food, grow, reproduce, and carry out other energy-requiring functions (Eckert and Randall 1983). Respiration is regulated, not only by internal processes within the fish, but also by the medium it is living in and any stresses that the environment may impose (Bligh et al. 1976) and both respiration rates and gill ventilation rates have been identified as sensitive indicators which can be used to describe the response of an organism to environmental conditions (Cech 1990).

Thermal diffusion is an order of magnitude more rapid than molecular diffusion and the large surface area and blood/water counter-current system which make the gills of fish very efficient for respiratory gas exchange also result in rapid branchial heat transfer. For most fishes, temperature throughout the body is in equilibrium with the environment to within a fraction of a degree, and therefore metabolic rate is highly

dependent on ambient water temperature. In contrast to birds and mammals (both of which increase metabolic rate and respiratory intensity in response to cold exposure), poikilotherms increase metabolic rate and respiratory intensity in response to an increase in ambient temperature. The amount of oxygen dissolved in water decreases as the temperature is increased, but is partially offset by a decrease in the viscosity of the water and a more rapid rate of gas diffusion. As temperature rises, however, there is an increasingly unfavourable balance between the cost of ventilation and rate of oxygen uptake (Cossins and Bowler 1987).

Early in the investigation of fish physiology, the observation was made that many species of fish living in polar oceans remained active at temperatures that rendered other animals sluggish or torpid (Krogh 1914). The first studies indicated that resting metabolic rates of polar fishes were higher at low temperatures than the metabolic rates of fishes adapted to higher temperatures but acclimatised to similar low temperatures (Scholander et al. 1953; Wohlschlag 1960; Wohlschlag 1964; Ralph and Everson 1968). This phenomenon came to be known as metabolic cold adaptation and has since received both support (Scholander et al. 1953; Brett and Groves 1979; De Vries and Eastman 1981; Torres and Somero 1988b; Torres and Somero 1988a), and opposition (Holeton 1974; Clarke 1983; Bushnell et al. 1994; Drud Jordan et al. 2001; Peck 2002). Many of the high oxygen consumption rates reported in the early literature are now thought to reflect the effects of handling stress (Holeton 1974; Forster et al. 1987), and the current view is that some degree of metabolic cold adaptation may occur, but that it does not amount to complete compensation (Forster et al. 1987; Wells 1987). Resting metabolism represents a cost to an organism, in that the energy utilised in maintenance must be met from food or reserves, and it is therefore unlikely that there is any evolutionary advantage to maintaining an elevated resting metabolic rate at low temperatures (Somero et al. 1968; Holeton 1974). Biochemical investigations have shown adaptive changes in the oxidative capacity of Antarctic fish (Smith and Haschemeyer 1980; Haschemeyer 1985; Torres and Somero 1988b; Torres and Somero 1988a; Crockett and Sidell 1990; Sidell 1991; Kawall et al. 2002), suggesting that the maximum capacity for aerobic energy production may be enhanced and that metabolic cold adaptation may be apparent in active metabolic rates, rather than standard or resting rates (Forster et al. 1987; Hardewig et al. 1998).

Fry (1947) first introduced the concept of scope for metabolic activity (defined as the difference between an organism's maximum metabolic rate under any given

environmental conditions, and its resting metabolic rate under the same conditions), and determinations of the metabolic scope of Antarctic notothenioid fishes at environmental temperatures have yielded values comparable to temperate fishes of similar ecotype (Forster et al. 1987; Johnston et al. 1991). Both resting oxygen consumption rate and ventilation frequency of Antarctic notothenioids have been shown to increase with an elevation in ambient temperature (Johnston et al. 1991; Wilson et al. 2002), although the maximum ventilation frequency of *P. borchgrevinki* is reported to be thermally insensitive (Wilson et al. 2002). The thermal sensitivity of maximum oxygen consumption rate and metabolic scope for activity of the Antarctic notothenioid fishes has not yet been investigated.

The main objective of this study was therefore to determine the effect of an acute increase in temperature on the maximum oxygen consumption rate and aerobic scope of two Antarctic nototheniid fishes of differing eco-physiology. *Pagothenia borchgrevinki* is a relatively active cryopelagic species (Andriashev 1970), while *Trematomus bernacchii* is of the ancestral benthic ecotype and is a relatively inactive ambush predator (Montgomery and Wells 1993).

MATERIALS AND METHODS

Pagothenia borchgrevinki (mass 83.7 ± 15.9 g, range 72 – 112 g, total length 205.2 ± 11.4 mm, range 180 – 230 mm (mean ± SD), n = 16) and *Trematomus bernacchii* (mass 87.9 ± 28.2 g, range 38 – 128 g, total length 187.6 ± 25.8 mm, range 140 – 210 mm (mean ± SD), n = 8) were caught in McMurdo Sound, Antarctica in late November / early December 2002, as described in Chapter One. The fish were held in a continuously flowing aquarium system (water temperature -1.0 ± 0.3°C) in the Wet Laboratory of Scott Base for at least three days prior to transportation to New Zealand. The flight to New Zealand was on board a LC130 Hercules aircraft in insulated bins of aerated, iced seawater and the survival rate for the 7-8 hour journey was 100%. In Christchurch, the fish were held at the University of Canterbury in a re-circulating seawater system (water temperature 0.0 ± 0.3°C) under a 24-hour daylight regime. A large proportion of the water in the system was replaced fortnightly with fresh seawater obtained from Lyttleton Harbour. A protein skimmer, biological filter, and the addition of calcium carbonate crystals maintained the concentrations of dissolved substances within a physiological

range and aeration of the water was provided by turbulence within the system. The fish were fed mussels (*Perna canaliculus*) weekly both before and between the experimental runs, but were fasted for three weeks prior to each experiment. The respirometry experiments were conducted from mid-February until mid-June 2003, and fish were randomly selected from the available pool for each experiment. Due to the limited number of fish, each individual was used several times but was fed and fasted for three weeks between consecutive experiments.

SERIES 4.1. RESTING OXYGEN CONSUMPTION RATES

The resting oxygen consumption rates (V_{O_2} rest) of *P. borchgrevinki* and *T. bernacchii* were determined at water temperatures of 3, 0 and 6°C, in that order. The respirometry chambers used for the investigation were opaque polyurethane cylinders with clear perspex lids and volumes ranging from 1410-1470 mL. Individual fish were introduced to the chambers 24-hours prior to commencement of recording, in order to allow recovery from the stress of handling. The 24-hour daylight regime (from which the fish had been removed in Antarctica) was maintained, and a tinfoil shield positioned to prevent the fish from being unduly disturbed by human activities. The chambers were immersed in water-baths at the appropriate temperature, and a continuous flow of oxygenated seawater maintained during the 24-hour settling period. At the end of this period, one of two methods of closed-box respirometry was employed to determine the rate of oxygen consumption over 90 minutes, or until oxygen tension within the chamber had been reduced to 100 mmHg. In both methods, the respirometry chamber was sealed from the reservoir circuit by closing taps on tubing adjacent to the chamber with no disturbance to the fish. As previous studies have found animal movements and water currents generated by ventilation sufficient to ensure adequate mixing of water in respirometry chambers, no additional mixing apparatus was used (Davison 1984; Sayer and Davenport 1987; Wells 1987).

In the first method, 1 mL samples were withdrawn by syringe from the respirometry chamber at 20 minute intervals, and the fluid volume replaced with fully saturated seawater by suction from a second syringe. Each sample was immediately injected into the measuring chamber of a Strathkelvin cell (MC 100) containing an IL 1302 oxygen electrode. This electrode was connected to a Strathkelvin oxygen meter

(model 781) and the oxygen tension value was recorded after 2 minutes, by which time the reading had stabilised.

The second method involved the use of a peristaltic pump to continuously draw water from the respirometry chamber past a water-jacketed IL 1302 oxygen electrode, and return it to the chamber. This oxygen electrode was connected to another Strathkelvin oxygen meter (model 781) which fed data directly into a Powerlab Data Acquisition System. The Powerlab was connected to a laptop computer (Compaq Armada 1500c) and the display was recorded by ADInstruments Chart for Windows version 4.0.2. software. The oxygen meters were calibrated at the beginning and end of each experimental run using air-saturated seawater (at the same temperature and salinity as that used in the subject respirometer) and the zero level checked daily using a solution of 3.81 g L^{-1} sodium borate into which crystalline sodium sulphite was added and stirred to partially dissolve. Salinity of the reservoir seawater was monitored regularly, and samples of seawater within the chambers were taken at the conclusion of 3 experimental runs at each temperature to assess ammonia (phenol nitroprusside method), nitrate (Hagen test kit #A-7845), and nitrite (Hagen test kit #A-7825) concentrations. All measurements fell within physiological limits, with maximum values of 0.25, < 5, and 0.1 mg L^{-1} recorded for ammonia, nitrate and nitrite, respectively, and a mean salinity of 35.4 ‰.

The set-up for recording at 0°C was located in an insulated room with an air temperature of -1.5 ± 0.5°C, while the 3 and 6°C respirometers were set up in a laboratory with a room temperature of 12-18°C. The respirometry chambers were thoroughly cleaned between fish and the background oxygen consumption was negligible over a 90-minute period at each temperature, as previously reported by Gordon et al. (1989) at temperatures below 20°C. No detectable change in oxygen tension over 90 minutes within an oxygen-depleted respirometer confirmed the absence of leaks in the system. No attempt was made to remove carbon dioxide from the system and so as oxygen levels were depleted, carbon dioxide levels rose. Ventilation frequencies were determined visually by counting opercular movements for 1 minute.

SERIES 4.2. MAXIMUM OXYGEN CONSUMPTION RATES

The maximum oxygen consumption rates (V_{O_2} max) of both *P. borchgrevinki* and *T. bernacchii* were determined at 3, 6, and 0°C, in that order, using exercise to elicit the maximal response. The relatively active cryopelagic *P. borchgrevinki* swam well in a swim tunnel, while the more sedentary benthic *T. bernacchii* could not be induced to swim in the tunnel and was chased with a hand-net to exhaustion.

P. borchgrevinki was subjected to an incremental swimming test, similar to that employed by Forster et al. (1987), in an 80 L Blazka-type swimming tunnel (Blazka et al. 1960). The swimming tunnel contained a respirometer box constructed from 10 mm clear perspex, with plastic screens at both upstream and downstream ends of the tunnel to prevent fish from escaping. The characteristics of the velocity profile in a swimming tunnel are important in the consideration of swimming speed and the tunnel used in this study incorporated fine-meshed grids and concave baffles in order to produce a rectilinear front of uniform turbulence. Water flow was produced by revolutions of an impeller, and speeds from 1 to 60 cm s^{-1} were possible using a variable speed controller. The swimming tunnel was filled with air-saturated seawater at the appropriate temperature prior to each experimental run, and the set-up located in an insulated room with an air temperature of -1.5 ± 0.5°C for swimming trials at all three temperatures. Preliminary measurements indicated that the seawater temperature within the tunnel varied by less than 0.2°C over the duration of a typical run. Turbulence at the surface of the tunnel did not affect the linear flow adjacent to the fish, but did maintain adequate oxygenation of the water.

The fish were placed in the swimming tunnel individually, and the water flow adjusted to 7.6 cm s^{-1} for a period of 10 minutes. This flow rate was too low to require the fish to swim, but sufficient to allow orientation within the tunnel. After the adjustment period, the flow rate was increased by 6.6 cm s^{-1} at ten minute intervals up to 27.3 cm s^{-1}, above which the flow was increased by 3.3 cm s^{-1} every ten minutes. The experiment was terminated after the fish had fallen back against the rear restraining grid twice. The time and speed at which the fish became exhausted was recorded in order to calculate the critical swimming speed (U_{crit}). The fish was then quickly removed from the swimming tunnel, and placed into a respirometry chamber (described in Series 4.1) filled with air-saturated seawater of the same temperature as that in the swimming tunnel. It was necessary to transfer fish from the swimming tunnel to individual respirometry

chambers as the tunnel volume was too large to permit accurate oxygen consumption recording from a single fish. The transfer was completed within 2 minutes of attaining U_{crit}, and the chamber then sealed and immersed in a water bath. Ventilation frequency was determined immediately and at 20, 40, 90 minutes, 4 and 24 hours post-V_{O_2} max. Oxygen tension measurements were made using one of the two methods described in Series 4.1 and readings were continued until the oxygen tension within the chamber was reduced to 100 mmHg. The taps were then opened, restoring the reservoir circuit, and the chamber flushed with air-saturated seawater for 15 minutes by the end of which the oxygen tension in the chamber had been restored to near air-saturation. The taps were closed and another series of measurements taken and this interrupted flow method was continued for 6 hours, after which the reservoir circuit was re-established and the fish left in the chamber overnight. A final determination of oxygen consumption rate was made 24-hours following the introduction of the fish to the chamber. The fish was then weighed, measured (nose to tip of tail), and fed before being released back into the 0°C aquarium system.

DATA ANALYSIS AND STATISTICAL METHODS

Oxygen Consumption

After correction for meter drift, the rate of oxygen consumption in units of mg O_2 kg^{-1} h^{-1} was calculated from:

$$V_{O_2} = \frac{\Delta P_{O_2} \times C \times V \times 31.9988}{t \times M}$$

where:

ΔP_{O_2} = change in oxygen partial pressure over the measurement period (mm Hg)

C = oxygen capacitance of seawater at a given temperature (μmol L^{-1} mm Hg^{-1})

V = volume of water in the respirometer (L)

31.999 = molecular weight of O_2

t = duration of measurement (h)

M = mass of the fish (g)

In order to determine the volume of water in the chambers, an approximation was made in that both species were assumed to be neutrally buoyant, allowing an estimate of mass = volume. The actual buoyancies (% body weight in -1.9°C seawater) of *P. borchgrevinki* and *T. bernacchii* are 2.75 and 3.37 respectively (Eastman 1993). The oxygen capacitance values used were 2.28 (0°C), 2.13 (3°C), and 1.99 (6°C) μmol L^{-1} $mmHg^{-1}$. Oxygen consumption rates were determined from the continuous Chart recordings by averaging the rate over consecutive 5 minute blocks.

Resting oxygen consumption was corrected to 100 g mass using the following formula:

$$V_{O_2}(100) = V_{O_2} \times (M/100)^{(1-A)}$$

where:

$V_{O_2}(100)$ = O_2 consumption of a 100 g animal

V_{O_2} = O_2 consumption of an animal with mass M

M = mass of the animal (g)

A = mass exponent describing the relationship between metabolic rate and body mass.

The mass exponent for resting oxygen consumption is generally independent of adaptation temperature, with the majority of values falling between 0.78 and 0.85 (Beamish 1978; Duthie 1982; Morris and North 1984; Schmidt-Nielsen 1984; Johnston et al. 1991; Clarke and Johnston 1999). Antarctic fish mass exponents are scarce, but range from 0.60 to 0.90 (Morris and North 1984). A value of 0.8 (identical to that used by Holeton in 1974) was used in this study to correct both resting and maximum oxygen consumption rates to 100 g mass and enable comparisons with previous studies. The active mass exponent is often higher than the resting value and has been demonstrated to increase with activity level (Brett 1965; Weibel 2000). In some fishes, the maximum metabolic rate is independent of mass (Brett and Glass 1973), but Forster et al. (1987) reported similar mass exponents for *P. borchgrevinki* at rest and during maximum activity and therefore maximum metabolic rates were scaled in this study.

Critical Swimming Speed

Critical swimming speed (U_{crit}), a special category of prolonged swimming, was first defined by Brett (1964) to identify the maximum velocity fish could maintain for a prescribed period of time. It is measured by interpolation for those fish that do not fatigue at exactly the beginning or end of a time period:

$$U_{crit} = U_i + (T_i/T_{ii} \times U_{ii})$$

where:

U_i = highest velocity maintained for the prescribed period (cm s^{-1})

U_{ii} = velocity increment (cm s^{-1})

T_i = time (min) fish swam at the "fatigue" velocity

T_{ii} = prescribed time period of swimming (min)

The rate of oxygen consumption measured at the critical swimming speed (*P. borchgrevinki*) or point of exhaustion (*T. bernacchii*) was designated V_{O_2} max. This is distinguished from the active metabolic rate which is defined as the rate of oxygen consumption during maximum sustained activity (Brett and Groves 1979). The scope for activity was calculated from V_{O_2} max - V_{O_2} rest, and the factorial scope for oxygen consumption, or factorial aerobic scope, was calculated from V_{O_2} max / V_{O_2} rest.

Condition Factor

Fulton's condition factor for each fish was calculated using the following formula (Ricker 1975):

$$\text{Condition Factor} = \frac{100 \times (\text{body mass, g})}{(\text{body length, cm})^3}$$

Q_{10}

Q_{10} values were calculated using the Van't Hoff equation (Hoar 1975):

$$Q_{10} = (R_2 / R_1)^{10/(T_2-T_1)}$$

where: R_1 and R_2 are the rates at temperatures T_1 and T_2, respectively.

Statistical Analysis

Within each species, data were compared by one-way analysis of variance (ANOVA). Where a treatment effect was indicated, inter-temperature differences were determined by Tukey-Kramer post-hoc tests and Dunnett's post-hoc analysis was used to compare values with baseline during recovery. Inter-specific comparisons were made using t-tests and the data were log transformed where indicated necessary by Bartlett's test to improve homogeneity of variance. All analyses were carried out using GraphPad Prism version 4.00 software and statistical significance was taken at the level of $p<0.05$. Data are presented as mean ± SEM, and all oxygen consumption rates have been corrected to 100 g mass, unless otherwise stated.

RESULTS

SERIES 4.1. RESTING OXYGEN CONSUMPTION RATES

The resting oxygen consumption rates and ventilation frequencies of *P. borchgrevinki* did not differ significantly from those of *T. bernacchii* at any temperature (Tables 4.1 and 4.2). Both species demonstrated thermal independence of resting oxygen consumption from 0 to 3°C, but a rapid increase in the rate of oxygen consumption from 3 to 6°C with Q_{10} values (3 to 6°C) of 3.2 and 8.0 for *P. borchgrevinki* and *T. bernacchii*, respectively (Fig. 4.1). Although ventilation frequencies of both species also attained maximum values at 3°C, the changes in ventilation frequency with temperature were not statistically significant (Fig. 4.2). The opercular movements of both species were

noticeably deeper at the higher temperatures, indicating that ventilatory stroke volume and thus total gill ventilation may not have been thermally independent.

There were no significant differences between the condition factors of *T. bernacchii* at the three temperatures, but the mean condition factor of *P. borchgrevinki* at 0°C was significantly higher than values at 3 and 6°C. As 0°C was the second of the three experimental temperatures, this is not significant in terms of any deterioration in condition of the fish during the study. Condition factor of the more laterally compressed *T. bernacchii* was consistently higher than that of the relatively streamlined *P. borchgrevinki*.

Table 4.1. The effect of an acute increase in temperature on resting oxygen consumption rate and ventilation frequency of *P. borchgrevinki*.

Temperature (°C)	V_{O_2} rest (mg O_2 kg^{-1} h^{-1})	V_{O_2} rest (100 g) (mg O_2 kg^{-1} h^{-1})	Ventilation frequency (min^{-1})	Condition Factor
0	33.7 ± 2.2	32.8 ± 1.8	19.1 ± 1.7	1.01 ± 0.02#
3	37.7 ± 2.3	36.4 ± 2.1	23.7 ± 2.9	0.96 0.03
6	54.9 ± 5.3*	51.6 ± 5.0*	20.8 ± 3.0	0.90 ± 0.03

Data obtained from 0°C-acclimated fish after 24 hours at the respective temperatures. N = 8 at 0°C and 6°C, N = 9 at 3°C. * Significantly different from values at both 0°C and 3°C. # Significantly different from values at both 3°C and 6°C.

Table 4.2. The effect of an acute increase in temperature on resting oxygen consumption rate and ventilation frequency of *T. bernacchii*.

Temperature (°C)	V_{O_2} rest (mg O_2 kg^{-1} h^{-1})	V_{O_2} rest (100 g) (mg O_2 kg^{-1} h^{-1})	Ventilation frequency (min^{-1})	Condition Factor
0	33.6 ± 2.6	32.0 ± 1.7	16.8 ± 1.3	1.30 ± 0.05
3	34.8 ± 2.1	32.8 ± 1.9	18.9 ± 1.0	1.36 ± 0.04
6	60.9 ± 6.2*	61.2 ± 7.2*	14.3 ± 1.4	1.26 ± 0.08

Data obtained from 0°C-acclimated fish after 24 hours at the respective temperatures. N = 6, 9 and 4, at 0°C, 3°C and 6°C, respectively. * Significantly different from values at both 0°C and 3°C.

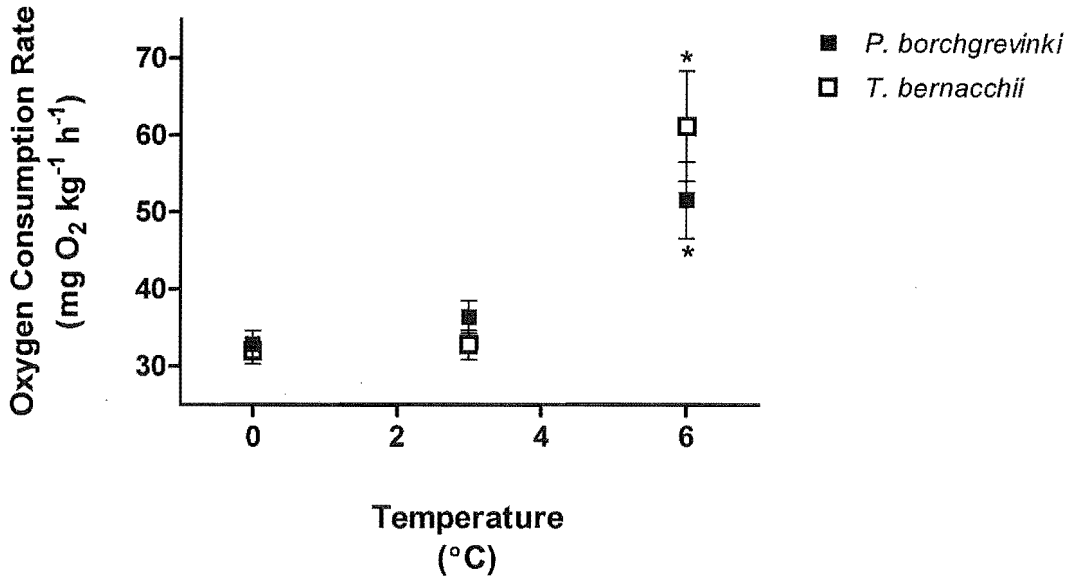


Fig. 4.1. The effect of an acute increase in temperature on resting oxygen consumption of two Antarctic notothenid fishes. Data obtained from 0°C-acclimated fish after 24 hours at each temperature. * Significantly different from values at 0°C and 3°C. There were no significant differences between the oxygen consumption rates of the two species at any temperature.

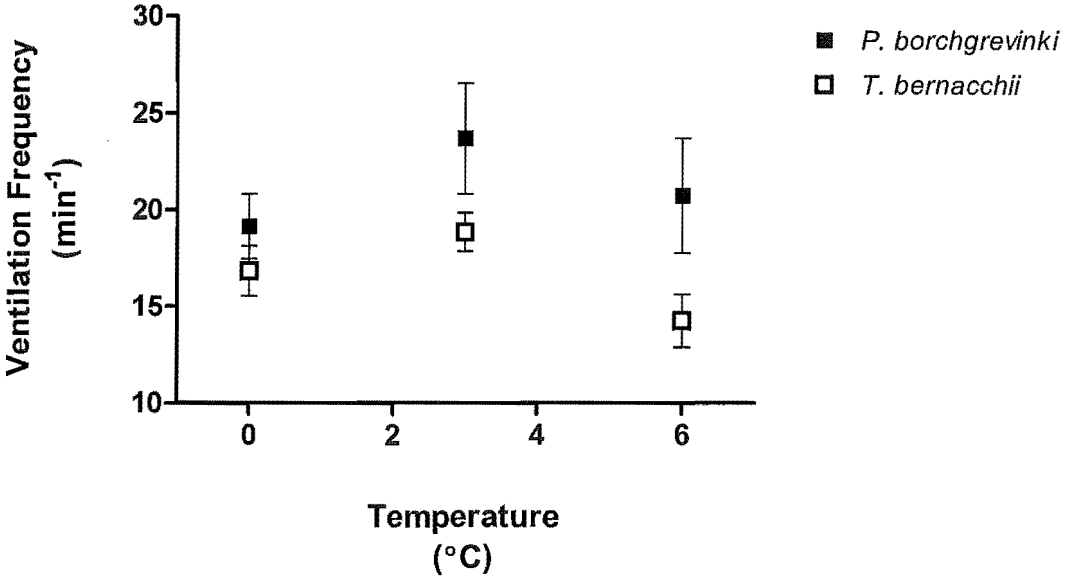


Fig. 4.2. The effect of an acute increase in temperature on ventilation frequency of two Antarctic notothenid fishes. Data obtained from 0°C-acclimated fish after 24 hours at each temperature. There were no significant differences in ventilation rates within or between species.

SERIES 4.2. MAXIMUM OXYGEN CONSUMPTION RATES

Pagothenia borchgrevinki

Exhaustive exercise elicited a significant increase in both oxygen consumption rate and ventilation frequency above resting levels at all three temperatures. The maximum oxygen consumption rate (V_{O_2} max) of *P. borchgrevinki* at 0°C was 221.5 ± 12.4 mg O_2 kg^{-1} h^{-1}, resulting in a scope for activity of 188.7 mg O_2 kg^{-1} h^{-1} and a factorial aerobic scope of 6.75. The pattern of thermal sensitivity of V_{O_2} max was different from that of resting oxygen consumption, with an increase from 0 to 3°C (Q_{10} = 2.8), and a marked decline from 3 to 6°C (Q_{10} = 0.1) (Fig. 4.3). This resulted in scope for activity and factorial aerobic scope both attaining maximum values at 3°C and minimum values at 6°C (Table 4.3). The ventilation frequency at V_{O_2} max, however, was independent of temperature from 0 to 6°C (Fig. 4.4).

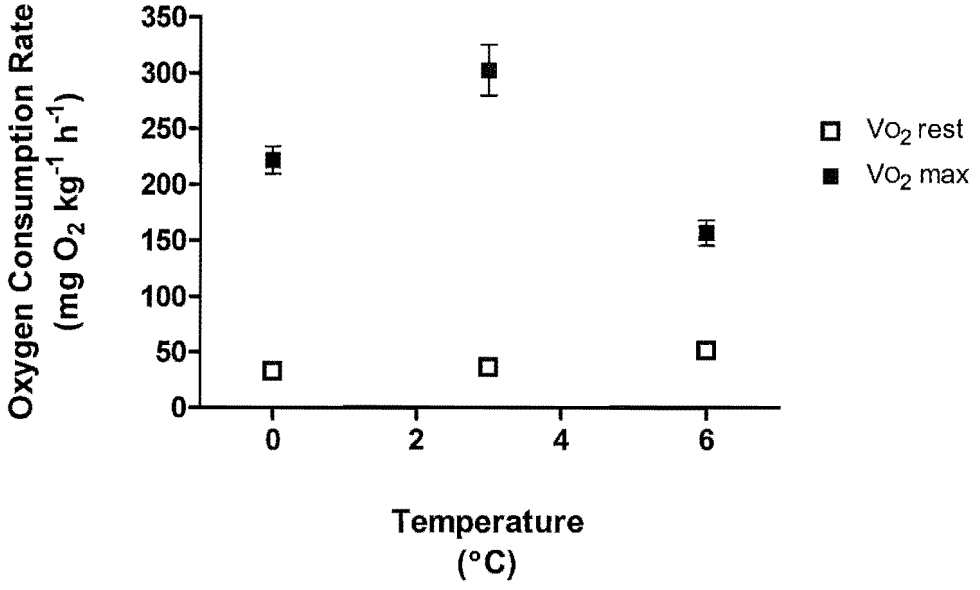


Fig. 4.3. The effect of an acute increase in temperature on resting and maximum oxygen consumption rates of *P. borchgrevinki*. Data obtained from 0°C-acclimated fish after 24 hours at each temperature. Error bars of the resting rates are obscured by the symbols.

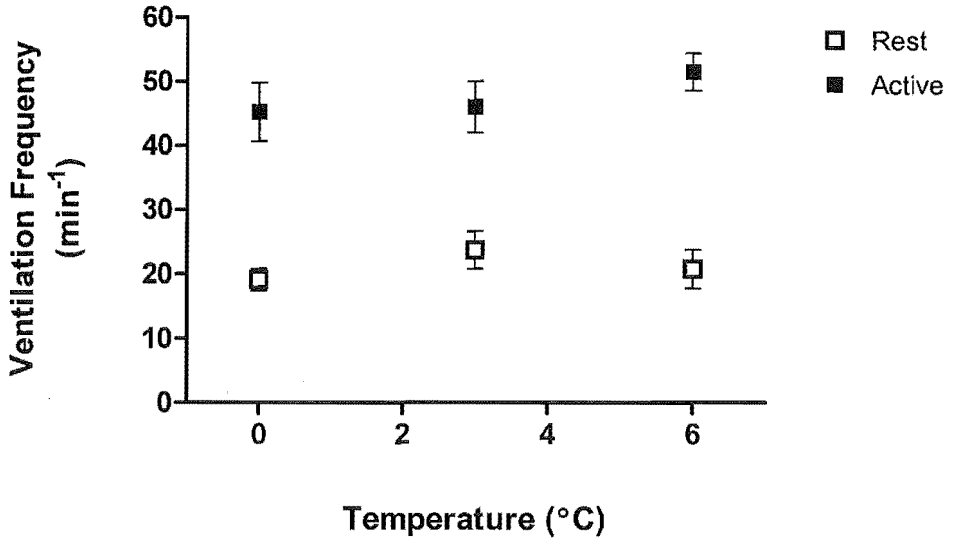


Fig. 4.4 Ventilation frequency of *P. borchgrevinki* at rest and at critical swimming speed at three different temperatures. Active rates are significantly higher than resting rates at all temperatures, but there is no significant effect of temperature on resting or active rates.

Table 4.3. The effect of an acute increase in temperature on maximum oxygen consumption rate and aerobic scope of *P. borchgrevinki*.

Temperature (°C)	V_{O_2} max (mg O_2 kg^{-1} h^{-1})	Scope for Activity (mg O_2 kg^{-1} h^{-1})	Factorial Aerobic Scope
0	221.5 ± 12.4	188.7	6.75
3	302.4 ± 22.8*	266.0	8.32
6	156.5 ± 11.3*	105.0	3.04

N = 7 at 0 and 3°C, N = 6 at 6°C. * Significantly different from value at 0°C.

The critical swimming speed of *P. borchgrevinki* mirrored the change in aerobic scope, attaining a maximum value at 3°C and decreasing from 3 to 6°C (Table 4.4, Fig. 4.5). At 0°C, the fish were able to maintain station in the tunnel by labriform swimming alone to a speed of 1.4 bl s^{-1}. Immediately following each increase in flow rate the fish were swept backwards, but they quickly regained position and adjusted the swimming speed. At speeds approaching U_{crit}, the fish had difficulty maintaining position using the pectoral fins alone and bursts of subcarangiform locomotion were employed to regain position. All fish were exhausted within several minutes of attaining a speed which could only be supported by a subcarangiform mode of swimming. *T. bernacchii* employed a

labriform mode of swimming right to the point of exhaustion and was not observed to recruit the myotomal musculature at any temperature.

The mean condition factor of *P. borchgrevinki* at 0°C was significantly higher than at 3 and 6°C (Table 4.4), although as 0°C was the third of the three treatments in this experiment, this is not significant in terms of any deterioration in condition of the fish during the study.

Table 4.4. The effect of an acute increase in temperature on the critical swimming speed of *P. borchgrevinki.*

Temperature (°C)	Mass (g)	Length (mm)	Condition Factor	n	U_{crit} (bl s^{-1})
0	83.1 ± 14.8	197.9 ± 12.9	1.07 ± 0.03#	7	1.86 ± 0.04
3	65.3 ± 16.2	193.3 ± 11.2	0.89 ± 0.03	7	1.68 ± 0.05
6	75.0 ± 6.3	202.5 ± 5.3	0.90 ± 0.04	6	1.24 ± 0.10*

* Significantly different from values at 0 and 3°C. # Significantly different from values at 3 and 6°C.

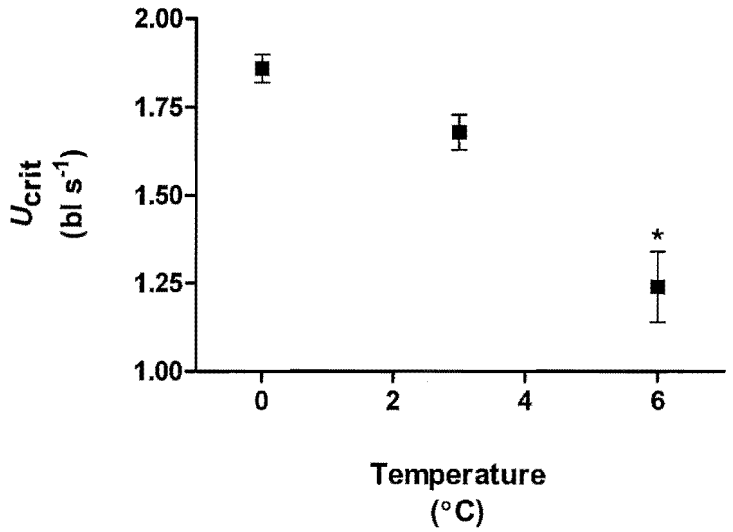


Fig. 4.5. Effect of an acute increase in temperature on critical swimming speed of *P. borchgrevinki.* * Significantly different from values at both 0 and 3°C.

Trematomus bernacchii

Both the oxygen consumption rate and ventilation frequency of *T. bernacchii* increased significantly from resting levels during exhaustive exercise at all experimental temperatures. The maximum rate of oxygen consumption of *T. bernacchii* was significantly lower than that of *P. borchgrevinki* at 0 and 3°C, although there was no significant difference at 6°C. The effect of an acute increase in temperature on V_{O_2} max of *T. bernacchii* was similar to that exhibited by *P. borchgrevinki*, with an increase from 0 to 3°C (Q_{10} = 2.5), a peak at 3°C, and a decrease from 3 to 6°C (Q_{10} = 0.81) (Fig. 4.6), although the changes in rate of *T. bernacchii* were not statistically significant. As in *P. borchgrevinki*, the scope for activity increased from 0°C to a maximum value at 3°C and then declined from 3 to 6°C (Fig. 4.8). The scope for activity and factorial aerobic scope of *T. bernacchii* were considerably lower than those of *P. borchgrevinki* at 0 and 3°C, but not significantly different at 6°C (Table 4.3 and Table 4.5). At 6°C, the scope for activity of *T. bernacchii* was of similar magnitude to that at 0°C, but due to a greater resting oxygen consumption rate at the higher temperature, the factorial aerobic scope was considerably lower at 6°C (Table 4.5). The ventilation frequency of *T. bernacchii* at V_{O_2} max was independent of temperature (Fig. 4.7).

Table 4.5. The effect of an acute increase in temperature on maximum oxygen consumption rate and aerobic scope of *T. bernacchii*.

Temperature (°C)	V_{O_2} max (mg O_2 kg^{-1} h^{-1})	Scope for Activity (mg O_2 kg^{-1} h^{-1})	Factorial Aerobic Scope
0	129.9 ± 15.4	97.9	4.06
3	171.1 ± 30.0	138.4	5.22
6	160.8 ± 14.5	99.6	2.63

N = 6 at 3°C, n = 4 at 0°C and 6°C.

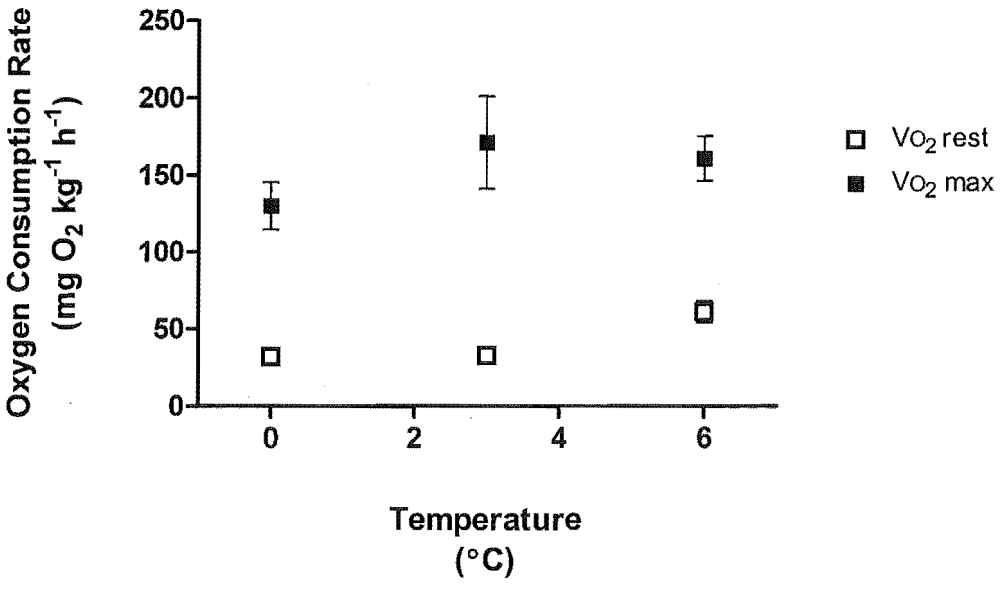


Fig. 4.6. The effect of an acute increase in temperature on resting and maximum oxygen consumption rates of *T. bernacchii.* Data obtained from 0°C-acclimated fish after 24 hours at each temperature. Error bars of the resting rates are obscured by the symbols.

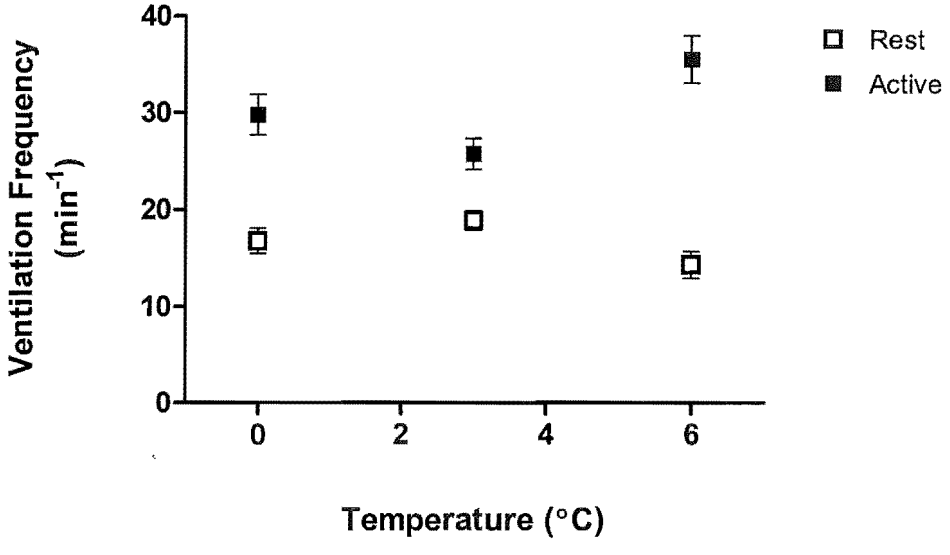


Fig. 4.7 Resting and active ventilation frequencies of *T. bernacchii* at three different temperatures. Active rates are significantly higher than resting rates at all temperatures, but there is no significant effect of temperature on resting or active rates.

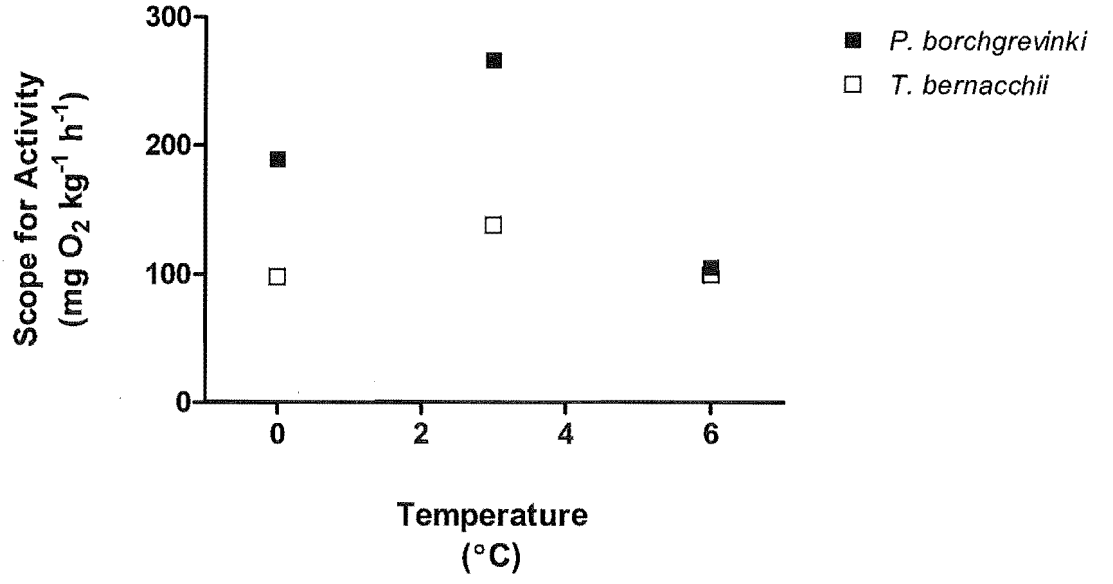


Fig. 4.8. The effect of an acute increase in temperature on metabolic scope for activity of two Antarctic notothenlid fishes of differing ecotype. Data obtained from 0°C-acclimated fish after 24 hours at each temperature.

Recovery

The recovery of oxygen consumption rates to resting levels was relatively rapid in both nototheniids at all three temperatures. In *P. borchgrevinki* at 0°C, a level of oxygen consumption not significantly different from V_{O_2} rest was restored 45 minutes post-V_{O_2} max (Fig. 4.9a). The time taken for oxygen consumption rates to return to resting levels was slightly shorter at both 3 and 6°C, with V_{O_2} not significantly different from the resting values after 25 and 30 minutes, respectively (Fig. 4.9b and c). The rate of oxygen consumption had also returned to the resting level at 0°C by 45 minutes post-V_{O_2} max in *T. bernacchii* (Fig. 4.10a). As in *P. borchgrevinki*, recovery was more rapid at 3 and 6°C, with V_{O_2} not significantly different from resting values after 20 and 10 minutes, respectively (Fig 4.10b and c).

The return of ventilation frequency to resting levels was also rapid following exhaustive exercise in both *P. borchgrevinki* and *T. bernacchii*. In both species, ventilation frequency had returned to resting levels by 20 minutes post-V_{O_2} max at 0°C and 3°C. The recovery at 6°C was slightly longer, with the rate elevated above resting levels 90 minutes post-V_{O_2} max but not significantly different from the resting value after

4 hours in *P. borchgrevinki*, and elevated above resting levels 4 hours post-V_{O_2} max but not significantly different from the resting value after 24 hours in *T. bernacchii*.

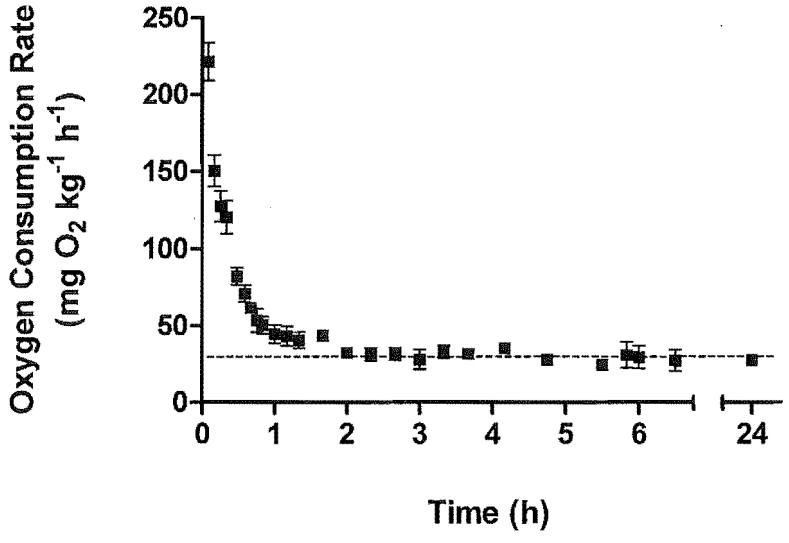


Fig. 4.9a. Recovery of *P. borchgrevinki* from exhaustive exercise at 0 °C. The dotted line represents the resting rate of oxygen consumption at 0°C.

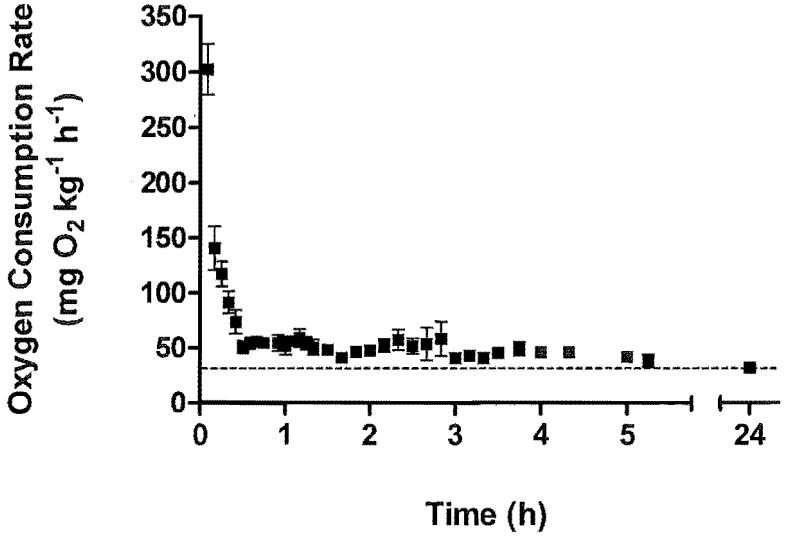


Fig. 4.9b. Recovery of *P. borchgrevinki* from exhaustive exercise at 3 °C. The dotted line represents the resting rate of oxygen consumption at 3°C.

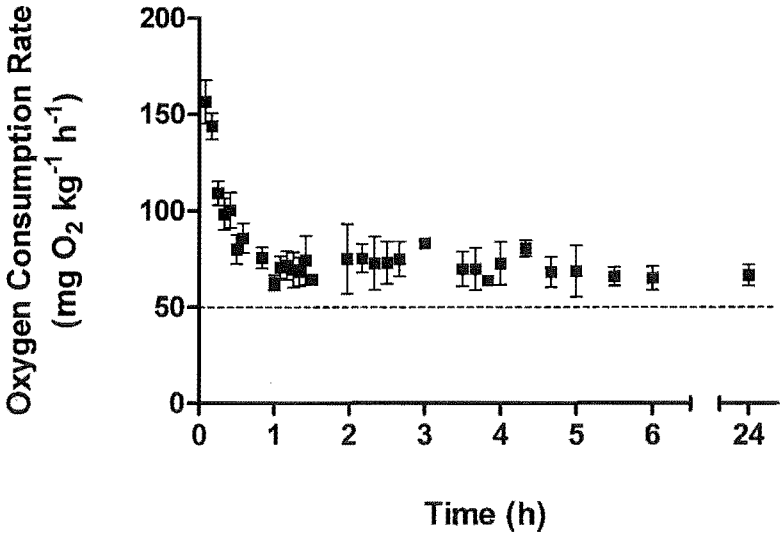


Fig. 4.9c. Recovery of *P. borchgrevinki* from exhaustive exercise at 6 °C. The dotted line represents the resting rate of oxygen consumption at 6°C.

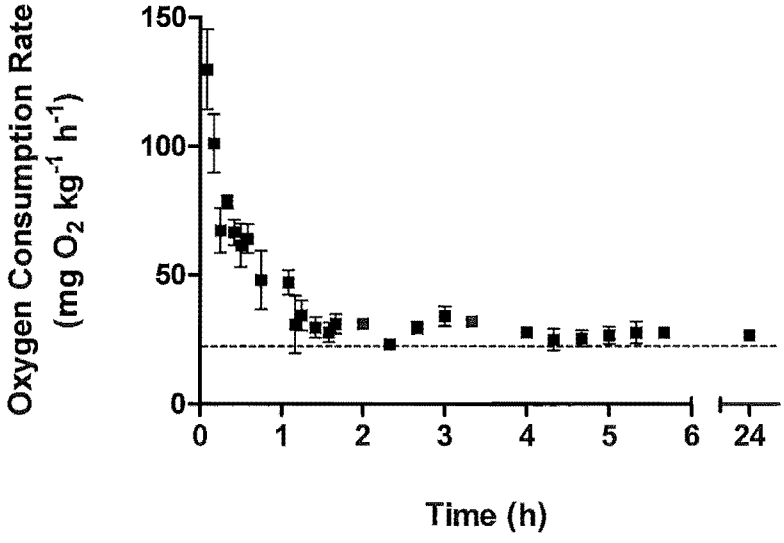


Fig. 4.10a. Recovery of *T. bernacchii* from exhaustive exercise at 0°C. The dotted line represents the resting rate of oxygen consumption at 0°C.

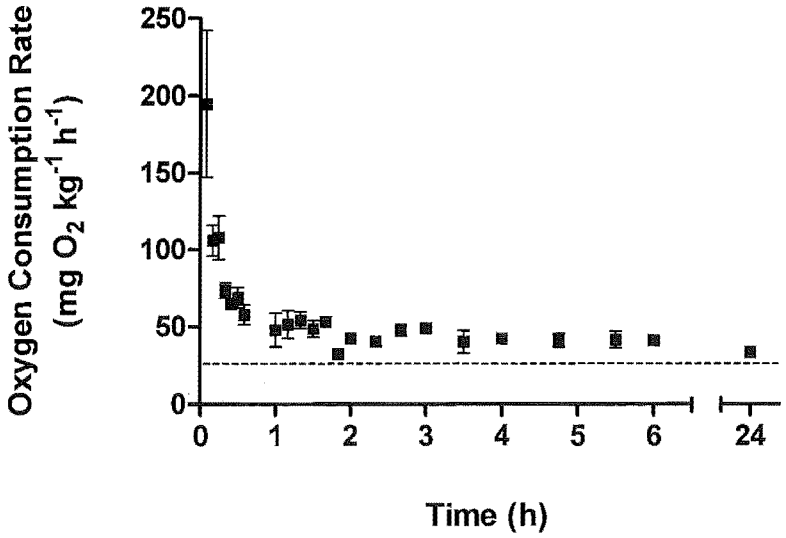


Fig. 4.10b. Recovery of *T. bernacchii* from exhaustive exercise at 3°C. The dotted line represents the resting rate of oxygen consumption at 3°C.

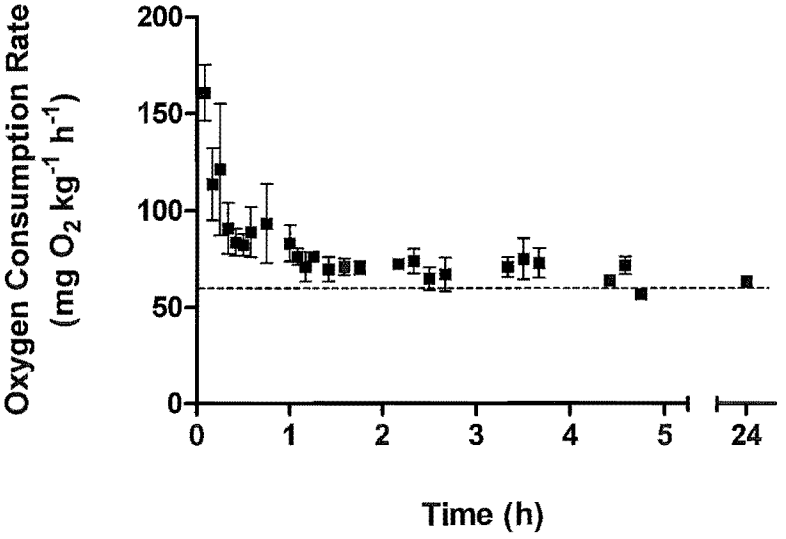


Fig. 4.10c. Recovery of *T. bernacchii* from exhaustive exercise at 6°C. The dotted line represents the resting rate of oxygen consumption at 6°C.

DISCUSSION

Resting oxygen consumption rates at 0°C were very similar in the two Antarctic nototheniids, with values of 32.8 ± 1.8 and 32.0 ± 1.7 mg O_2 kg^{-1} h^{-1} determined for *Pagothenia borchgrevinki* and *Trematomus bernacchii,* respectively. These values are considerably lower than those reported by early studies, but compare well with the results of more recent investigations. The resting oxygen consumption rate (100 g mass) of *P. borchgrevinki* varies in the literature from 27.0 to 56.2 mg O_2 kg^{-1} h^{-1} (Wohlschlag 1964; Forster et al. 1987; Wells 1987; Wilson et al. 2002), while published values for *T. bernacchii* vary from 28.0 to 42.3 mg O_2 kg^{-1} h^{-1} (Wohlschlag 1964; Wells 1987; Davison 2001), at temperatures from -1.5 to 0.5°C. The value for another Antarctic notothenid, the sedentary benthic *Notothenia neglecta*, has been reported as 22.6 mg O_2 kg^{-1} h^{-1} at 0°C (Johnston et al. 1991). The high values measured in the majority of early studies are now thought to reflect the effects of handling stress and it is generally accepted that a period of recovery is required to enable polar fish to regain normal physiological states and resting metabolic rates following handling (Holeton 1974; Morris and North 1984; Wells et al. 1984; Forster et al. 1987). Preliminary investigations carried out as part of this study indicated that both *P. borchgrevinki* and *T. bernacchii* attained stable rates of oxygen consumption 15-16 hours after the handling stress of introduction to the respirometry chamber which is in agreement with previous reports of stable oxygen consumption rates and full biochemical recovery of these fish 2-12 hours following handling stress (Wells et al. 1984; Wells 1987). The oxygen consumption rate of *N. neglecta* reaches a steady level 10 hours after handling (Johnston et al. 1991), and this is comparable to recovery rates of other Arctic and Antarctic fishes (Holeton 1974; Morris and North 1984; Saint-Paul et al. 1988). The 24-hour recovery period employed in this study should therefore have been sufficient to minimise the effects of handling-induced stress. The fish in the current study had been maintained in captivity and acclimated to a temperature of 0.0 ± 0.3°C for at least two months prior to the first experiment, in contrast to the majority of early metabolic studies which measured rates in fish 24 hours to 14 days post-capture. Alteration of both haematological and cardiovascular parameters have been observed in captive fish (Davison et al. 1995), but whether this represents a decrease in stress levels, or is indicative of a chronic response to captivity remains to be determined. It is, however, possible that the elevated

acclimation temperature may result in metabolic rates and thermal sensitivities which differ from those of fish acclimatised to -1.86°C in the wild (see Chapter Nine).

The nutritional status of an organism is another factor which has a marked effect on metabolic rates, with an increase in the rate of oxygen consumption following feeding, known as the specific dynamic action or SDA, and a decrease in the metabolic rate during prolonged starvation. The elevation of metabolic rate during the SDA is thought to reflect the energy requirements of a number of processes including digestion, absorption and storage of nutrients, de-amination of amino acids, synthesis of excretory products, and the increased synthesis of protein and lipid associated with growth (Jobling 1992; Wieser 1994). Fasting periods of 2-4 times the duration of the SDA are recommended prior to determination of resting oxygen consumption in fish (Johnston and Battram 1993; Boyce and Clarke 1997) and, with the SDA of Antarctic fish having been estimated at between 5 and 16 days (Johnston and Battram 1993; Boyce and Clarke 1997; Ware 1999; Boyce et al. 2000; Brodeur et al. 2002), the three-week fast of fish used for this investigation should have been sufficient to reduce this source of variation.

Due to practical constraints on fish numbers, the same fish were used for several experimental runs and therefore fasted several times. There was, however, no decrease in condition factor of either species from the beginning to the end of the study, and the condition factor values calculated for *P. borchgrevinki* fell within the range for freshly caught fish of this species (Davison 1998). Many fishes have the ability to withstand long periods of starvation, and periodic fasting is a common feature in the life histories of many species (Love 1970). Changes in condition factor may underestimate the effects of starvation as fish have been shown to increase tissue hydration during prolonged periods of starvation (Lambert and Dutil 1997), but the fact that critical swimming speeds of *P. borchgrevinki* measured at 0°C at the conclusion of the experiment were comparable to values from freshly-caught fish (Montgomery and Macdonald 1984; Forster et al. 1987; Davison et al. 1988; Wilson et al. 2002) suggests that fasting did not have a significant effect on critical swimming speed.

Another factor complicating the comparison of oxygen consumption rates between fish is the relationship between body mass and metabolic rate (Clarke and Johnston 1999) in which mass-specific oxygen consumption decreases as body mass is increased (Schmidt-Nielsen 1984). The Antarctic fish obtained for this study were all of similar size and weighed close to 100 g. Although this factor precluded an estimation of the mass exponent for metabolism, it also minimised the effect of scaling on the results.

The resting oxygen consumption rate has been defined as the rate for quiescent fish, but not necessarily the lowest rate during the 24-hour cycle (Fry 1971) as metabolic rates, along with many other physiological processes, generally exhibit a diurnal rhythm. Cycling of oxygen consumption has not been observed in a 24-hour daylight regime (Wells 1987) but, in order to minimise this possible source of error in the comparisons between different temperatures, both resting and maximal oxygen consumption evaluations were made at the same time each day (mid-morning). The rate of oxygen consumption was determined by interrupted-flow respirometry, which proved to be the most practical method given the inherent oxygen capacity of seawater at these temperatures and the low metabolic rates of Antarctic fish. The oxygen uptake rate of fish is independent of available oxygen at relatively high oxygen tensions, but linearly related to available oxygen at low tensions (Fry 1947). The critical P_{O_2} (oxygen tension at which fish can no longer regulate oxygen consumption and change from being an oxygen regulator to an oxygen conformer) of *P. borchgrevinki* is reported as 30 - 60 mmHg at temperatures from -1.5 to 0°C (Forster et al. 1987; Wells 1987; Davison et al. 1990) and increases with increasing temperature (Chapter 8), and therefore oxygen consumption rates in this study were only measured between air-saturation and an oxygen tension of 100 mmHg.

The standard metabolic rates of fish in general (mainly temperate fishes) are reported to have a mean of 89 ± 34 mg O_2 kg^{-1} h^{-1}, and range from 26 to 229 mg O_2 kg^{-1} h^{-1} (Brett and Groves 1979). The standard rate of oxygen consumption cannot be measured directly and is usually assessed during exercise tests by extrapolation to zero activity (Wohlschlag 1964; Brett and Groves 1979), although for resting benthic species it can be assumed that the terms resting and standard are essentially equivalent (Wieser 1985). The resting oxygen consumption rates of *P. borchgrevinki* and *T. bernacchii* at 0°C are thus at the lower end of the range of standard rates, falling below the majority of temperate fishes. Rates of oxygen consumption, however, vary widely with size, maturation state, activity and environmental variables, and the only meaningful comparisons are those made between fishes of similar activity level, size, maturation and physiological state. The values determined in this study still provide no evidence for the traditional concept of metabolic cold adaptation (a 200%+ increase in rates of cold-adapted species) when compared to the rates of temperate-water notothenioids (see Chapter Five), or other sedentary temperate and tropical species (Johnston et al. 1991; Johnston and Battram 1993; Steffensen et al. 1994).

Animals with higher levels of activity or costs of locomotion are expected to have higher resting metabolic rates than more sluggish species in order to attain high rates of metabolism during exercise (Pörtner et al. 1998). Within the notothenioids and among other Antarctic fishes there has been a relationship demonstrated between resting metabolic rate and activity level, and it has been suggested that evidence for metabolic cold adaptation may only be present among the more active species (Montgomery and Wells 1993). The cryopelagic *P. borchgrevinki* is one of the more active Antarctic nototheniids, and the similarity in resting oxygen consumption rates of this species and the more sedentary *T. bernacchii* provides no evidence for the theory. To the contrary, the comparable resting oxygen consumption rates of the two species could be taken to reflect relatively greater thermal compensation of resting metabolic rate of the more sedentary species. The similarity of resting oxygen consumption rates in the two species relates well to the similar levels of both aerobic and anaerobic enzymatic activities in brain tissue of Antarctic notothenioids of differing ecotype (Kawall et al. 2002).

The resting rate of oxygen consumption exhibited near thermal-independence from 0°C to 3°C in both *P. borchgrevinki* and *T. bernacchii* (Q_{10} values of 1.4 and 1.1, respectively), in contrast to the results of Wilson et al. 2002 which indicated a significant increase in resting oxygen consumption rate of *P. borchgrevinki* from -1 to 2°C ($Q_{10} \approx 7$) and from 2 to 4°C ($Q_{10} \approx 10$). A rapid increase in resting oxygen consumption rate was, however, observed in both species in the current study from 3 to 6°C. Wilson et al. (2002) did not measure resting oxygen consumption at 6°C, stating that the fish did not survive prolonged exposure to this temperature. Data provided in Chapter Two of this thesis confirms that -1°C-acclimated *P. borchgrevinki* do not survive long-term exposure to 6°C, although there were no mortalities among the 0°C-acclimated fish kept at 6°C for 48 hours in this investigation, and none of the fish showed any signs of distress. This suggests that acclimation to 0°C, a temperature less than 2°C above the environmental level of these fishes, has a significant effect on thermal sensitivity (see Chapter Nine).

The thermal insensitivity of the Antarctic nototheniids from 0 to 3°C relates well to the region in the middle of the thermal range where metabolic rate is relatively independent of temperature in other fishes (Cossins and Bowler 1987; Jobling 1994; Taylor et al. 1997). Deviation from this optimal range tends to result in increased thermal sensitivity, particularly as temperature rises toward the upper limit for the species, and the rapid increase in oxygen consumption rate from 3 to 6°C of the two nototheniids in this study compares favourably with results from *N. neglecta* in which resting metabolic

rate has been shown to be more thermally sensitive above the physiological range (Q_{10} 3 to 6°C = 6.4) (Johnston et al. 1991). Such a rapid increase in oxygen consumption rate generally indicates close proximity to the upper thermal limit (Hochachka and Somero 2002), and is in agreement with the 4°C upper viable limit (Guynn et al. 2002), and 4-6°C upper lethal limit (Somero and De Vries 1967) previously reported for these fishes. The increased demand for oxygen at elevated temperatures is thought to be due to an acute increase in the metabolic cost of maintenance (Cossins and Bowler 1987). Starting from an estimated cost of 30% of standard metabolic rate for ventilation and circulation (Johnston and Dunn 1987), an increase in temperature is likely to result in a considerable increase in the cost of ventilation as greater volumes of water need to be presented to the branchial exchange surfaces. Although there was no change in the resting ventilation rate of either species with temperature, the deepening of opercular movements at higher temperatures suggests that there may have been an increase in ventilatory stroke volume which would have contributed to an increase in total gill ventilation. An acute increase in temperature has also been shown to raise the level of spontaneous activity in fish (Peterson and Anderson 1969; Stevens and Fry 1972), thereby increasing metabolic rate, although the only evidence comes from relatively active pelagic fishes. In the current study, there was no increase in activity observed at the higher temperatures, with very little activity observed at all. This is in contrast to the previous report of considerable variability in the resting oxygen consumption rate of *P. borchgrevinki*, which was attributed primarily to spontaneous activity (Forster et al. 1987). Both *T. bernacchii* and *P. borchgrevinki* perched on their pelvic fins on the base of the chambers and made no attempts to swim. In the wild, the former has been observed to utilise sponges as an elevated perch on the sea floor (Moreno 1980), while the latter perches within the layers of platelet ice (Ekau 1991; Gutt 2002). Another theory suggests that spontaneous activity may be lowest within the preferred temperature range, increasing as metabolic scope is decreased to enable fishes to select optimal thermal habitats (Bryan et al. 1990; Holker 2003), although there was no evidence provided for this in the current study. Both species did, however, make occasional sweeping movements with their pectoral fins. Wells (1987) determined the resting metabolic rate of spinalectomized *P. borchgrevinki* and found very similar results to un-operated resting fishes indicating that these movements have no significant effect on metabolic rate, but he proposed that the fin movements may assist with mixing of the water in the respirometry chambers and reduce the boundary layer at the skin surface, promoting oxygen transfer.

The maximum rate of oxygen consumption of *P. borchgrevinki* at 0°C (221.5 ± 12.4 mg O_2 kg^{-1} h^{-1}) is slightly higher than the 193.4 mg O_2 kg^{-1} h^{-1} previously measured at the same temperature (Forster et al. 1987), while the V_{O_2} max of *T. bernacchii* at 0°C (129.9 ± 15.4 mg O_2 kg^{-1} h^{-1}) is near the lower end of the previously reported range of 140-180 mg O_2 kg^{-1} h^{-1} (Wohlschlag 1960). The maximum metabolic rates of relatively inactive temperate flatfish measured at their environmental temperatures vary from 164-218 mg O_2 kg^{-1} h^{-1} (Duthie 1982; Mallekh and Lagardere 2002), while the highest metabolic rates of active fish reach 1000 mg O_2 kg^{-1} h^{-1} (Fry 1947; Brett 1964). With the comparatively low metabolic rates of flatfish attributed to their limited gill areas as well as their sedentary lifestyles, more useful comparisons could be made between the maximum metabolic rates of temperate-water and Antarctic nototheniids, although data from the temperate-water species is not yet available. The factorial scopes of *P. borchgrevinki* and *T. bernacchii* at 0°C (6.8 and 4.1, respectively), compare well with previous studies which suggest that the value is 4-7 times the fasting metabolic rate in notothenioid fishes (Forster et al. 1987; Davison et al. 1990; Johnston et al. 1991). These values compare favourably with those of temperate flatfish (Duthie 1982), turbot (Mallekh and Lagardere 2002), and tropical wrasses that use a labriform mode of swimming (Gordon et al. 1989) at their environmental temperatures, and the factorial scope of *P. borchgrevinki* at 3°C compares well with the metabolic scope of 7.5 reported for rainbow trout at 4°C (Wieser 1985). The current study therefore indicates compensation of metabolic scope and provides no evidence to support the theory that low aerobic scopes of these fishes may necessitate the seasonal switching of energy allocation between growth and reproduction (Johnston and Battram 1993). A high factorial scope may be a misleading indicator of performance capacities (Clarke and Johnston 1999; Pörtner 2002), as the lower standard metabolic rates of polar species result in a significant reduction in absolute metabolic power compared with temperate and tropical fishes (Clarke and Johnston 1996; Pörtner 2002). The maximum metabolic scope of the relatively sedentary turbot, however, was 176 mg O_2 kg^{-1} h^{-1} at 18°C (Mallekh and Lagardere 2002), which is similar to the maximum value of *T. bernacchii*. The greater metabolic scope of the mobile, semi-pelagic *P. borchgrevinki* confirms the relevance of metabolic scope as an indicator of locomotory ability in Antarctic nototheniids, and is in support of the theory that the behavioural and physiological options available to ectotherms in any given environment are a direct function of their scope for activity in that environment (Fry 1947; Johnston et al. 1991). This is also in support of the findings of Crockett and Sidell (1990), who

reported a relationship between maximum enzyme activities and activity levels of Antarctic nototheniids, with higher rates in the semi-pelagic *T. newnesi* than in the sedentary *Gobionotothen gibberifrons*. The fact that *T. bernacchii* could not be induced to swim in a swimming tunnel and was therefore chased to exhaustion, may, however, have had some influence on the lower magnitude of V_{O_2} max, as the maximum oxygen consumption rate may not have been attained.

In both Antarctic nototheniids, maximum oxygen consumption rates and scopes for activity were attained at 3°C. It has been suggested that the conditions at which metabolic scope is maximal correspond to the optimal environmental parameters for a given species (Kelsch and Neill 1990), and the temperature at which the highest oxygen uptake occurs is close to the optimal temperature for growth in some species (Jobling 1981; Waller 1992). This indicates that 3°C may be close to the thermal optimum for 0°C-acclimated fish, but with the differing thermal responses of 0°C and -1.86°C-acclimated fish (see Chapter 9), this result may not be relevant to fish in the wild.

From 3 to 6°C a reduction in the maximum oxygen consumption rate, combined with a marked increase in the resting rate, resulted in a marked decrease in aerobic scope of both species, similar to the decline in factorial aerobic scope of the Antarctic teleost *Pachycara brachycephalum* near 6°C (Mark et al. 2002). The aerobic scope of Atlantic cod is also reduced at temperatures near the upper lethal limit (Schurmann and Steffensen 1997; Claireaux et al. 2000). The thermal sensitivity of maximum metabolic rate thus differs from that of resting metabolic rate, with the maximum metabolic rate of most species attaining a peak at an optimum temperature and then decreasing above this level (Newell 1973). There must therefore be some cellular mechanisms which have differing thermal sensitivities in active and resting animals. Newell (1973) studied the intertidal gastropod *Littorina littorea* and found that rates identical to the active rates could be induced by supplying high substrate levels, whereas low and temperature-independent rates, identical to the standard rate of inactive animals, could be induced at low substrate concentrations. Positive or negative modulation of enzyme-substrate affinity with temperature has also been shown to maintain reaction rates of a variety of enzymes relatively independent of temperature at low substrate concentrations in several other ectotherms (Hochachka and Somero 1971). The differences in thermal responses of resting and maximal oxygen consumption rates may therefore be the result of variations in the availability of substrates to the mitochondria.

The decrease in aerobic scope near the upper thermal limit has not been fully explained, although it has been hypothesized that the energy demands of ventilation and associated circulation become excessive at some point, reducing the scope for activity (Jones 1971; Pörtner et al. 2000). As indicated in Chapter Seven, there is a marked decrease in the scope for change in cardiac output with increasing temperature in -1°C-acclimated fish, which relates well to the decrease in aerobic scope. The scope for change in ventilation frequency, however, was maintained to 6°C in both species, although this is not necessarily representative of maintenance of scope for total gill ventilation. Along with the increased demands of the heart and respiratory muscles and the increased costs of osmoregulation (Webb 1971), there is a considerable increase in red muscle activity from resting to maximal rates (Driedzic and Hochachka 1978). With red muscle representing only 1.6-2.9% of the total body weight of Antarctic nototheniids, depending upon activity level (Eastman 1993), the increase in oxygen consumption of this tissue alone must be very high.

The resting ventilation rates of *P. borchgrevinki* and *T. bernacchii* at 0°C compare well with the majority of previous values which range from 12.3 ± 1.4 to 25.4 ± 1.3 min^{-1} in the former, and 17.8 ± 0.9 to 22 ± 4 min^{-1} in the latter, at temperatures from -1.5 to 0.5°C (Tetens et al. 1984; Wells 1987; Forster et al. 1998; Davison 2001; Wilson et al. 2002). Forster et al. (1987), however, reported a considerably higher resting ventilation rate of *P. borchgrevinki* (44.3 ± 1.6 min^{-1} at 0°C) after 2-12 hours in respirometry chambers. The thermal insensitivity of resting ventilation rate observed in this study, however, is in contrast to previous reports. An increase in ventilation rate of both *P. borchgrevinki* and *T. bernacchii* has been reported during acute 10°C exposure (29.3 ± 2.0 min^{-1} and 55.0 ± 5.0 min^{-1}, respectively) (Forster et al. 1998), and increases have been reported from *P. borchgrevinki* after 18 hours at 4°C (43 min^{-1}) (Wilson et al. 2002), and after 8-13 days of 4.5°C acclimation (32.3 ± 7.8 min^{-1}) (Tetens et al. 1984). Another notothenid fish, *Lepidonotothen nudifrons*, exhibits a normal Q_{10} relationship between ventilation and temperature, with a progressive deviation from this relationship only approaching the upper critical limit (Hardewig et al. 1999). The frequency of opercular movements in carp has, however, been demonstrated to increase with an acute change in temperature, but to decrease with a longer period of acclimation to the higher temperature (Cai and Adelman 1990). The temperature-dependence of ventilation frequency of freshly-caught *P. borchgrevinki* (Chapter 8) suggests that the insensitivity observed in the current investigation may have been influenced by the 2-3 month period

of captivity at 0°C. The mechanisms controlling ventilation in fishes are still unclear. It has been suggested that elevated levels of circulating catecholamines do not play a role in the stimulation of ventilation (Gilmour 1998), and Egginton et al. (unpubl. data) have shown that ventilation rate is affected by bilateral vagotomy and therefore under some degree of cholinergic control.

An increase in total ventilation would be expected in association with the substantial increase in resting oxygen consumption from 3 to 6°C. The absence of an increase in ventilation frequency may either be due to a widening gap between oxygen supply and demand as the cost of increasing ventilation becomes excessive nearing the upper thermal limit, or due to increased reliance on stroke volume to modulate total gill ventilation at higher temperatures. Increased ventilation rates are thought to result in a reduction in the effectiveness of oxygen uptake (Hughes and Shelton 1962; Taylor et al. 1997), and stroke volume is therefore reported to be the most economical method by which to increase total gill ventilation (Fernandes and Rantin 1989; Perry and Wood 1989). Patterns of gill perfusion may also be actively regulated (Nilsson and Pettersson 1981) increasing the number of lamellae perfused at higher temperatures. Both *P. borchgrevinki* and *T. bernacchii* have been shown to utilise the gills as the major site of gas exchange at environmental temperatures, with minimal contribution from cutaneous respiration (Wells 1987; Davison et al. 1990; Davison 1998; Davison 2001). No studies have been carried out to investigate the effect of increasing temperature on the relative importance of cutaneous respiration, although no compensatory shift is observed in fish with gills damaged by X-cell disease (Davison et al. 1990).

The thermal independence of ventilation rate at V_{O_2} max from 0 to 6°C in both species compares well with the thermal insensitivity reported by Wilson et al. (2002) from 2 to 8°C. The magnitude of ventilation frequency of *P. borchgrevinki* at V_{O_2} max also compares well with the values reported by Wilson and co-workers, but are lower than values reported by Forster et al. (1987) from a study which also found a higher resting ventilation rate. The lower active ventilation frequency of *T. bernacchii* at all temperatures relates well to the lower V_{O_2} max of this species.

The critical swimming speed (U_{crit}) attained by *P. borchgrevinki* at 0°C (1.86 ± 0.04 bl s^{-1}) falls in the middle of previously reported values which range from 1.0 to 2.7 bl s^{-1}, at temperatures from 0 to -1.5°C (Wohlschlag 1964; Montgomery and Macdonald 1984; Forster et al. 1987; Davison et al. 1988; Archer and Johnston 1989; Johnston et al.

1991; Franklin and Johnston 1997; Wilson et al. 2002). Some of the higher values from previous studies were from fish acclimated to temperatures nearer their -1.86°C environmental temperature, indicating that acclimation temperature may have had an influence on the values measured in this study. Critical swimming speeds of fish kept at -1°C for only three days following capture (Chapter Six), however, were not significantly different from the values obtained in this investigation.

The comparison of swimming speeds between fish from different temperatures is difficult, not only because of differences in the physical properties of the media, but also because of differences in modes of swimming, body size, form and ecology. Notothenioids, both demersal and pelagic, routinely perform labriform (Lindsey 1978) swimming, engaging in subcarangiform swimming only for burst activity (Montgomery and Macdonald 1984; Archer and Johnston 1989). The failure of *T. bernacchii* to recruit the myotomal musculature in subcarangiform locomotion at any temperature may either be a feature of the eco-physiology of the species, or may be due to the fact that chasing failed to elicit the maximum response. In labriform locomotion, thrust is produced by a sculling movement of the pectoral fins with the tail used as a rudder (Montgomery and Macdonald 1984) and the majority of these fish thus have large pectoral fins (Andriashev 1965) which are powered mainly by oxidative red fibres (Davison and Macdonald 1985; Johnston and Harrison 1985). Typically, white muscle supports anaerobic activity through phosphocreatine and glycogen reserves, whereas red muscle is engaged in oxidative metabolism and sustains aerobic swimming. Notothenioid species possess large amounts of white muscle and the rapid exhaustion of *P. borchgrevinki* following recruitment of the myotome, is likely to be due to low activity levels of enzymes involved in the glycolytic pathway and a low anaerobic capability (Dunn and Johnston 1986; Johnston 1987; Davison et al. 1988). Lactate accumulation and phosphocreatine hydrolysis are more limited at lower temperatures (Weiser et al. 1986; Via et al. 1989), but it has not yet been determined whether the low anaerobic capacity of Antarctic notothenioids is a consequence of the low environmental temperature and a lack of thermal compensation of the enzymes of the glycolytic pathway (Holeton 1974), a result of the switch from myotomal-based slow speed swimming to pectoral-fin based swimming (Davison 1988), or a phylogenetic trait of the family. One theory is that glycolytic enzymes may be reduced in species which have compensated mitochondrial (oxidative) enzyme activity at low temperatures (Guderley 1998).

P. borchgrevinki is reported to employ labriform locomotion for velocities up to 1.8 bl s^{-1} (Montgomery and Macdonald 1984), although in this study the fish were only able to maintain station in the tunnel using their pectoral fins alone at speeds up to 1.4 bl s^{-1}, similar to the maximum labriform swimming speed of *N. neglecta* (Archer and Johnston 1989). Critical swimming speeds for temperate labriform swimmers vary between 3 and 4 bl s-1 (Webb 1973; Webb 1984; Davison 1988), indicating that the U_{crit} of Antarctic species is slightly lower than temperate species, but not unduly compromised by the low environmental temperatures.

Critical swimming speed can be affected by training (Beamish 1978) and as the majority of fish swam twice in this study there was a possibility that the final swimming trial (at 0°C) may have been influenced by a training effect. Consecutive swims in this study were at least 3 weeks apart, and with the results of Chapter Six indicating no influence of training over a shorter time-period, training does not appear to be a complicating factor. The swimming temperatures were not assigned randomly due to the practical difficulties associated with altering water-bath temperatures between consecutive fish, and swimming speed was not corrected for blocking effects as the maximum cross-sectional area of the fish was less than 5% of the cross-sectional area of the tunnel resulting in negligible error from this source (Bell and Terhune 1970). There is an effect of mass on relative swimming speed in fish, with absolute U_{crit} values (cm s^{-1}) for large fish being greater than for small, but relative values (body length s^{-1}) demonstrating the reverse trend. This is due to decreasing mass-specific power output as size increases (Hammer 1995), but as there was little variation in size among the fish involved in this study, scaling was not employed.

The critical swimming speed of *P. borchgrevinki* was independent of temperature from 0 to 3°C, but declined significantly at 6°C. Early studies suggested that *P. borchgrevinki* reached its maximum swimming speed at -0.8°C and was unable to swim above 2°C (Wohlschlag 1964), although this has been refuted by more recent results. Wilson et al. (2002) reported similar results to this study with a slight (17%) decline in critical swimming speed accompanying an increase in temperature from -1 to 4°C, and a much greater decrease in performance from 4 to 8°C. They reported a thermal performance breadth of 5°C for critical swimming speed, slightly greater than the 3°C performance breadth of this study, but considerably less than the 11°C value for burst swimming (Wilson et al. 2001). Critical swimming speed is typically reduced at low temperatures, increases to a maximum speed at an optimum temperature (which varies

between species and with acclimation temperature), and is reduced at temperatures close to the upper thermal limit for the species, resulting in a bell-shaped curve (Brett 1964; Brett and Glass 1973; Geist et al. 2003). Although only the upper half of the curve is able to be described for *P. borchgrevinki*, the effect of an increase in temperature on U_{crit} fits this pattern and mirrors the change in aerobic scope.

Recent research has indicated that critical swimming speed is not necessarily a measure of an animal's aerobic performance, as originally proposed by Beamish in 1978. In some fish, attainment of U_{crit} implies that V_{O_2} max has been exceeded, and that the organism has entered a state of anaerobic metabolism prior to fatigue (Lee et al. 2003). In both Atlantic and Greenland cod, the highest oxygen consumption is recorded during the first 10 minutes of recovery (Bushnell et al. 1994), and a delayed rise, with a peak at 20 minutes post-exercise, has previously been reported in *P. borchgrevinki* (Davison et al. 1990), although this was in contrast to the results of Forster et al. (1987). In this study, the maximum rate of oxygen consumption was recorded at U_{crit} in both species, and the rapid recovery of oxygen consumption rates to resting levels suggests that there was no sizeable oxygen debt to repay. Prolonged swimming ability is therefore likely to be a good measure of aerobic performance for *P. borchgrevinki*. With *P. borchgrevinki* observed to recruit the myotomal musculature in attainment of U_{crit}, in contrast to *T. bernacchii*, recovery of the former may have been expected to be more prolonged but this was not observed with recovery rapidly achieved by both species.

The oxygen consumption rates of both Antarctic nototheniids were restored to resting levels 10 to 45 minutes after exhaustive exercise at all temperatures, with the shortest recovery periods at 6°C. From graphs of the data (Fig. 4.9 and 4.10), it appears as though the duration of recovery was actually increased at 6°C, but due to greater inter-individual variation at the higher temperature the significance of differences between post-exercise and resting values was reduced. Other researchers have found recovery processes to be largely independent of temperature (Kieffer et al. 1994; Franklin et al. 1996), although tilapia chased to exhaustion demonstrate a greater oxygen debt and increase in oxygen uptake during recovery at higher temperatures (McKenzie et al. 1996).

The recovery of ventilation rates to resting levels was also achieved rapidly (within 20 minutes) in both Antarctic nototheniids at 0°C and 3°C, relating well to the rapid return to resting oxygen consumption rates at these temperatures. Recovery at 6°C was more prolonged in both species, in support of the observation that the rate of oxygen

consumption at 6°C appears to be elevated for a longer period at this temperature. Even at 6°C, however, recovery was still relatively rapid, with a return to resting ventilation frequency within 24 hours. Immediately following the exhaustive exercise, an increase in the magnitude of opercular movements was observed in both species. This was accompanied by frequent "gulps" which involved an excessive movement of the operculum and contributed to a reduction in average ventilation rate, but presumably increased flow through the gills.

The brief recovery periods of the current study lend no support to the theory that the major effect of low temperature on prey capture and predator avoidance in polar fish would be through a reduction in the rate at which mitochondrial activity or gluconeogenesis could clear the accumulated products of anaerobic utilisation of substrates (Clarke 1998). After notothenioids have been forced into periods of subcarangiform swimming the levels of lactate in red and white muscle remain relatively low (Davison et al. 1988, Davison unpubl. data) as the anaerobic energy production in these fibres is supplied by hydrolysis of phosphocreatine, rather than by anaerobic glycolysis (Dunn 1988). The short recovery period is therefore likely to be required predominantly for the regeneration of creatine phosphate stores (Walesby and Johnston 1979; Dunn and Johnston 1986), although there may be a relatively small requirement for the repletion of glycogen stores (Scarabello et al. 1992), correction of ion and fluid volume shifts which occur during exercise (Wood 1991), and a non-specific stress reaction to forced exercise (Scarabello et al. 1991). Phosphocreatine is completely rephosphorylated within 3 hours in the relatively inactive Antarctic eelpout, which is more rapid than observed in cold-adapted eurythermal eelpout, and in support of the idea that cold-adaptation favours rapid recovery (Hardewig et al. 1998). This restoration of pre-exercise conditions within reasonable time enables bouts of activity to take place in succession, and may be crucial in predator-prey interactions.

In conclusion, the maximum metabolic rates and aerobic scopes of both *P. borchgrevinki* and *T. bernacchii* were found to increase with temperature but decrease rapidly approaching the upper lethal limit. The decline in aerobic scope from 3 to 6°C contributed to a similar decline in the predominantly aerobic prolonged swimming ability of *P. borchgrevinki*. There was evidence provided for a correlation between lifestyle and aerobic scope, but no significant effect of lifestyle on resting oxygen consumption rates. Comparison of the whole-animal maximum aerobic capacities of Antarctic and temperate-water species indicated that there had been some thermal compensation of

active rates in the Antarctic species, but not to the degree predicted by the theory of metabolic cold adaptation.

Chapter Five

The effect of an acute increase in temperature on resting oxygen consumption rate of temperate-water notothenioid fishes

INTRODUCTION

The original concept of metabolic cold adaptation, which suggested that the resting metabolic rates of polar fishes should be higher at their environmental temperatures than those of fish adapted to higher temperatures but acclimated to similar low temperatures (Krogh 1914) has since received both support (Scholander et al. 1953; Wohlschlag 1960; Wohlschlag 1964; Ralph and Everson 1968), and opposition (Holeton 1973; Holeton 1974; Clarke 1983; Wells 1987; Clarke 1991; Bushnell et al. 1994; Clarke and Johnston 1999; Drud Jordan et al. 2001; Peck 2002). The majority of recent investigations which have compared fishes of similar lifestyle provide no evidence for the theory (Johnston et al. 1991; Johnston and Battram 1993; Bushnell et al. 1994; Steffensen et al. 1994), although there are a minority which conclude that it cannot be dismissed (Wells 1987; Torres and Somero 1988b; Torres and Somero 1988a). One of the factors which complicated early comparisons of the metabolic rate of Antarctic fishes with warmer water species is that most of the data for temperate and tropical species was from active freshwater fishes. There is an inherent problem in comparing species with different levels of locomotory activity in that metabolic rate varies as a function of lifestyle (De Vries and Eastman 1981). Comparisons are therefore only meaningful when made between fish with similar feeding strategy, locomotory ability, size, and dietary state (De Vries and

Eastman 1981; Wells 1987). One possible way to minimise this problem is to use related species from different latitudes where habitat temperature is the major variable.

Inhabiting the temperate waters off New Zealand coasts are two species which belong to the perciform suborder Notothenioidei: *Bovichtus variegatus* (Richardson, 1864) from the Bovichtidae (thornfish) family, and *Notothenia angustata* (Hutton, 1875) (also known as *Paranotothenia angustata*) from the Nototheniidae family which includes the Antarctic species *Pagothenia borchgrevinki* and *Trematomus bernacchii.*

The bovichtids have long been regarded as the most primitive notothenioid family (Regan 1914, cited in Eastman 1993), a view which has been sustained by modern systematic (see Gon and Heemstra 1990) and karyological work (Prirodina 1986). An area cladogram for notothenioid families, produced by mapping broad geographic distributions onto the taxa produced by cladistic analysis, suggests that the ancestral notothenioid stock initially split into two clades with one containing the bovichtids and a second containing all other notothenioids (Fig. 5.1). The phylogenetic trees drawn from the oxygen-transport system of temperate-water notothenioids place the divergence of Bovichtidae prior to the formation of the Antarctic Convergence (Fago et al. 1992).

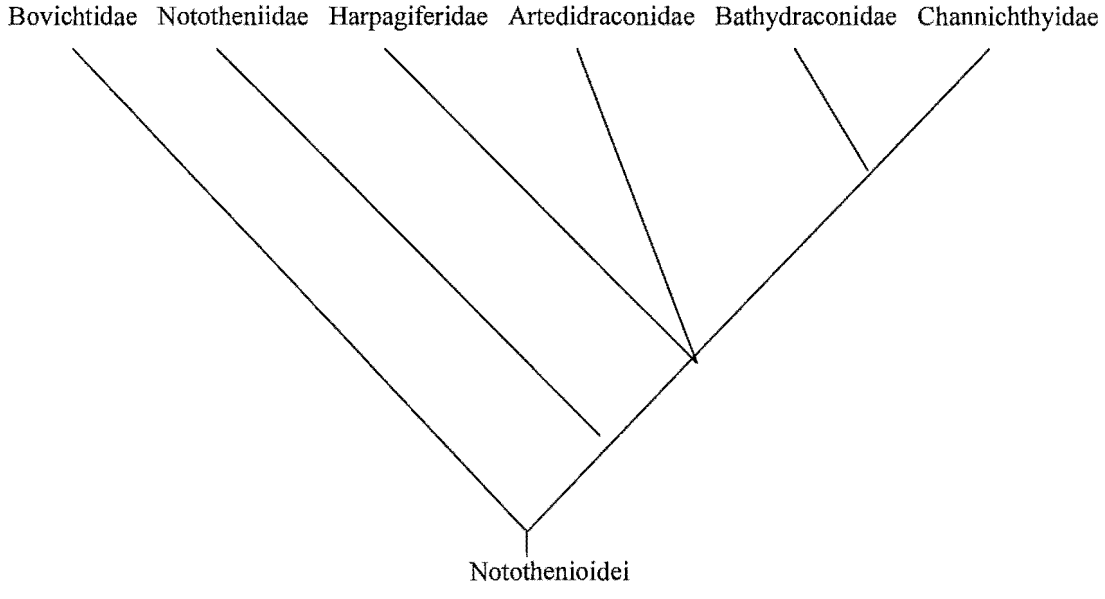


Fig. 5.1. Area cladogram of hypothesized relationships among families of the suborder Notothenioidei. Adapted from Eastman 1993.

The bovichtid family is unusual among the notothenioids in that it has a largely non-Antarctic distribution. There is only one species found south of the Antarctic Polar Front, with the remainder inhabiting waters around South America, Southeastern Australia, New Zealand, and a few islands near the Subtropical Convergence (Eastman 1993). In common with the majority of bovichtids, *B. variegatus* has a heavy body and bony, spiny head. The species is endemic to New Zealand (Hardy 1988) and has been recorded from the lower half of the North Island (from New Plymouth on the west and Tokomaru Bay on the east) to the Auckland Islands in the Subantarctic, a latitudinal range of over 12°. *B. variegatus* is an intertidal fish, common in tidal pools and on shallow rock reefs to a depth of 9m, with a diet of crustaceans and worms (Hardy 1988). In its natural environment this species encounters water temperatures from 5 to 19°C (Eastman 1993). The juveniles pass through a pelagic distribution phase (Robertson and Mito 1979), which may explain the wide range of this genus around the northern margin of the Southern Ocean, while the adults have a dorsally directed exit for water from the operculum, an indication of a sedentary lifestyle (Eastman 1993). Like the Antarctic notothenioids, *B. variegatus* lacks a swim bladder, but in contrast to the more southern species it lacks the genes for anti-freeze glycoprotein synthesis (Cheng et al. 2003) and possesses a functional glomerular kidney (Eastman 1993).

The nototheniids are the most diverse notothenioid family with respect to size, body form, habitat, and distribution. They are found throughout the Antarctic and Subantarctic regions, as well as in the coastal waters of New Zealand and South America (Eastman 1993). The notothenid *N. angustata* is commonly found in both the New Zealand and Patagonian regions and is very similar to the Antarctic nototheniids with respect to haemoglobin multiplicity (D'Avino and di Prisco 1997), amino acid composition (di Prisco and D'Avino 1989; di Prisco et al. 1990) and oxygen binding properties (di Prisco et al. 1991). This species also possesses a pauciglomerular kidney (Eastman 1993) and the genes for glycopeptide antifreeze production (Cheng 2000), and recent results have demonstrated that the transcribed antifreeze glycopeptide genes of *N. angustata* are translated into small amounts of circulatory protein, with expression augmented to slightly higher levels by acclimation of the fish to 2-4°C (Cheng et al. 2003). Phylogenetic trees drawn using the oxygen-transport system (Fago et al. 1992), antifreeze glycopeptide genes (Cheng et al. 2003), and morphological analysis and other molecular approaches (Bargelloni et al. 1994) support an Antarctic evolutionary history for this species. It is thought that *N. angustata* moved northwards from Antarctic waters

when the Antarctic Convergence advanced 300 km to the north during the late Miocene (6.5 – 5.0 million years ago) and cold water reached as far north as New Zealand (Kennett 1982). *N. angustata* is described as a demersal benthic species, although juveniles are found in rock pools (Froese and Pauly 2004). This species feeds on squid, invertebrates and small fishes and is found to depths of 200 m (Gon and Heemstra 1990), at water temperatures from 4 to 14°C (Guynn et al. 2002).

The major objective of this study was to investigate the effect of an acute change in temperature on resting oxygen consumption rate of two temperate-water notothenioids (*B. variegatus* and *N. angustata*), with the intention of comparing the responses with those of the Antarctic nototheniids (*P. borchgrevinki* and *T. bernacchii*) studied in Chapter Four. A secondary investigation involved determining the thermal sensitivity of the scaling coefficient for the relationship between resting oxygen consumption rate and mass of *B. variegatus*.

MATERIALS AND METHODS

Bovichtus variegatus (mass 69.5 ± 84.9 g (mean ± SD), range 8.1 – 185 g, n = 5), and *Notothenia angustata* (mass 653.3 ± 181.4 g (mean ± SD), range 400 – 810 g, n = 6) were caught by staff at the Portobello Marine Laboratory on the Otago Peninsula (water temperature approximately 10°C) and transported by road in aerated insulated bins to Christchurch. The survival rate for transportation was 100%. The fish were held in a recirculating seawater aquarium system (water temperature 12.3 ± 0.5°C) at the University of Canterbury and fed mussels (*Perna canaliculus*) twice weekly for 6 weeks prior to the study. The fish were fasted for one week prior to each experiment as the specific dynamic action of the relatively sedentary temperate-water sculpin is reported to be 152 hours (Johnston and Battram 1993) and most temperate organisms show a suppressed rate of oxygen consumption after a longer period of starvation (Wieser 1973). A large proportion of the seawater in the aquarium was replaced weekly with fresh seawater from Lyttleton Harbour. A protein skimmer filtered the water and regular assessment was made of both salinity and the concentration of nitrogenous waste products. A 12/12 hour light/dark regime was maintained during both the acclimatisation period and experimental runs.

Bovichtus variegatus

The set-up for measuring resting oxygen consumption of *B. variegatus* was similar to that used for the two Antarctic nototheniid species (see Chapter Four), using the same cylindrical perspex respirometry chambers (volumes 1410-1470 mL). The fish were placed into the chambers individually 24 hours prior to the commencement of recording in order to allow recovery from the stress of handling, and a tinfoil collar was used to shield the fish from adjacent human activities. The chambers were immersed in thermostatically-controlled water-baths at 12°C, and a continuous flow of air-saturated seawater maintained from a water-jacketed reservoir. At the end of the settling period, each chamber was isolated from the reservoir circuit by closing taps without disturbing the fish. A peristaltic pump was used to draw water from the chamber continuously past a water-jacketed IL 1302 oxygen electrode, and a Powerlab Data Acquisition System fed the information into a laptop running ADInstruments Chart for Windows version 4.0.2. recording software, as described in Chapter Four. The oxygen tension of the water was recorded continuously for 120 minutes, or until the oxygen tension of the water had dropped to 120 mm Hg (approximately 90 minutes in the largest fish at the highest temperature). Ventilation frequency was determined at the commencement of recording by counting opercular movements over the duration of one minute. Due to limited numbers, the same fish were then subjected to identical protocols at 18, 6, and 3°C, in that order. The fish were fed and then fasted for a week between consecutive experiments. To determine the resting rate of oxygen consumption, the change in oxygen tension over the initial 20 minute period was disregarded as this was typically variable, and the change in oxygen tension averaged over the following hour. The rate of oxygen consumption was found to be very stable over this period at all temperatures.

Notothenia angustata

In order to determine resting oxygen consumption of *N. angustata* a much larger respirometry set-up was required. A rectangular perspex respirometry chamber (8.08 L capacity) was immersed in a thermostatically-controlled water-bath, with continuous seawater circulation from a 250 L thermostatically-controlled reservoir. A large proportion of the seawater in this reservoir was changed after each alternate experimental run and a pump was used to circulate water within the chamber, both during and between

measurements. During measurements, the respirometry system was isolated from the reservoir by closing taps on adjacent tubing and 1 mL samples were removed by syringe from the chamber at 10 minute intervals with the water volume replaced by suction from a second syringe filled with air-saturated seawater. The samples were immediately injected into a water-jacketed Strathkelvin cell (MC 100) connected to a Strathkelvin oxygen meter (model 781). Measurements continued until the oxygen tension within the respirometer had fallen to between 110-120 mm Hg, which took about 1 hour at the lower temperatures and about 30 minutes at the highest temperature. Unfortunately the fish were unable to be left in the system overnight, due to the relative sizes of the fish and the seawater reservoir, but were introduced to the respirometer 6-8 hours before recording began. Ventilation frequency was determined following the initial oxygen tension measurement by counting opercular movements. As with *B. variegatus*, the same fish were used for each run although one fish died between trials due to a problem in the aquarium system and was replaced in the final experimental run with another individual. Resting oxygen consumption of each fish was determined individually at 6, 12, 18, and 3°C, in that order. The fish were fed and fasted for 1 week between experiments. In calculation of the rate of oxygen consumption the first 10 minute interval was excluded and the mean oxygen consumption rate determined over the remainder of the measurement period.

As described in Chapter Four, the oxygen meters were calibrated at the beginning and end of each experimental run using air-saturated seawater (at the same temperature and salinity as that used in the subject respirometer), and the zero level checked daily using a solution of 3.81 g L^{-1} sodium borate into which crystalline sodium sulphite had been added and stirred to partially dissolve. The salinity of the reservoir seawater was monitored regularly, and samples of seawater within the chambers were taken at the conclusion of 2 experimental runs at each temperature to assess ammonia (phenol nitroprusside method), nitrate (Hagen test kit #A-7845), and nitrite (Hagen test kit #A-7825) concentrations. All concentrations were within the normal physiological range. The respirometry chambers were thoroughly cleaned between fish in order to prevent microbial build-up and the background oxygen consumption over 90 minutes in a blank (empty) respirometer was found to be negligible at all temperatures. An oxygen-depleted control respirometer confirmed the absence of leaks in the system. No attempt was made to remove carbon dioxide from the system and so as oxygen levels were depleted, carbon dioxide levels rose.

DATA ANALYSIS AND STATISTICAL METHODS

Oxygen Consumption

After correction for meter drift, the rate of oxygen consumption in units of mg O_2 kg^{-1} h^{-1} was calculated from:

$$V_{O_2} = \frac{\Delta P_{O_2} \times C \times V \times 31.999}{t \times M}$$

where:

ΔP_{O_2} = change in oxygen partial pressure over the measurement period (mm Hg)

C = oxygen capacitance of seawater at a given temperature (μmol L^{-1} mm Hg^{-1})

V = volume of water in the respirometer (L)

31.999 = molecular weight of O_2

t = duration of measurement (h)

M = mass of the fish (g)

To determine the volume of water in the chambers, an approximation was made in that both species were assumed to be neutrally buoyant. The buoyancies (% body weight in seawater at -1.9°C) of *B. variegatus* and *N. angustata* are 5.87 and 4.43 respectively (Eastman 1993). The oxygen capacitance values used were 2.13 (3°C), 1.99 (6°C), 1.77 (12°C), and 1.59 (18°C) μmol L^{-1} mm Hg^{-1}.

Resting oxygen consumption was corrected to 100 g mass (*B. variegatus*) or 500 g mass (*N. angustata*) using the following formula:

$$V_{O_2}(X) = V_{O_2} \times (M/X)^{(1-A)}$$

where:

X = 100 (*B. variegatus*) or 500 (*N. angustata*) g

$V_{O_2}(X)$ = O_2 consumption for an animal with mass X (g)

V_{O_2} = O_2 consumption for an animal with mass M (g)

A = mass exponent describing the relationship between metabolic rate and body mass. A value of 0.8 was used (see discussion).

Q_{10}

Q_{10} values were calculated using the Van't Hoff equation (Hoar 1975):

$$Q_{10} = (R_2 / R_1)^{10/(T_2-T_1)}$$

where R_1 and R_2 are the rates at temperatures T_1 and T_2, respectively.

Scaling Exponent

Due to the order of magnitude variation in size of *B. variegatus* obtained for this study, it was possible to make an estimate of the scaling exponent by which resting metabolic rate varied as a function of body mass at 3, 6, 12, and 18°C. The relationship between oxygen consumption and body mass was calculated from logarithmically ($\log_{10}$) transformed data. Using the equation: $\log \dot{V}_{O_2}$ rest = $\log a + b$ (log mass), the mass exponent (b) was determined from the slope of the regression line.

Statistical Analysis

Data from *B. variegatus* at different temperatures were compared by repeated-measures one-way analysis of variance (ANOVA). Where a treatment effect was indicated, inter-temperature differences were determined using the Tukey-Kramer post-hoc test. A standard ANOVA was used to compare data from *N. angustata*, due to the fact that one fish died during the experiment. All oxygen consumption data for *B. variegatus* have been corrected to 100 g mass, and data for *N. angustata* corrected to 500 g mass, unless otherwise stated. Least squares linear regression was used to determine the slope of the $\log_{10}$ $\dot{V}_{O_2}$ versus $\log_{10}$ mass graphs. Analyses were carried out using GraphPad Prism version 4.00 software, and data are presented as mean ± SEM, unless otherwise stated. Statistical significance was taken at the level of $p<0.05$.

RESULTS

In the aquarium and in the respirometry chambers, both *N. angustata* and *B. variegatus* exhibited very little movement and remained perched on the base of the chambers.

Bovichtus variegatus

The oxygen consumption rate of *B. variegatus* at the 12°C acclimation temperature was 35.8 ± 1.9 mg O_2 kg^{-1} h^{-1}. There was a plateau in the rate of resting oxygen consumption from 6 to 12°C (Q_{10} = 1.4), with a significant increase from 12 to 18°C (Q_{10} = 3.0) attaining a maximum value of 69.4 ± 2.5 mg O_2 kg^{-1} h^{-1} at 18°C. The minimum rate (22.4 ± 1.5 mg O_2 kg^{-1} h^{-1}), measured at 3°C, was significantly lower than the rate at 12°C. (Table 5.1, Fig. 5.2). Ventilation frequency was also thermally insensitive near the acclimation temperature, with a plateau in rate from 3 to 12°C, and an increase in frequency accompanying the increase in oxygen consumption rate from 12 to 18°C (Table 5.1, Fig. 5.3).

Table 5.1. The effect of an acute change in temperature on oxygen consumption rate and ventilation frequency of 12°C-acclimated *B. variegatus*.

Temperature (°C)	V_{O_2} (mg O_2 kg^{-1} h^{-1})	V_{O_2} (100 g) (mg O_2 kg^{-1} h^{-1})	Ventilation frequency (min^{-1})	n
3	29.5 ± 2.4#	22.4 ± 1.5#	22.0 ± 2.2	5
6	39.5 ± 7.8	27.6 ± 5.5	28.6 ± 3.4	5
12	48.9 ± 8.1	35.8 ± 1.9	19.2 ± 1.1	5
18	94.6 ± 14.5*	69.4 ± 2.5*	44.0 ± 3.5*	5

Data obtained from 12°C-acclimated fish after 24 hours at the respective temperatures. * Significantly different from values at 3, 6 and 12°C. # Significantly different from values at 12 and 18°C.

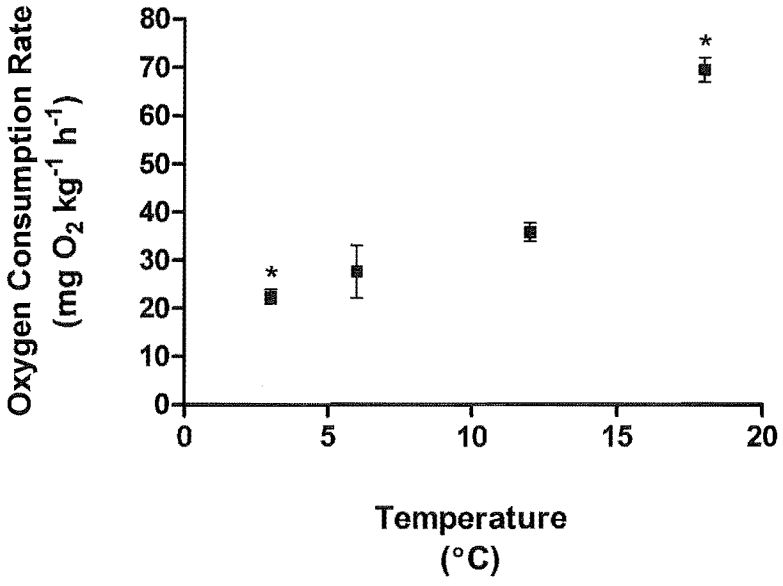


Fig. 5.2. The effect of an acute change in temperature on the oxygen consumption rate of 12°C-acclimated *B. variegatus.* Data obtained from fish following 24-hour exposure to each temperature. Values have been corrected to 100 g mass. N = 5.
* Significantly different from rate at 12°C acclimation temperature.

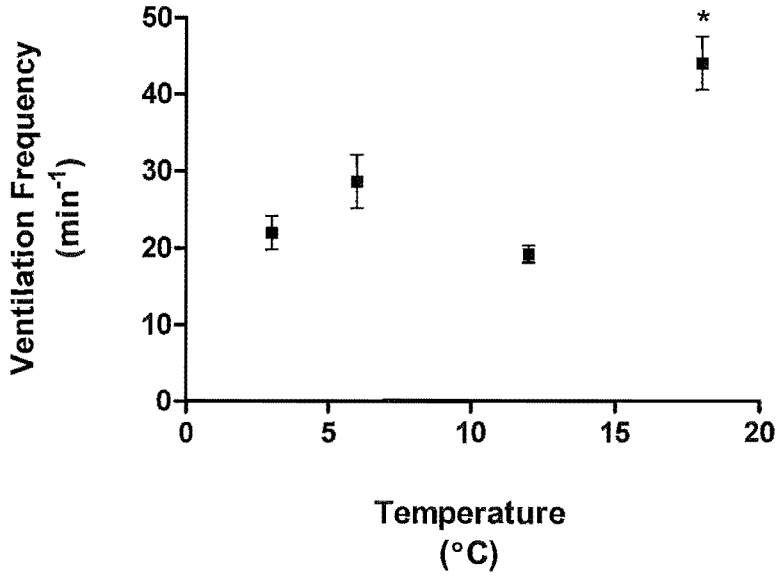


Fig. 5.3. The effect of an acute change in temperature on the ventilation frequency of 12°C-acclimated *B. variegatus.* Data obtained from fish following 24-hour exposure to each temperature. N = 5. * Significantly different from rate at 12°C acclimation temperature.

Notothenia angustata

Both oxygen consumption rate and ventilation frequency of *N. angustata* were considerably higher than those of *B. variegatus* at the 12°C acclimation temperature (Table 5.1 and 5.2). The rate of oxygen consumption of *N. angustata* declined below the acclimation temperature, although the decrease was not statistically significant. From 12 to 18°C, however, V_{O_2} increased significantly (Q_{10} = 4.0) to attain a maximum value of 120.9 ± 14.5 mg O_2 kg^{-1} h^{-1} at 18°C (Table 5.2, Fig. 5.4). Ventilation frequency increased slightly from 12 to 18°C with the increase in oxygen consumption, although the change was not significant, but there was a significant decrease in the ventilation rate at 3°C (Table 5.2, Fig 5.5).

Table 5.2. The effect of an acute change in temperature on oxygen consumption rate and ventilation frequency of 12°C-acclimated *N. angustata*.

Temperature (°C)	V_{O_2} (mg O_2 kg^{-1} h^{-1})	V_{O_2} (500 g) (mg O_2 kg^{-1} h^{-1})	Ventilation frequency (min^{-1})	n
3	25.9 ± 1.6	27.1 ± 1.7	19.5 ± 1.6#	6
6	30.3 ± 2.8	31.0 ± 2.9	30.3 ± 3.2	6
12	50.5 ± 3.3	51.7 ± 3.7	33.4 ± 2.2	5
18	116.5 ± 14.1*	120.9 ± 14.5*	38.4 ± 1.8	5

Data obtained from 12°C-acclimated fish after 24 hours at the respective temperatures. * Significantly different from values at 3, 6 and 12°C. # Significantly different from values at 6, 12 and 18°C.

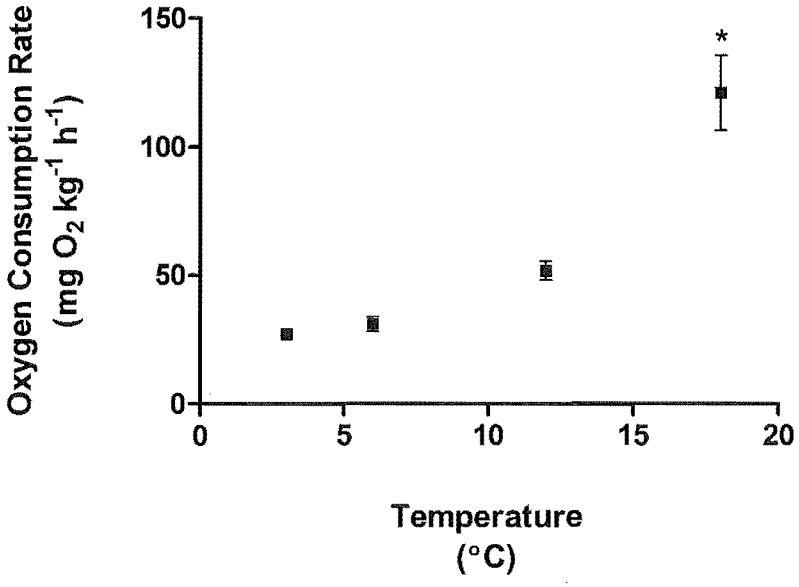


Fig. 5.4. The effect of an acute change in temperature on the oxygen consumption rate of 12°C-acclimated N. *angustata*. Data obtained from fish following 24-hour exposure to each temperature. Values have been corrected to 500 g mass. N = 6 at 3 and 6°C, N = 5 at 12 and 18°C. * Significantly different from rate at 12°C acclimation temperature.

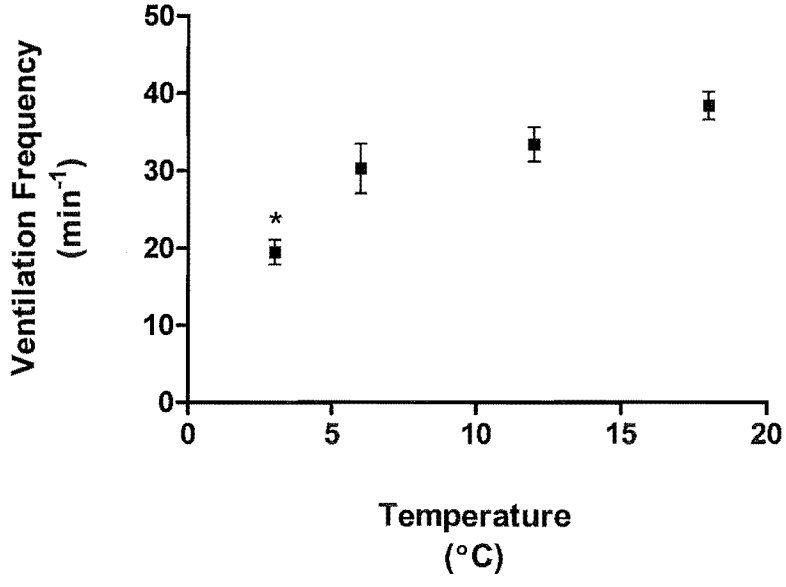


Fig. 5.5. The effect of an acute change in temperature on ventilation frequency of *N. angustata*. Data obtained from 12°C-acclimated fish after 24 hours at each temperature. Values corrected to 500 g mass. N = 6, at 3 and 6°C. N = 5, at 12 and 18°C. * Significantly different from rate at 12°C acclimation temperature.

Comparison of Temperate and Antarctic Notothenioid Species

Comparing the four species at their relative acclimation temperatures, the oxygen consumption rate of *B. variegatus* was not significantly different from the rates of the Antarctic species, while the rate of *N. angustata* was significantly higher. The minimum oxygen consumption rates of *N. angustata* and *B. variegatus* (both at 3°C) were lower than the minimum V_{O_2} of both Antarctic species (measured at 0°C), although the difference was only significant with regard to *B. variegatus* (Table 5.3, Fig. 5.6). The thermal sensitivity of oxygen consumption followed a broadly similar pattern in all four species, with a plateau near the acclimation temperature and a marked increase in thermal sensitivity at temperatures approaching the upper thermal limit (Antarctic species) or maximum habitat temperature (temperate species).

Table 5.3. The effect of change in temperature on resting oxygen consumption rates of Antarctic and temperate notothenioid fishes.

Temperature (°C)	*Pagothenia borchgrevinki*	*Trematomus bernacchii*	*Notothenia angustata*	*Bovichtus variegatus*
0	**32.8 ± 1.8**	**32.0 ± 1.7**		
3	36.4 ± 2.1	32.8 ± 1.9	27.1 ± 1.7	22.4 ± 1.5
6	51.6 ± 5.0	61.2 ± 7.2	31.0 ± 2.9	28.6 ± 3.4
12			**51.7 ± 3.7**	**35.8 ± 1.9**
18			120.9 ± 14.5	69.4 ± 2.5

Data corrected to 100 g mass, except *N. angustata* which is corrected to 500 g mass.
Numbers in bold represent values at the acclimation temperature respective species.

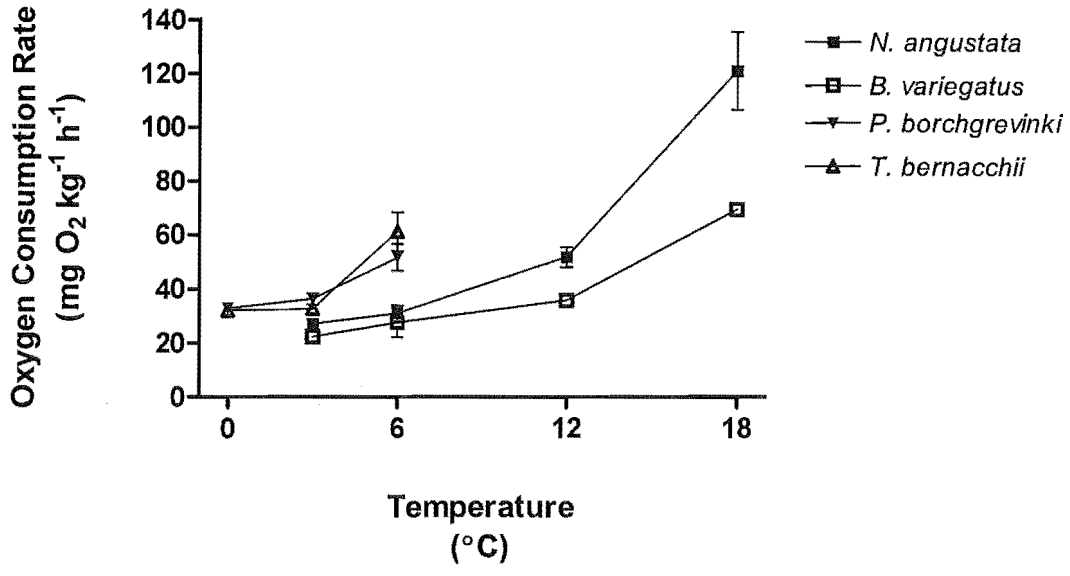


Fig. 5.6. Effect of an acute change in temperature on oxygen consumption rate of two temperate-water and two Antarctic notothenioid fishes. Temperate water species acclimated to 12°C, and Antarctic species acclimated to 0°C. Data corrected to 100 g mass, except for *N. angustata* which is corrected to 500 g mass. Note: the absence of error bars for some points is due to the small magnitude of error.

Scaling Coefficient

Resting metabolic rate is related to body size by the equation:

$$V_{O_2} = a \times M^b \quad \text{(Schmidt-Nielsen 1984)}$$

where:

V_{O_2}	=	resting rate of oxygen consumption
a	=	proportionality constant
M	=	mass of the fish (g)
b	=	the scaling exponent

Both V_{O_2} and M were logarithmically ($\log_{10}$) transformed, resulting in the relationship:

$$\log V_{O_2} = \log a + b \, (\log M)$$

The scaling coefficient *b* (slope of the graph) was determined at each temperature by the method of least squares linear regression (Fig. 5.7).

The scaling coefficient (*b*) at the 12°C acclimation temperature was 0.75 ± 0.03, and there was no significant difference between values at any of the four temperatures (Table 5.4). The magnitude of the y-intercept (*a*), however, increased significantly with increasing temperature.

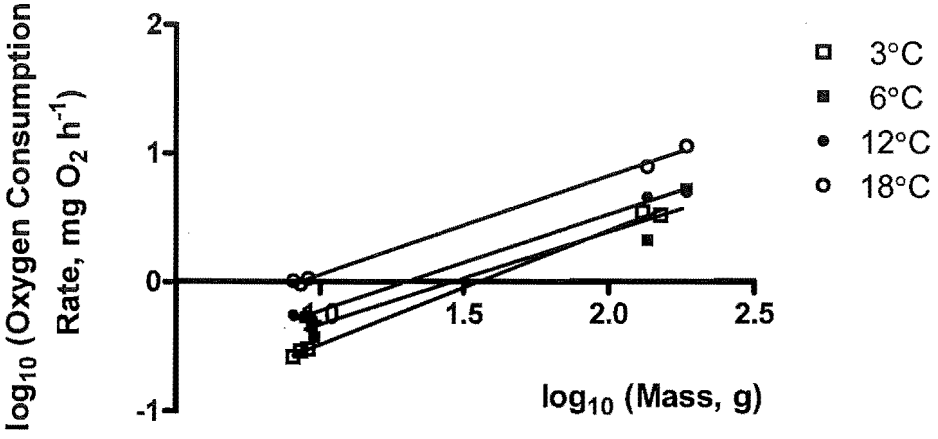


Fig. 5.7. Logarithmically transformed relationship between oxygen consumption rate and body mass of *B. variegatus* at different temperatures. N = 5. The equations of the linear regression lines are detailed in Table 5.4.

Table 5.4. Least squares regression analysis of oxygen uptake (mg h^{-1}) as a function of fish mass (g) for *B. variegatus* at four different temperatures.

Temperature (°C)	Slope (*b*) (± SEM)	y intercept (*a*)	Coefficient of Determination (r^2)
3	0.88 ± 0.03	-1.37	0.998
6	0.72 ± 0.11	-1.05	0.939
12	0.75 ± 0.03	-0.97	0.995
18	0.77 ± 0.02	-0.71	0.998

Body mass range 8.1 – 185.0 g. N = 5.

DISCUSSION

Oxygen Consumption Rates – Thermal Sensitivity and Compensation

The oxygen consumption rates of both *B. variegatus* and *N. angustata* decreased slightly as the temperature decreased below their 12°C acclimation temperature, although the difference was only significant from 12°C to 3°C in *B. variegatus*. The response to an increase in temperature above the acclimation level, however, was more marked, with significant increases in the rate of oxygen consumption from 12 to 18°C in both species. These results are in support of the general trend for an increase in temperature to be more perturbing of enzymes than a decrease (Somero et al. 1996). For processes such as enzyme activity, Q_{10} values are typically near 2 when thermal effects are studied within a species' normal (physiological) range of body temperatures, with increased thermal sensitivity outside the optimal range especially as temperature rises toward the upper limit for the species (Hochachka and Somero 2002). The resting metabolic rates of the majority of fishes are relatively temperature-independent near their acclimation temperature (Cossins and Bowler 1987; Jobling 1994; Taylor et al. 1997; Hochachka and Somero 2002; Mallekh and Lagardere 2002), but increase above and/or below, particularly in response to acute changes in temperature (Peck 1989; Johnston et al. 1991; Claireaux et al. 2000; Hochachka and Somero 2002). The resting metabolic rate of the Antarctic notothenid *Notothenia neglecta*, for example, is more temperature-dependent above its normal thermal range (Q_{10} 3-6°C = 6.4), while thermal sensitivity of the tropical species *Paracirrhites arcatus* increases below its physiological range (Q_{10} 13.5-20°C = 7.9) (Johnston et al. 1991). The Antarctic notothenids *Pagothenia borchgrevinki* and *Trematomus bernacchii* exhibit a similar trend with increased thermal sensitivity from 3 to 6°C (Chapter Four) although, due to the freezing point of seawater, only the response above their acclimation temperature can be described. The responses of organisms to acute thermal changes differ from those of species acclimated to the same thermal change and with the fish in this study exposed to each temperature for 24 hours the response can be considered to be acute. It has been demonstrated in Atlantic cod that fish acclimated to different temperatures have similar metabolic rates, whereas fish acutely exposed to different temperatures exhibit greater thermal sensitivity (Steffensen et al. 1994).

A significant effect of seasonality on growth and feeding, particularly of Antarctic species, has also been suggested, although no evidence of a seasonal effect was detected in comparing the oxygen consumption rates of -1 to -0.5°C (winter) and 0 to 0.9°C (summer) acclimated *N. neglecta* (Johnston and Battram 1993). In this study, the Antarctic species were collected in early summer, and the temperate-water species caught in late summer, both times of relatively high productivity, and therefore metabolic rates of both groups should have been near maximum levels.

The relatively greater thermal sensitivity of *N. angustata* from 12 to 18°C, compared with *B. variegatus*, relates well to the lower thermal range of the former. As the latter is an intertidal species, it is likely that it would have a greater tolerance of higher and more variable temperatures, with the significant risk of isolated rock pools warming during the day. The increase in thermal sensitivity of *B. variegatus* from 6 to 3°C, however, indicates that this species may be less tolerant of cooler temperatures. Further support is provided by the observation that two individuals showed severe signs of stress at 3°C, with one fish losing equilibrium towards the end of the respirometry investigation, and a second fish dying overnight following the respirometry experiment at 3°C. *N. angustata* will also not survive prolonged periods at temperatures much below 4°C (De Vries pers. comm.), although acclimation to 2-4°C for several weeks has been reported (Cheng et al. 2003). The changes in ventilation frequency of *B. variegatus* mirrored the changes in oxygen consumption rate with temperature, but the ventilation frequency of *N. angustata* did not follow the same pattern as oxygen consumption, with a significant decrease from 6 to 3°C and no significant increase associated with the rapid increase in oxygen consumption from 12 to 18°C. This mis-match of ventilation frequency with oxygen consumption may indicate either a break-down in the relationship between the two parameters nearing the critical thermal limit, or a shift toward modulation of gill ventilation via changes in stroke volume.

Both the oxygen consumption rate and ventilation frequency of *B. variegatus* at the 12°C acclimation temperature were similar to those of the similarly sized Antarctic nototheniids at their 0°C acclimation temperature, indicating thermal compensation of resting metabolic rate of the Antarctic species. These values also compare well with values from sedentary North Sea fishes acclimated to 4 - 7°C (Johnston et al. 1991), and from the sedentary sculpin at acclimation temperatures from 4.5 to 15°C (Johnston and Battram 1993; Steffensen et al. 1994). The resting oxygen consumption rate of *N. angustata*, even when corrected to 500 g rather than 100 g, was considerably higher than

that of the other three species, as was ventilation frequency. The size range of temperate-water fishes obtained for the current investigation was not ideal and the oxygen consumption rates of *N. angustata* were not corrected to 100 g mass, due to the fact that this was well outside the experimental range. With mass-specific oxygen consumption decreasing with an increase in body mass (Schmidt-Nielsen 1984), this factor complicates the comparison of oxygen consumption rates between this species and the other notothenioids. The oxygen consumption rate of *N. angustata* compares well with the 54.3 ± 4.1 mg O_2 kg^{-1} h^{-1} consumption rate of the more active Atlantic cod (mass 950-1850 g) at its 10°C acclimation temperature (Claireaux et al. 2000). It is possible that the oxygen consumption rates of this species may have been influenced by the shorter settling time, although preliminary investigations at 12°C indicated that oxygen consumption rates of *B. variegatus* reached stable levels 4 hours after the handling stress of introduction to the chambers, and stable oxygen consumption values have been reported 2-3 hours following handling in Greenland cod at 4.5°C (Steffensen et al. 1994). The 6-hour minimum recovery period employed for *N. angustata* should therefore have been sufficient to minimise the effects of handling stress.

Extrapolation of the oxygen consumption rates of the two temperate-water species from their 12°C acclimation temperature down to 0°C (using a Q_{10} of 2.0) gives oxygen consumption rates of 22.5 mg O_2 kg^{-1} h^{-1} (500 g mass) for *N. angustata* and 15.6 mg O_2 kg^{-1} h^{-1} (100 g mass) for *B. variegatus*, close to the theoretically-determined 20 mg O_2 kg^{-1} h^{-1} minimum metabolic rate to sustain life in free-living fish of the 10-100 g range (Brett and Groves 1979). Comparing these values with those of the Antarctic nototheniids at 0°C indicates a 31-52% elevation of resting oxygen consumption rate in the Antarctic species, which is considerably less than the 200+% increase predicted by the theory of metabolic cold adaptation (Wohlschlag 1960; Wohlschlag 1964), and is in agreement with the results of other studies carried out on confamilial species from different thermal zones (Steffensen et al. 1994; van Dijk et al. 1999). Extrapolation of metabolic rates to temperatures outside the thermal range of an organism is subject to error in that a Q_{10} value must be arbitrarily assigned. Application of a temperature coefficient (Q_{10}) of 2 to the decrease in temperature 12 to 3°C, however, gives oxygen consumption rates of 27.7 for *N. angustata* and 19.1 for *B. variegatus*, indicating that this value is very close to the observed Q_{10} for an acute change in temperature. With acute increases in temperature resulting in greater thermal sensitivity of rates than acclimatory changes, the ~40% elevation of oxygen consumption rate in the Antarctic species is

likely to be reduced by acclimation of the temperate-water species to the lower temperatures. A direct comparison between the oxygen consumption rates of the Antarctic and temperate-water species at the common experimental temperatures of 3 and 6°C is not likely to provide useful information, due to the increase in thermal sensitivity of the Antarctic fishes nearing their upper lethal limit at 6°C, and increase in thermal sensitivity of the temperate-water species nearing their lower thermal limit at 3°C. Comparisons of resting metabolic rates should ideally be made between cold-adapted species and fishes adapted to higher temperatures but acclimated to similar low temperatures (Wohlschlag 1964). In fact, undisputable demonstration of metabolic cold adaptation would require the acclimation of temperate-water fish to subzero temperatures (Houlihan and Allen 1982), which is clearly not possible. The overlap of respective thermal ranges, which has been utilised in the study of metabolic rates of northern temperate and Arctic species (Steffensen et al. 1994), has not previously been thought to exist between McMurdo Sound and New Zealand notothenioids. The results from Chapters 6 and 7, however, suggest that long-term acclimation of the Antarctic nototheniids may be possible to 4°C, the lowest temperature to which temperate-water notothenioids have been successfully acclimated (De Vries pers. comm.), providing a basis for further studies. In this study, both Antarctic and temperate-water species were acclimated to temperatures approximately 2°C above that at which they had been caught, which could potentially have had a greater effect on metabolism of the stenothermal Antarctic species. The oxygen consumption rate of *P. borchgrevinki* was, however, not significantly different from that of fish held at -1°C for three days following capture (Chapter Eight).

With resting metabolism representing a cost to the organism, the evolutionary advantage of maintaining an elevated rate at cold temperatures has been questioned (Somero et al. 1968; Montgomery and Wells 1993; Clarke and Johnston 1999) and it has been suggested that some of the specific adaptations to freezing temperatures in the Antarctic species may incur an additional metabolic cost which is incorporated into the resting metabolic rate (Wells 1987). These adaptations include the synthesis of glycopeptide antifreezes in the liver (Cheng and De Vries 1991), tubular secretion in the aglomerular kidneys (De Vries and Eastman 1981), and the maintenance of high mitochondrial volume densities in the swimming muscles (Johnston 1987; Dunn 1988; Archer and Johnston 1991; O'Brien et al. 2003). Another cost may be associated with the upward adjustment of ion pump densities to maintain cellular potential at the lower

temperatures (Hochachka 1988). *N. angustata* retains some of these characteristics, possessing a pauciglomerular kidney which produces insignificant filtrate (Eastman 1993), and the genes for glycopeptide antifreeze production (Cheng 2000; Cheng et al. 2003) which are translated at low levels (Cheng et al. 2003). *B. variegatus,* in contrast, has well-developed, fully-functional glomeruli (Eastman and De Vries 1986) and does not possess the genes for antifreeze production (Cheng et al. 2003). The retention of some of the cold-adaptive characteristics of its Antarctic ancestors may contribute to the slightly higher basal metabolic costs of *N. angustata*, compared with *B. variegatus*, at its 12°C acclimation temperature.

The full cost of cold-adaptation in the Antarctic species is, however, likely to be compensated for by reductions in other aspects of basal metabolism, such as the reduction in muscle fibre number (Johnston 2003; Johnston et al. 2003). Antarctic notothenioids have large muscle fibres (Dunn et al. 1989; Johnston 1989; Battram and Johnston 1991), resulting in a lower surface-to-volume ratio and thus decreasing basal energy requirements as fewer energy-utilising pumps are required to maintain equilibria (Hochachka 1986). With 20-40% of routine energy expenditure associated with the maintenance of ionic gradients across membranes (Jobling 1994), the reduction in muscle fibre numbers could represent a significant energy saving (Johnston 2003). With twenty percent of the mitochondrially-located oxygen consumption of standard metabolic rate due to proton leak through a non-specific cation channel in the inner mitochondrial membrane in temperate ectotherms (Brookes et al. 1998), it has also been suggested that there may be some compensation for the higher densities of Antarctic fishes in the form of a reduction in proton leakage across the inner mitochondrial membrane in the cold (Pörtner et al. 1998; Hochachka and Somero 2002), and it is even possible that antifreeze proteins may act to block ion channels, reducing leakage and thereby decreasing the energy turnover of Na^+/K^+-ATPase (Rubinsky et al. 1991). The high osmolarity of body fluids in Antarctic fishes (Dobbs and De Vries 1975; O'Grady and DeVries 1982) may also contribute towards a decrease in costs, with both *N. angustata* and *B. variegatus* having plasma osmolarities typical of temperate-water species (341-361 mOsm L^{-1}) (Eastman 1993).

The results of this study are thus in agreement with the statements that cold adaptation, with the exception of the requirement to avoid freezing, is merely a specific example of the more general temperature compensation needed by all marine organisms to maintain homeostasis (Clarke 1991), and that the slightly elevated metabolic rates are

probably the consequence of an increased energy flux, rather than a metabolic adaptation to the low temperature itself (Macdonald et al. 1987; Montgomery and Wells 1993). Within the expanding evidence for cold adaptation emerging from biochemical studies (Smith and Haschemeyer 1980; Haschemeyer 1985; Torres and Somero 1988b; Torres and Somero 1988a; Crockett and Sidell 1990; Sidell 1991; Kawall et al. 2002) it is apparent that there is considerable variation in the level of compensation of different processes. This has been demonstrated by the greater cold adaptation of fatty acid metabolic pathways (Torres and Somero 1988a; Crockett and Sidell 1990; Sidell 1991), compared with other aerobic and anaerobic pathways. Zimmerman and Hubold (1998) highlighted the difficulty of quantifying metabolic cold adaptation in species with differing activity levels, and in the brain, a tissue which is likely to be minimally affected by different activity levels (Somero 1998), Kawall et al. (2002) reported some cold adaptation of both aerobic (citrate synthase) and anaerobic (LDH) activities in Antarctic notothenioids, but not complete compensation. As emphasized by Clarke (1980, 1983, 1991, 1998), metabolic rate is a complex entity, incorporating a variety of different components all of which may differ in their response to temperature. Down regulation of some pathways and up-regulation of others makes the overall assessment of metabolic cold adaptation difficult. Although there are a myriad of problems associated with comparisons of metabolic rates at different temperatures, even between related species, the information provided by whole-animal studies is important in the larger picture. Enzymatic investigations yield valuable information, but enzymatic activity normally increases with temperature to temperatures well above the organism's lethal temperature and the temperature yielding the maximum rate of activity in vitro is not necessarily inhabitable by the organism (Somero 1991).

The biochemical investigations suggest that the maximum capacity for aerobic energy production may be enhanced in Antarctic species (Crockett and Sidell 1990) and therefore metabolic cold adaptation may be apparent as an elevation of the active metabolic rate, rather than standard or resting rates. Further research, comparing the maximum metabolic rates of Antarctic and temperate notothenioids preferably following thermal acclimation to a common temperature (4°C), will provide useful information in the continuing investigation of cold adaptation.

In conclusion, this study provides no evidence for the traditional concept of metabolic cold adaptation of resting oxygen consumption rates in Antarctic nototheniids, although there may be slightly higher basal metabolic costs associated with adaptation to

the Antarctic environment. The effect of an acute increase in temperature on resting oxygen consumption rate of both stenothermal Antarctic nototheniids and the more eurythermal temperate-water notothenioids follows a similar pattern, with a plateau of relative thermal independence surrounding the acclimation temperature and considerably greater thermal sensitivity towards the extremes of the thermal range.

The Scaling Coefficient (Mass Exponent)

The scaling coefficient, describing the relationship between resting oxygen consumption rate and mass, was found to be independent of temperature from 3-18°C in the temperate-water notothenioid *B. variegatus*. Johnston et al. (1991) also found no evidence for systematic change in the scaling coefficient with temperature in comparing temperate and Antarctic species. It has, however, been suggested that the scaling coefficient is species-specific, and that temperature effects need to be assessed species by species (Glass 1969). In support of this theory, a significant reduction in the mass exponent of southern catfish has been reported with increasing temperature (Xie and Sun 1990). Differences have also been detected between mass exponents of summer and winter-acclimatised *N. neglecta* (Johnston et al. 1991), and between the scaling coefficients of *T. bernacchii* at different times of the year (Wohlschlag 1964), although these are unlikely to be the result of temperature in the stable Antarctic thermal environment and more likely to be due to seasonal changes in metabolism, possibly due to gonad development (Johnston et al. 1991). Recently, a significant effect of temperature on the mass coefficient of roach has been described in which the mass coefficient of the fish was at its lowest at 10°C, and increased both above and below (Holker 2003). Although there were no significant differences between values in the current study, the data follow a similar pattern, with the lowest mass coefficient at 6°C and increases at temperatures both above and below. The resting metabolic rate of fish may therefore increase more rapidly with increasing mass at the temperature extremes, although more data are required to determine whether this is a general trend. The slightly higher scaling coefficient of *B. variegatus* at 3°C, may indicate a relatively greater depressing effect of the low temperature on metabolic rate of smaller fish. At this temperature it was two of the smallest fish which demonstrated the severe stress effects described above.

The magnitude of the scaling exponent of *B. variegatus* varied from 0.72 ± 0.11 to 0.88 ± 0.03 which compares well with regressions between resting oxygen consumption and body mass of other fishes in which the mass coefficient varies from 0.6 to 0.9, with the majority of values falling between 0.76 and 0.86 (Glass 1969; Beamish 1978; Duthie 1982; Morris and North 1984; Johnston et al. 1991; Johnston and Battram 1993; Holker 2003). The value determined in the current study also compares favourably with the value of 0.79 ± 0.11 reported by Clarke and Johnston (1999) after averaging data from 69 species of post-larval teleost fish. A value of 0.83 has been determined for the mass coefficient of resting oxygen consumption at 0°C in *P. borchgrevinki* (Forster et al. 1987), and it has been suggested that the mass exponents of Antarctic fishes may be higher than average values from temperate waters (Wohlschlag 1964), although comparison with the mass exponents of the temperate-water *B. variegatus* provide no evidence for this.

Within species, there are a variety of factors, such as reproductive and nutritional status and age, which are known to affect the value of mass exponents (Wohlschlag 1964; Morris and North 1984; Wells 1986). The relationship may differ from juvenile to adult stages (Laurence 1975), although Morris and North (1984) found no difference in mass exponents between post-larval specimens and larger fish. Despite the large size range, all fish in this study were of adult morphology. Some of the variation between mass exponents in the literature is thought to be due to the use of a weight range which is too narrow (Hemmingson 1960, cited in Morris and North 1984). With only five individual fish in the current study, and their mass values clustered at either end of the scale, the spread of raw data points in the current study was not ideal, although the resulting mass exponents were similar to those of other species.

Comparing the regression parameter *a* (the y intercept of the oxygen consumption versus mass graph) between species has previously revealed a positive relationship with activity level (Morris and North 1984) which is thought to be related to the higher inherent resting metabolic rate of the more active species. In the current study, there was a positive relationship between the intercept value and temperature, due to the increase in resting metabolic rate with increased temperature.

In summary, the mass exponent of *B. variegatus* is independent of temperature across the thermal range of the species, and is of similar magnitude to a variety of other temperate-water fishes.

Chapter Six

The effect of an acute increase in temperature and warm-acclimation on the prolonged swimming ability of *Pagothenia borchgrevinki*

INTRODUCTION

With the formation of the Drake Passage between the Antarctic Continent and South America approximately 25 million years ago, the Southern Ocean was cut off from large-scale seawater exchange with the warmer northern water masses. Seawater temperatures began to fall and gradually reached their current, near-freezing levels some 14 million years ago (Hochachka and Somero 2002), although there are reports of more recent warming episodes, involving temperature increases of up to several degrees Celsius (Kennett 1977). The present-day Antarctic marine ecosystem is regarded to be extremely thermally stable (Clarke 1983; Clarke and Johnston 1996) and inhabitation of this environment has led to marked stenothermality in Antarctic fishes of the perciform suborder Notothenioidei. These fishes dominate McMurdo Sound in both number and biomass (Hochachka and Somero 2002) and their thermal tolerance ranges are extremely narrow, with heat death occurring at temperatures above ~6°C (Somero and De Vries 1967; Somero 1991; Somero et al. 1998; Hofmann et al. 2000). Cold adaptation has taken place in a variety of physiological pathways of the notothenioids (see Chapter One), and the uniquely stable environment is thought to have obviated the need to retain the functional plasticity required in more variable ecosystems (Somero 1995). It has been suggested that evolution in such an environment would have impaired the abilities of these fish to cope with higher temperatures, and that even prolonged acclimation could

not broaden their thermal tolerances (Eastman 1993; Knox 1994; Somero et al. 1998; Hofmann et al. 2000). Recently, however, it has been demonstrated that at least one of these stenothermal Antarctic nototheniids (*Notothenia coriiceps*) can be gradually acclimated in the laboratory to Subantarctic temperatures (Egginton unpubl. data, cited in Egginton et al. 2002).

Temperature acutely affects the rates of most biological processes, from enzyme activities to whole-animal behaviour. Rates typically increase with increasing temperature to some maximum level and then rapidly decline, although the shapes of the response functions are often able to be modified during temperature acclimation/acclimatisation (Cossins and Bowler 1987; Johnson and Bennett 1995). This malleability of an organism's phenotype (physiology, morphology or behaviour) in response to changes in environmental conditions is referred to as phenotypic plasticity (Scheiner 1993). Acclimation responses are physiological examples of phenotypic plasticity (Huey and Berrigan 1996), and thermal acclimation is defined as the process by which an organism adjusts its physiology or performance in response to an imposed change in environmental temperature (Wakeling et al. 2000). The beneficial acclimation hypothesis predicts that acclimation to a particular temperature should give an organism a performance advantage over another organism that has not had the opportunity to acclimate to that particular environment (Zamudio et al. 1995), although it has been proposed that stenothermal habitats may favour stronger acute and reduced acclimation effects, as the physiological trade-offs of specialist phenotypes restrict performance across a wide variety of temperatures (Huey and Hertz 1984), and fish from stenothermal environments have been reported to demonstrate less plasticity in muscular performance with changes in temperature than eurythermal species (Johnston and Ball 1997). The amount of phenotypic plasticity available to allow acclimatisation to local temperature changes, and thereby to enable species to remain in their current habitats through these changes, is assuming greater importance as a result of the current interest in global warming (Wood and McDonald 1997).

Swimming ability is considered to be a major determinant of survival in most fish (Jones et al. 1974; Spouge and Larkin 1979; Taylor and McPhail 1986; Rome et al. 1992; Stobutzki and Bellwood 1994; Plaut 2001) as the majority of these animals lack defences and escape by swimming is the main method to avoid predation (Videler 1993; Reidy et al. 1995). Swimming is also the means by which food and mates can be obtained, and unfavourable environmental conditions avoided (Drucker 1996). Although heat stress of

the swimming muscles is unlikely to be a common immediate cause of death, with tissues such as the gill epithelium and respiratory neurones having greater sensitivity and being more likely to fail (Cossins and Bowler 1987), swimming capability is likely to be a significant factor in determining Darwinian fitness (Reidy et al. 2000). Fishes, in general, have evolved a number of adaptive strategies to minimise the deleterious effects of changing temperature on swimming performance, but the majority of studies to date have investigated cold rather than heat tolerance (Taylor et al. 1997). Much of the past work has also focussed on the biochemical and sub-organismal physiological processes (Kleckner and Sidell 1985; Sidell and Moerland 1989; Johnston et al. 1990; Hochachka and Somero 2002), although acclimation of whole-animal locomotor performance has been demonstrated (Griffiths and Alderdice 1972; Taylor et al. 1993; Johnson and Bennett 1995; Temple and Johnston 1998; O'Steen and Bennett 2003).

The swimming of fish has been classified into three major categories: burst, prolonged, and sustained (Brett 1964). Burst swimming refers to the highest speeds at which fish are capable of swimming, and typically can only be maintained for less than 20 seconds. Prolonged swimming can be maintained for longer periods (20 seconds - 200 minutes) but always results in fatigue, while sustained swimming applies to speeds which can be maintained for very long periods (greater than 200 minutes) without muscular fatigue. Critical swimming speed, also referred to as the maximum sustained swimming speed, is a special category of prolonged swimming (Brett 1964).

The Antarctic nototheniid *Pagothenia borchgrevinki* is an active cryopelagic fish (Andriashev 1970) which has been observed to utilise both burst and prolonged swimming in the avoidance of predators (Davis et al. 1999; Ponganis et al. 2000). The burst swimming performance of this species is independent of temperature and unaltered by warm-acclimation (Wilson et al. 2001), in support of the idea that stenothermality has resulted in a loss of ability to restructure its phenotype. The predominately aerobic prolonged swimming ability of this species is highly temperature-dependent, with a rapid decline in swimming performance above 4°C (Wilson et al. 2002), but whether performance at higher temperatures is altered by warm-acclimation has not been determined and is the question that this study was designed to answer. Taking into account the stenothermality of the species, the extremely stable thermal environment, and the lack of warm-acclimation of burst swimming performance, it was not expected that significant acclimation would be observed.

A second objective was to investigate the effect of an acute increase in temperature on the rank order performance of *P. borchgrevinki*. Significant correlations have been demonstrated in both fish and lizards between the rank order performances of a number of individuals before and after an acute temperature change (Huey and Hertz 1984; Kolok 1992), suggesting that individuals do not specialise at being good performers at either cold or warm temperatures, but that the best performers remain so regardless of the temperature at which they perform. Whether this trend also applies to the prolonged swimming ability of Antarctic nototheniids is unknown.

MATERIALS AND METHODS

SERIES 6.1. EFFECT OF ACUTE TEMPERATURE INCREASE AND WARM-ACCLIMATION ON CRITICAL SWIMMING SPEED

Pagothenia borchgrevinki (post-acclimation mass 67.7 ± 16.7 g (mean ± SD), range 47.1 – 112.0 g, length 209.2 ± 15.5 mm (mean ± SD), range 189 – 239 mm, n = 20) were caught in McMurdo Sound, Antarctica in late November 2003, as described in Chapter One. The fish were randomly divided into two groups of ten, with both groups kept in the Wet Laboratory of Scott Base for a 4-5 week acclimation period. A natural photoperiod (24-hour daylight) was maintained and the fish were not fed during either the acclimation period or the swimming trials. One group of fish (cold-acclimated) was kept in a 65 L tank in the flow-through aquarium system at a water temperature of -1.0 ± 0.3°C, while the second group (warm-acclimated) was placed in a 100 L tank isolated from the flow-through system. The seawater temperature in this tank was maintained at 4.0 ± 0.5°C, using a heat exchanger linked to an adjacent freshwater tank which contained a thermostatically controlled heater. A large proportion of the seawater in the static tank was able to be replaced daily without altering the temperature by more than 0.5°C.

At the end of the acclimation period, both groups of fish were subjected to an incremental swimming test in an 80 L Blazka-type swimming tunnel (Blazka et al. 1960), described in Chapter Four. The fish were placed in the swimming tunnel individually and the velocity of water flow set at 14.1 cm s^{-1} for a period of 30 minutes to allow the fish to become accustomed to the surroundings. This flow rate was too low to require the fish to

swim continuously, but sufficient to cause orientation into the direction of the current. At the end of the adjustment period, the flow rate was increased by 6.6 cm s^{-1} increments at ten minute intervals until the fish was exhausted. The experiment was terminated after the fish had fallen back against the rear restraining grid twice. The time and speed at which the fish were exhausted was recorded in order to calculate the critical swimming speed (U_{crit}). The -1°C-acclimated fish swam at -1, 2, 4 and 6°C, in that order, whereas the 4°C-acclimated fish swam at 4, -1, 2, 6, 8 and then 10°C, with 48-hour recovery periods between swimming trials for both groups. The -1°C-acclimated fish participated in fewer trials as they were unable to swim at temperatures above 6°C. Identification of individual fish was possible by the placement of a short length of coloured cotton through the dorsal fin. The swimming tunnel was filled with air-saturated seawater of the appropriate temperature prior to each experimental run, and the set-up was located in an insulated laboratory with an air temperature of 3-6°C. A large proportion of the seawater in the tunnel was replaced between each fish. Preliminary measurements indicated that any heat produced by the impeller had a minimal effect on the temperature of seawater within the tunnel, with an increase of less than 0.5°C over the duration of a typical run. Turbulence at the surface of the tunnel ensured adequate oxygenation of the water without affecting the linear flow adjacent to the fish. At the conclusion of the experiment all fish were weighed and measured (nose to tip of tail).

SERIES 6.2. EFFECT OF TRAINING ON CRITICAL SWIMMING SPEED

P. borchgrevinki (length 217.1 ± 13.1 mm (mean ± SD), range 189 – 238 mm, n = 10) were caught in McMurdo Sound, Antarctica in early December 2003, as described in Chapter One. The fish were held in the flow-through aquarium system (water temperature -1.0 ± 0.3°C) of the Wet Laboratory at Scott Base for three days to allow recovery from the stress of capture. The fish were unfed and a natural photoperiod (24-hour daylight) maintained. After the three-day recovery period, the fish were individually subjected to an incremental swimming test at -1°C, using the protocol described in Series 6.1. The swimming trial was repeated after three days, and again two days later, giving a total of three performances. The fish were individually identified by coloured cotton as described above. The total length of each fish (nose to tip of tail) was determined at the conclusion of the final swimming trial in order to calculate the relative swimming

performances and the mean critical swimming speeds were compared across the three trials in order to identify any effect of training.

SERIES 6.3. RANK ORDER PERFORMANCE

The rank order performance (relative performance ranking of individual fish within each group) of critical swimming speed was compared at each of the three training swims (Series 6.2), and at each experimental temperature for the cold and warm-acclimated fish (Series 6.1).

DATA ANALYSIS AND STATISTICAL METHODS

Critical Swimming Speed

Critical swimming speed (U_{crit}) was first defined by Brett (1964) to identify the maximum velocity fish could maintain for a prescribed period of time. It is measured by interpolation for those fish that do not fatigue at exactly the beginning or end a time period:

$$U_{crit} = U_i + (T_i/T_{ii} \times U_{ii})$$

where:

U_i	=	highest velocity maintained for the prescribed period (cm s^{-1})
U_{ii}	=	velocity increment (cm s^{-1})
T_i	=	time (min) fish swam at the "fatigue" velocity
T_{ii}	=	prescribed time period of swimming (min)

Thermal Performance Breadth

Thermal performance breadth was defined as the total range of temperatures over which performance was at least 80% of its peak value (Huey and Stevenson 1979).

Condition Factor

Fulton's condition factor for each fish was calculated using the following formula (Ricker 1975):

$$\text{Condition Factor} = \frac{100 \times (\text{body mass, g})}{(\text{body length, cm})^3}$$

Statistical Analysis

Within acclimation groups and the training study, data were analysed by repeated-measures analysis of variance (ANOVA), with Dunnett's post-hoc test employed to compare performance with control values. Differences between the acclimated groups were assessed by t-test with Welch's correction for unequal variances where indicated necessary by Bartletts test. Linear regression was used to identify relationships between swimming speed and body length, and Spearman rank correlation coefficients were used to test for correlation of rank order performance across temperatures and between training trials. Analyses were carried out using GraphPad Prism version 4.00 software. Statistical significance was taken at the level of $p<0.05$ and data are presented as mean ± SEM, unless otherwise stated.

RESULTS

SERIES 6.1. EFFECT OF ACUTE TEMPERATURE INCREASE AND WARM-ACCLIMATION ON CRITICAL SWIMMING SPEED

The critical swimming speed of -1°C-acclimated *P. borchgrevinki* was highly temperature-dependent, with a thermal performance breadth of only 3°C (Fig. 6.1). The maximum critical swimming speed (2.03 ± 0.08 bl s^{-1}) was measured at the -1°C acclimation temperature, with performance maintained at 2°C but a significant reduction in critical swimming speed above 2°C (Table 6.1).

The thermal performance breadth for critical swimming speed of 4°C-acclimated fish was 9°C, three-times that of the cold-acclimated fish (Fig. 6.2). The maximum

critical swimming speed (2.07 ± 0.04 bl s^{-1}) of the warm-acclimated fish was recorded at 2°C, with no significant change in performance from -1 to 8°C, but a decrease at 10°C (Table 6.1). The critical swimming speed of warm-acclimated fish at their 4°C acclimation temperature (1.98 ± 0.03 bl s^{-1}) was not significantly different from that of -1°C-acclimated fish at their respective acclimation temperature. There was also no significant difference in critical swimming speed of the two acclimation groups at -1 or 2°C, but above 2°C the speed of -1°C-acclimated fish was significantly slower (Fig. 6.3). There were no significant differences in condition factor or mass between the cold and warm-acclimated fish, but the mean body length of the 4°C-acclimated fish was greater than that of -1°C-acclimated fish (Table 6.1).

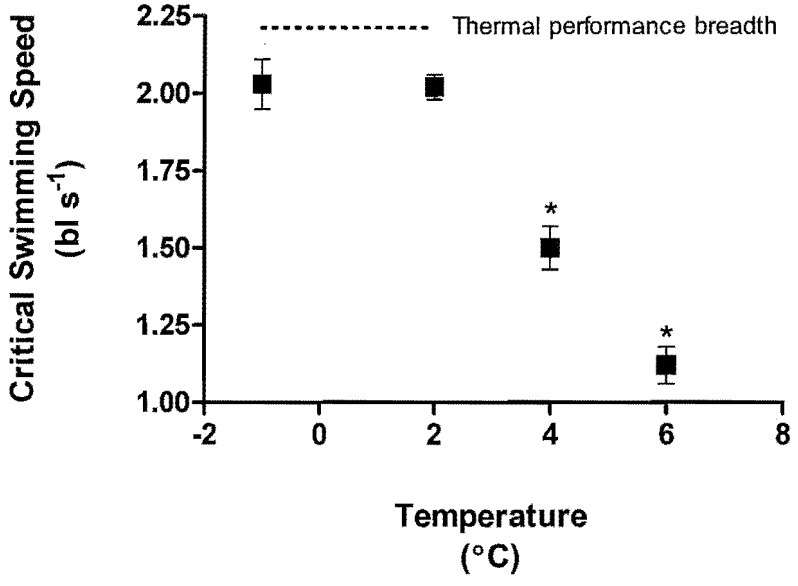


Fig. 6.1. Effect of an acute increase in temperature on critical swimming speed of -1°C-acclimated *P. borchgrevinki.* * Significantly different from performance at acclimation temperature.

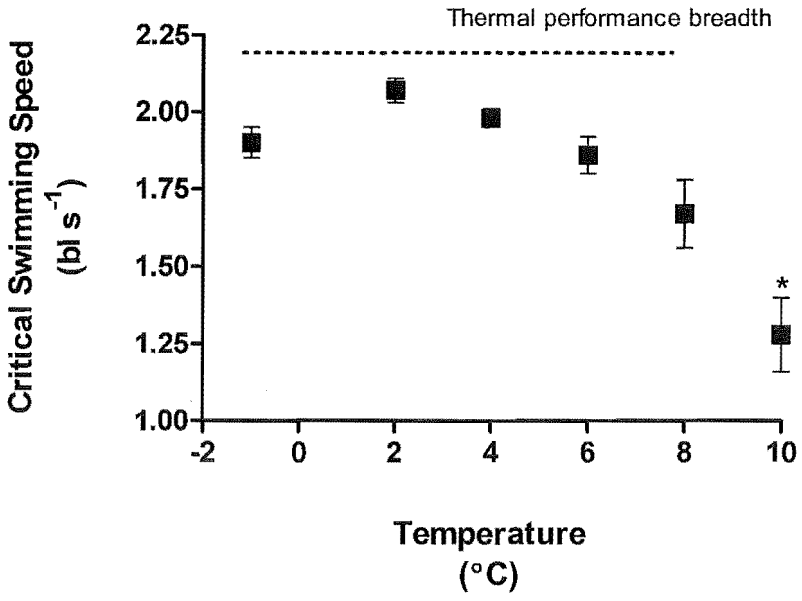


Fig. 6.2. Effect of an acute increase in temperature on critical swimming speed of 4°C-acclimated *P. borchgrevinki.* * Significantly different from performance at acclimation temperature.

Table 6.1. The effect of an acute increase in temperature on the critical swimming speed of *P. borchgrevinki* acclimated to two different temperatures.

Acclimation Temperature (°C)	-1	4
Length (mm)	200.9 ± 3.8	217.5 ± 4.5#
Mass (g)	60.7 ± 3.8	74.7 ± 5.8
Condition Factor	0.74 ± 0.02	0.71 ± 0.02
Critical Swimming Speed (bl s^{-1})		
At -1°C	**2.03 ± 0.08**	1.90 ± 0.05
2°C	2.02 ± 0.04	2.07 ± 0.04
4°C	1.50 ± 0.07*	**1.98 ± 0.03#**
6°C	1.12 ± 0.06*	1.86 ± 0.06#
8°C	-	1.67 ± 0.11
10°C	-	1.28 ± 0.12*

Figures in bold represent fish swimming at their respective acclimation temperatures. * Significantly different from performance at the acclimation temperature. # Significantly different from -1°C-acclimated fish at the same temperature. N = 10 for both groups.

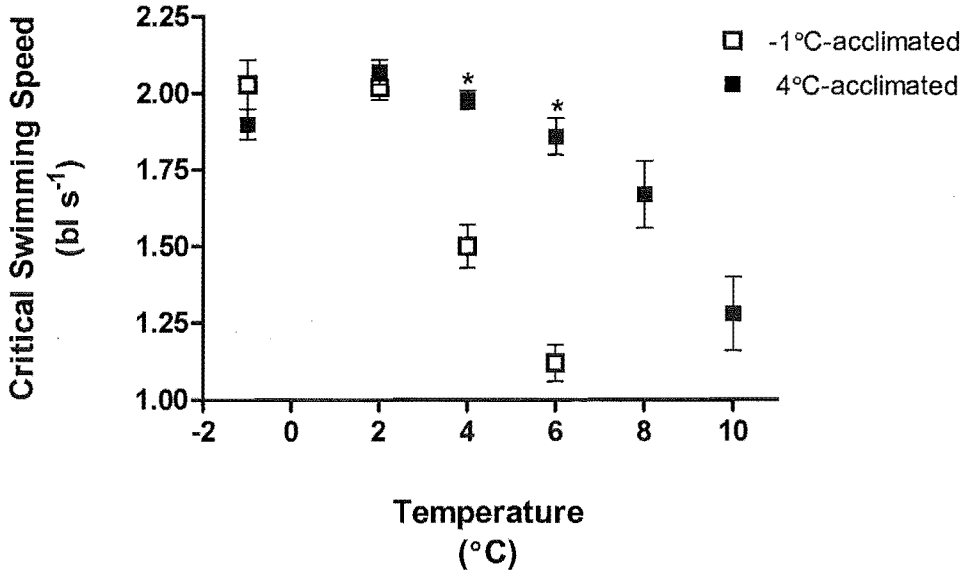


Fig. 6.3. Effect of an acute increase in temperature on critical swimming speed of *P. borchgrevinki* acclimated to two different temperatures. * Significantly different from -1°C-acclimated group

There was a slight decrease in critical swimming speed (bl s^{-1}) of both -1°C and 4°C-acclimated fish with increasing total body length, although neither trend was statistically significant (Fig. 6.4 and 6.5).

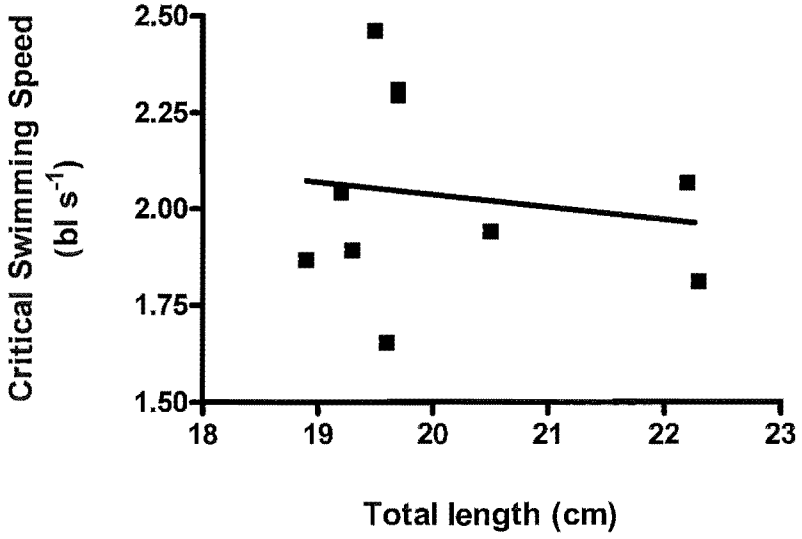


Fig. 6.4. Critical swimming speed of -1°C-acclimated *P. borchgrevinki* as a function of total body length.

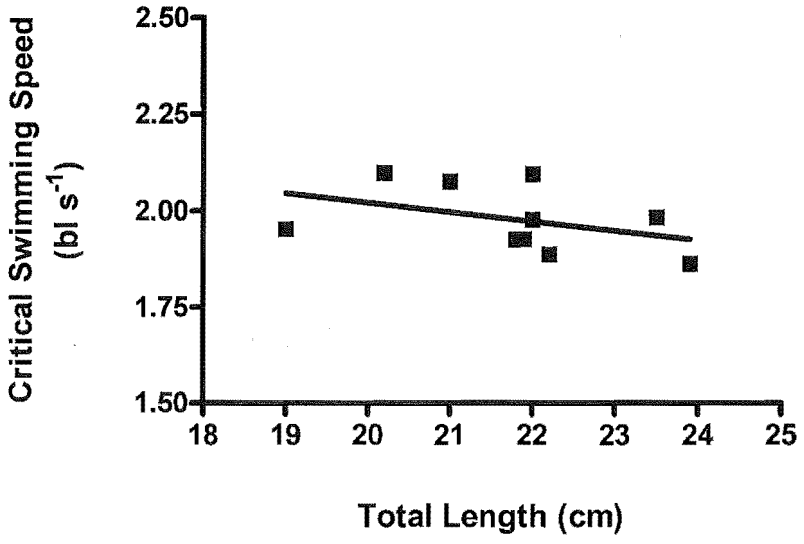


Fig. 6.5. Critical swimming speed of 4°C-acclimated *P. borchgrevinki* as a function of total body length.

SERIES 6.2. EFFECT OF TRAINING ON CRITICAL SWIMMING SPEED

There was no significant effect of training on the critical swimming speed of *P. borchgrevinki* (Fig. 6.6). The mean critical swimming speeds on days 1, 4, and 7 were 1.92 ± 0.07, 1.91 ± 0.06, and 1.90 ± 0.07 bl s^{-1}, respectively.

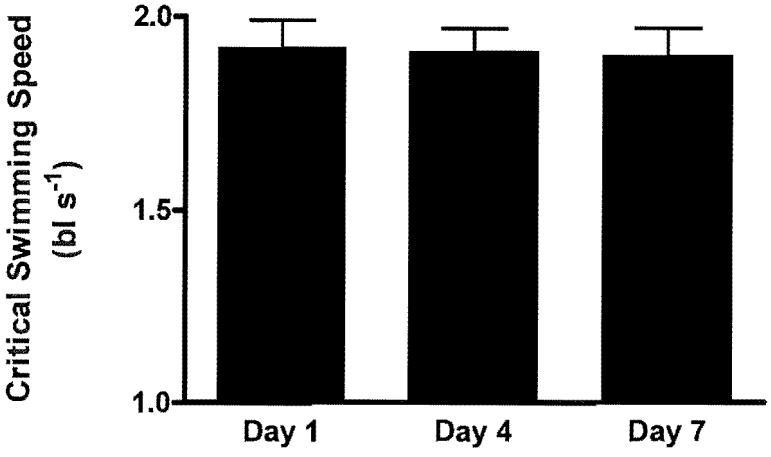


Fig. 6.6. Effect of training on critical swimming speed of *P. borchgrevinki.* Fish swam three times over the duration of a week.

As in the acclimated fish, there was a decrease in critical swimming speed with increasing body length, although the slope of the regression line was not significantly different from zero (Fig. 6.7).

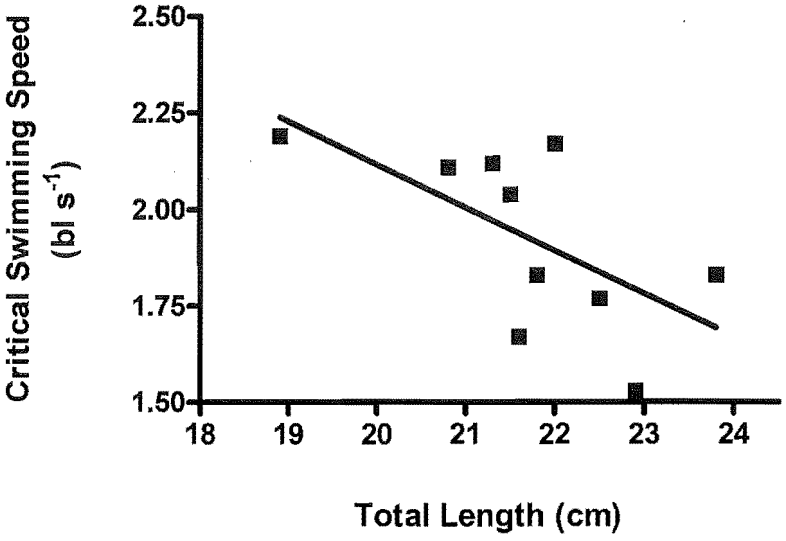


Fig. 6.7. Critical swimming speed of *P. borchgrevinki* as a function of total body length.

SERIES 6.3. RANK ORDER PERFORMANCE

There was significant correlation of rank order performance across the three swimming trials of the training investigation (Series 6.2) with Spearman rank correlation coefficients of 0.8415 ($p = 0.0037$) for the second trial, and 0.9058 ($p = 0.0008$) for the third, when compared with the rank order performance of the first trial (Fig 6.8).

In -1°C-acclimated fish, the correlation of rank order performance was maintained from -1 to 2°C (Spearman rank correlation coefficient = 0.6970, $p = 0.0306$), but the ranking at 4 and 6°C was not significantly correlated with that at either -1 or 2°C (Fig. 6.9). In 4°C-acclimated fish, there was no correlation between rank order performance at the acclimation temperature and that at any other experimental temperature. There was, however, significant correlation of rank order performance between the critical swimming speeds at -1 and 2°C (Spearman rank correlation coefficient of 0.6606, $p = 0.0438$) (Fig. 6.10).

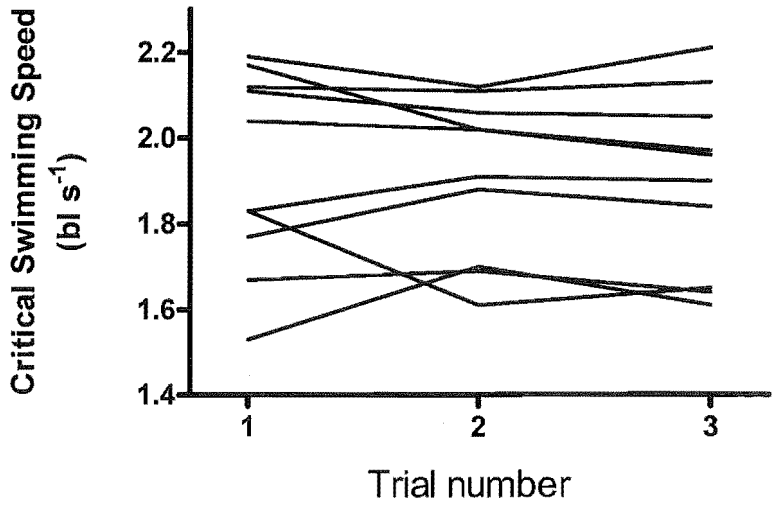


Fig. 6.8. Individual critical swimming speeds of individual *P. borchgrevinki* over three separate swimming trials at -1°C.

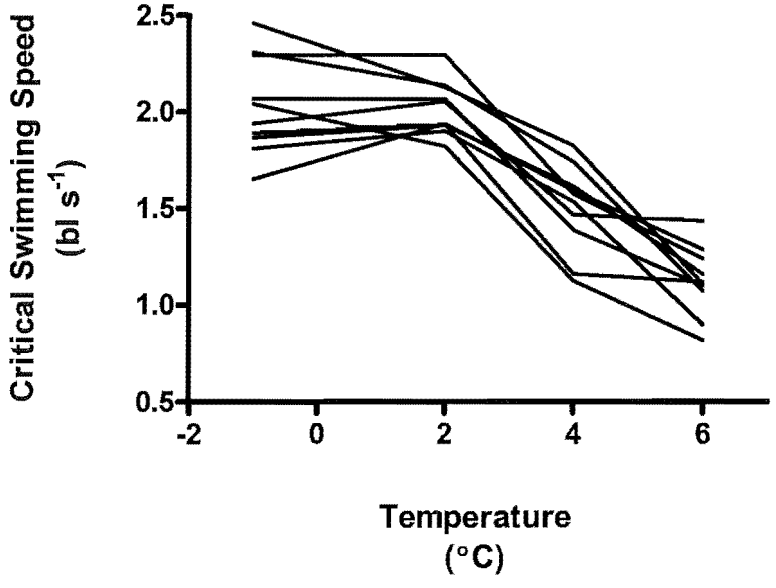


Fig. 6.9. Individual critical swimming speeds of -1°C-acclimated *P. borchgrevinki* at different temperatures.

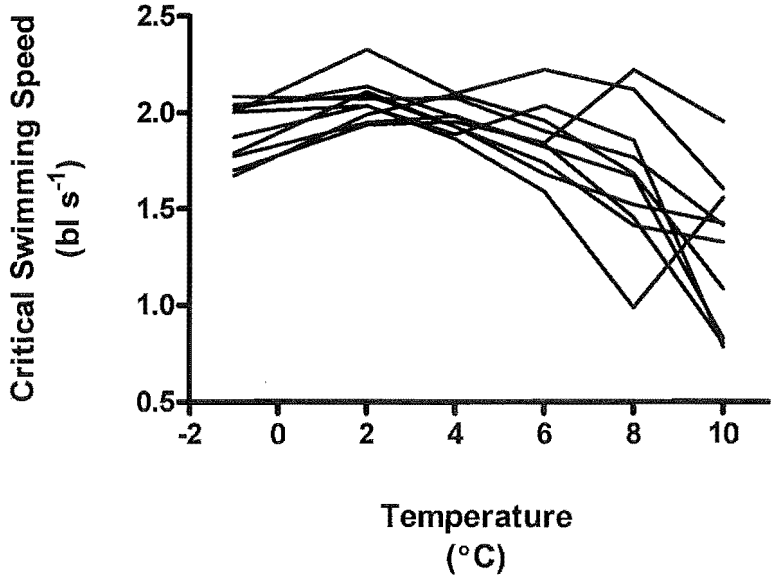


Fig. 6.10. Individual critical swimming speeds of 4°C-acclimated *P. borchgrevinki* at different temperatures.

DISCUSSION

The prolonged swimming performance of -1°C-acclimated *Pagothenia borchgrevinki* was found to be highly sensitive to temperature, with a significant reduction in performance at temperatures above 2°C. The thermal performance breadth for critical swimming speed was only 3°C, which is identical to the performance breadth of 0°C-acclimated fish (Chapter Four), and compares well with the 5°C performance breadth reported by Wilson et al. (2002) for this species. A similar pattern of thermal sensitivity has been observed in the prolonged swimming performance of other fishes, with a reduction at low temperatures, an increase to a maximum speed at an optimum temperature, and a decrease at temperatures approaching the upper thermal limit (Fry and Hart 1948; Brett 1967; Brett 1971; Brett and Glass 1973; Beamish 1978; Myrick and Cech 2000; Ojanguren and Brana 2000). In Antarctic fish, only the upper half of the curve is able to be described due to the proximity of their environmental temperature to the freezing point of seawater. With thermal perturbations to most biochemical or physiological processes considerably greater above than below an organism's normal body temperature (Somero et al. 1996), and the total performance ranges for Antarctic organisms only able to be assessed at temperatures above normal environmental levels,

comparisons between stenothermal Antarctic and eurythermal temperate or tropical species are inherently biased. Thermal performance breadths for critical swimming speed are, however, at least 10°C for several eurythermal fish species (Fry and Hart 1948; Johnston 1993). Comparison of the narrow thermal breadth for prolonged swimming of this stenothermal Antarctic species with those of temperate eurythermal organisms, lends support to the "Jack-of-all-temperatures should be a master of none" theory (Huey and Hertz 1984). The aforementioned theory predicts that ectotherms from highly stable thermal environments should possess specialist phenotypes that maximise performance, while ectotherms from more variable environments should possess generalist phenotypes that maximise performance breadth (Van Berkum 1988).

The decline in aerobic swimming performance above and below the acclimation temperature could stem from changes in the viscosity or dissolved oxygen availability of the medium (Fuiman and Batty 1997; Johnson et al. 1998). At low velocities, such as those of prolonged swimming in *P. borchgrevinki*, resistance to movement is dominated by frictional resistance which is due predominately to viscosity. An increase in temperature could thus improve swimming performance as the viscosity of water is reduced (Vogel 1981; Sidell and Moerland 1989; Macdonald and Wells 1991) and the diffusion rates of gases and metabolites to and from working muscles are increased (Taylor et al. 1997), but it is more likely that the changes are due primarily to the internal environment (i.e. oxygen delivery to or power produced by the muscle) (Taylor et al. 1997) as changes in viscosity and dissolved oxygen content are relatively minor over the range of temperatures tolerated by fish (Randall and Brauner 1991), particularly Antarctic fish.

Prolonged swimming performance is predominately aerobic (Webb 1971) and therefore involves an increase in the activity of many organ systems, including the muscular system which provides the propulsive mechanism and the respiratory and cardiovascular systems which provide the oxygen and metabolites required (Hughes et al. 1988). It is thought that oxygen limitation may be the predominant factor determining performance limits, as excess capacity of any component of the oxygen delivery system appears to be avoided in an evolutionary context with oxygen delivery systems set to a minimum level of functional capacity just sufficient to meet maximum oxygen demands between the average highs and lows of environmental temperature, a concept known as symmorphosis (Taylor and Weibel 1981; Taylor et al. 1997). The limitation of oxygen capacity is likely to occur at a high level of organisational complexity, involving the

respiratory and cardiovascular systems, which then contributes to cellular and molecular disturbances at the lower levels (Pörtner 2002b; Pörtner 2002a). In *P. borchgrevinki*, a decrease in the scope for change in cardiac output (Chapter Seven) has been associated with the decrease in aerobic scope (Chapter Four) at temperatures near the upper lethal limit.

The strong thermal dependence of prolonged swimming performance demonstrated by *P. borchgrevinki* is in contrast to the thermal insensitivity of burst swimming performance of this species (Wilson et al. 2001), with a thermal performance breadth of at least 11°C reported for burst swimming. The physiological mechanisms vary with the category of swimming, and therefore it is not surprising that the thermal responses should differ. Prolonged and maximum efforts are supported by different muscle fibre types (Johnston and Ball 1997), with prolonged swimming involving predominantly slow twitch fibres which are concentrated in the red muscle (Johnston 1981; Altringham and Johnston 1988). These slow fibres depend on aerobic metabolism and therefore rely on complex respiratory and circulatory support systems to supply the required oxygen and substrates (Bone 1966; Johnston et al. 1977). They also have a rich blood supply and considerable stores of lipid (Bone 1978). Maximal or burst performance, in contrast, involves the recruitment of the entire white muscle mass (Johnston et al. 1993). The fast twitch fibres of the white muscle depend on fewer physiological systems than the slow fibres, and as the fast fibres utilise anaerobic metabolism and endogenous fuel stores of high energy phosphates they can, recovery excluded, function largely independently of the circulation and complex thermally-sensitive enzyme chains (Johnston and Altringham 1991; Johnston et al. 1991b). Anaerobic use of energy is therefore generally less thermally dependent than aerobic use of energy (Brett 1964; Weiser et al. 1985; Weiser et al. 1986; Bennett 1990). It has been suggested that the trade-offs in thermal performance breadth resulting from thermal specialisation may occur in just a few key physiological processes (Huey and Hertz 1984; Van Berkum 1988; Bennett et al. 1992; Gilchrist 1996; Wilson et al. 2001) and may as a result only be apparent in whole-animal performance measures which incorporate a large number of physiological and biochemical steps, such as prolonged swimming ability.

The critical swimming speed of -1°C-acclimated *P. borchgrevinki* at their acclimation temperature (2.03 ± 0.08 bl s^{-1}) compares well with the more recent critical swimming speeds of this species reported in the literature. Previously measured U_{crit}

values range from 1.0 to 2.7 bl s^{-1}, at acclimation temperatures from 0 to -1.5°C (Wohlschlag 1964; Montgomery and Macdonald 1984; Forster et al. 1987; Davison et al. 1988; Archer and Johnston 1989; Johnston et al. 1991a; Franklin and Johnston 1997; Wilson et al. 2002). The 1.0 bl s^{-1} measured by Wohlschlag in the earliest investigation, however, is a speed at which fish in the current investigation were not required to swim and could maintain station in the swimming tunnel by gripping the floor with their pelvic fins. The critical swimming speed of *P. borchgrevinki* at 0°C reported in Chapter Four of this thesis (1.86 ± 0.04 bl s^{-1}) was slightly lower than the speed determined in this experiment, a difference which is likely to be due either to the 1°C difference in temperature or to the fact that those fish had been kept in captivity for several months. A critical swimming speed of 2 bl s^{-1} has been identified as a realistic figure for many average sized temperate-water fishes of different species (Hammer 1995), and the prolonged swimming performance of Antarctic fishes can therefore been interpreted as adequately cold-compensated, compared with temperate fish of similar lifestyle and body size (van Dijk et al. 1998; Peck 2002).

Forced exercise of fish in swim tunnels and the determination of critical swimming speed is a commonly employed method by which to investigate the thermal plasticity of locomotory activity (Fry and Hart 1948; Johnston 1993; Rome and Swank 2001), but its ecological relevance has been questioned and caution has been advised in applying conclusions based on the analysis of steady swimming to fish swimming in the natural environment in an unsteady manner (Hoar and Randall 1978). In the natural habitat, prolonged swimming tends to be a relatively uneven activity, comprising periods of cruising punctuated by the occasional rapid burst with frequent changes of direction (Getty and Pulliam 1991). Plaut (2000), however, reviewed the use of critical swimming speed and after identifying similar trends in a variety of other traits including routine daily activity level and standard metabolic rate concluded that its measurement was ecologically relevant and that it was an appropriate tool to assess the effects of different factors on the eco-physiological performance of fish. Strong currents are common under the sea ice adjacent to Ross Island, and it is likely that *P. borchgrevinki* would encounter conditions in its natural habitat which are similar to those produced within the swim tunnel (Davison et al. 1988). This species lacks a swimbladder (Dewitt 1971) and, although buoyancy has been increased by a variety of adaptations (De Vries and Eastman 1981; Eastman and De Vries 1982; Eastman and DeVries 1985), the fish are negatively buoyant (Eastman 1993) and must swim constantly to maintain station in the water

column (Montgomery and Macdonald 1984). They have, however, been observed in the wild resting within the platelet ice beneath the annual sea ice (Andriashev 1970; Gutt 2002).

There are a number of factors which need to be taken into account in the use of swimming tunnels. The first concerns flow characteristics as, even with grids producing theoretically laminar flow, the flow within swim tunnels is often micro-turbulent, a flow type rarely encountered in the natural environment (Gordon et al. 1989; Plaut and Gordon 1994). The Blazka-type swim tunnel used in this study contained fine-meshed grids and concave baffles in an effort to produce uniform flow, and as the main objective of this study was to compare performances at two different acclimation temperatures any unusual flow characteristics should have affected both groups equally and should not have influenced the conclusions. Another feature of swim-tunnels is the presence of a 'wall effect', a boundary layer near the tunnel walls where velocity is slower than that prescribed (Webb 1993). At the very lowest speeds, *P. borchgrevinki* would cling to the base of the tunnel using its pelvic fins, although at speeds at which constant swimming was required the fish remained in the centre of the tunnel, minimising this source of error. The third factor to be considered is the blocking effect due to the presence of a fish in the tunnel. This effect is only significant when the maximum cross-sectional area of the fish is greater than 10% of the tunnel cross-sectional area (Bell and Terhune 1970) and, as the maximum cross-sectional area of fish in this study was less than 5% of the tunnel area, this effect was not corrected for.

It has also been reported that critical swimming speed may be overestimated if the time interval between velocity increments is too short (Hammer 1995). A relationship has been demonstrated between the interval of velocity increments and magnitude of critical swimming speed in largemouth bass (Farlinger and Beamish 1977), although Beamish (1980) reported no difference in the critical swimming speeds of Arctic char swum using velocity increments of 5, 10 and 75 minutes. The magnitude of critical swimming speeds obtained in this study compare well with previous reports from this species, although most of those investigations utilised velocity increments of a similar duration to this study. Again, as the primary objective of this study was comparative it is the relative rather than absolute values which are of greatest importance.

Critical swimming speed is dependent upon the size, sex, maturity and nutritional status of fish, and on season, light, ambient gas concentrations, and training (Hammer 1995). The results of this study (Series 6.2) indicate no effect of a short period of training

on the critical swimming speed of *P. borchgrevinki*. The lack of a significant training effect is supported by the fact that the critical swimming speeds of -1°C-acclimated fish, which swam a total of four times, were very similar to the speeds reported by Wilson et al. (2002) from fish which swam at only one experimental temperature. There are reports in the literature both affirming and refuting an effect of training on critical swimming performance. The majority of studies in which positive effects were identified (MacLeod 1967; Beamish 1970; Otto and O'Hara Rice 1974; Nahas et al. 1982; Besner and Smith 1983; Farrell et al. 1990; Holk and Lykkeboe 1998) involved long periods (40+ days) of intensive training (up to 23 hours per day), while other, less intensive, training regimes have failed to detect an effect (Bainbridge 1962; Farrell et al. 1991; Thorarensen et al. 1993; Gallaugher et al. 2001).

Size is one of the most important constraints on swimming performance. Working on the assumption that fish volume and the proportionate amount of muscle increases as the square of length, Thompson (1917) proposed that sustained or prolonged swimming speeds should be proportional to the length of fish raised to the power of 0.5. This theory has been supported by more recent studies, with relative swimming performance (expressed as percentage of body length) declining as body size increases (Brett 1965; Jones et al. 1974; Beamish 1978; Hammer 1995). In this study, both acclimation groups and the training group exhibited a slight decline in critical swimming speed with increasing total length, although none of the relationships were significant. The lack of significance was probably due to a narrow range of lengths within each subject group, and a high level of inter-individual variation. A relationship has previously been identified between the length and critical swimming speed of *P. borchgrevinki* at 0°C, with fish of 14 cm length able to sustain speeds up to 2.5 bl s^{-1}, while fish of 23 cm length were only able to sustain 1.8 bl s^{-1} (Forster et al. 1987). The fish in the current study were toward the larger limit of those utilised in the previous study, but fitted the regression equation determined by that investigation very well. The mean total length of the 4°C-acclimated group was significantly greater than that of the cold-acclimated group in this study and therefore relative swimming performance of the former group may have been slightly underestimated. Mass also has an effect, with critical swimming speeds greater in absolute terms (cm s^{-1}) for larger fish but lower in relative terms (bl s^{-1}) due to decreasing mass-specific power output as size increases (Hammer 1995), although there was no significant difference in mass of any of the groups of fish in this study.

The relative health of individual fish can have a major impact on performance (Butler and Milleman 1971; Beamish 1978) and the condition factor values calculated after the swimming trial for both cold and warm-acclimated fish were low compared with values from freshly caught healthy fish, and even lower than values from diseased fish (Davison 1998). With critical swimming speeds determined for the fasted fish in this study (Series 6.1) not significantly different from those of the non-fasted fish (Series 6.2) it appears as though starvation had little effect on prolonged swimming ability. It is possible that fasting at 4°C may have had a greater effect on condition than at -1°C alone but without condition factor values for each group of fish prior to acclimation this cannot be determined.

Due to time constraints, resulting from a limited period of time in the field in Antarctica and the presence of a single swim tunnel, the fish were transferred to the tunnel 30 minutes prior to the commencement of each swimming trial. It is therefore possible that our results may have been complicated by the presence of a handling effect, although the fish were all rapidly transferred to the swim tunnel from an adjacent tank in order to minimise the duration of both handling and air exposure. Recent studies have shown no difference in critical swimming speed between fish left to recover overnight and those left to recover for 1-2 hours after the handling stress of placement in the swim tunnel (Kolok 1991; Peake et al. 1997) and the only other studies to determine critical swimming speed in this species have employed a similar settling period.

In contrast to expectation, the prolonged swimming ability of *P. borchgrevinki* was significantly greater at elevated temperatures following 4-5 weeks of warm-acclimation. The thermal performance breadth for the critical swimming speed of 4°C-acclimated *P. borchgrevinki* was 9°C, three times that of -1°C-acclimated fish, and the critical swimming speed of the warm-acclimated fish was significantly higher than that of cold-acclimated fish at all experimental temperatures above 2°C. With the critical swimming speed of warm-acclimated fish at 4°C not significantly different from the speed of -1°C-acclimated fish at their respective acclimation temperature, this is a good example of perfect thermal compensation (Precht et al. 1973). The results of the rank order performance investigation indicated, however, that the fish which performed best at the elevated temperatures were not those which had performed best at near environmental temperatures. Athletic ability at environmental temperatures is thus not a good predictor of swimming ability at higher temperatures in *P. borchgrevinki*, even in warm-acclimated fish. The relative swimming performances of individual fish at -1°C,

however, were highly repeatable, as indicated by the maintenance of rank order performance over three swimming trials in the training study (Series 6.2). At temperatures above 2°C *P. borchgrevinki* does not therefore provide support for the theory (Huey and Hertz 1984; Kolok 1992) that the best performers remain so regardless of the temperature at which they perform. The inter-individual variability in the effect of increased temperature on relative locomotory performance of this species indicates that there are different traits which come into play at higher temperatures, and these will provide material for natural selection to act upon if the rate of climate change is slow enough for genetic adaptation to occur.

The acclimation of prolonged swimming performance, in contrast to the lack of acclimation of burst swimming performance (Wilson et al. 2001), is in agreement with the work of O'Steen and Bennett (2003) who reported strong acclimation of critical swimming speed with no acclimation of maximum speed in two species of cyprinid fishes. As discussed above, aerobic swimming is dependent on a greater number of physiological pathways, and it may be that only some of these have retained sufficient plasticity for acclimatory change. With burst swimming performance independent of temperature from -1 to 10°C, it is possible that the lack of thermal acclimation may be the result of limited opportunities to improve performance by restructuring the phenotype (Wilson et al. 2001).

There is an obvious advantage to animals having optimal locomotory performance at the temperature to which they have been acclimated (Cossins and Bowler 1987), and the thermal optimum of aerobic swimming of a variety of fishes has been reported to vary with thermal acclimation status (Fry and Hart 1948; Brett 1967; Rome et al. 1984a; Sisson and Sidell 1987; Guderley and St Pierre 1996). The temperature of optimum performance occurs where acclimation and exposure temperatures coincide in several species (Fry and Hart 1948; Larimore and Duever 1968; Griffiths and Alderdice 1972; Beamish 1978; Schurmann and Steffensen 1997; Pörtner 2002b), although laboratory acclimation may produce a more pronounced response to changes in temperature than natural acclimatisation in the wild (Kleckner and Sidell 1985). With maximum critical swimming speed of the 4°C-acclimated fish at 2°C, rather than at the 4°C acclimation temperature, there is the possibility that the process of acclimation may be incomplete after 4-5 weeks.

The characteristics which are important in determining the functional plasticity of prolonged swimming have not yet been determined because, despite decades of extensive

research into the factors underlying aerobic muscle performance, the nature of the limits to aerobic swimming are still unclear. There is a wealth of information concerning the mechanisms of cold adaptation but, as the reasons for a decline in swimming performance above the thermal optimum are likely to be different from those below optimum, the mechanisms underlying warm-acclimation may also be qualitatively different from those forming the basis of cold-adaptation (Taylor et al. 1997). From the results of Chapter Seven, it is apparent that scope for change in cardiac output increases with rising temperature in 4°C-acclimated *P. borchgrevinki*, in contrast to a reduction in the cardiac scope of -1°C-acclimated fish as temperatures increase. The thermal sensitivity of aerobic scope of warm-acclimated *P. borchgrevinki* has not yet been investigated, but the aerobic scope of cold-acclimated fish exhibits a marked decrease at higher temperatures, mirroring swimming ability (Chapter Four). An increase in maximum metabolic rate at elevated temperatures has been described following warm-acclimation in fish (Beamish 1978; Schurmann and Steffensen 1997) and it is likely that long-term adaptation will increase the energy efficiency of most processes (Pörtner 2002b).

An improvement in muscle performance is another factor which may contribute to the increase in swimming ability of warm-acclimated fish, probably through a myriad of adjustments in structure and function at different levels of organisation, rather than through changes in just one or two adaptive parameters (Egginton and Ross 1992). Swimming is accompanied by an increase in the rate of energy conversion from the resting rate, and swimming capacity is therefore regulated by the metabolic capacity of fish to convert chemical energy into propulsive thrust through muscular contraction. Adenosine triphosphate (ATP), generated by the stepwise degradation of carbohydrate and lipid, is an essential prerequisite for muscle contraction, and oxidative capacity and mitochondrial function thus seem to be one likely target for thermal compensation (Guderley and St Pierre 1996; Guderley 2004). There is considerable evidence that thermal acclimation of fish results in compensatory modifications in mitochondrial properties designed to minimise the thermal variation of oxidative capacity (Guderley and St Pierre 2002), although cristae density appears to be unchanged (Tyler and Sidell 1984; Egginton and Sidell 1989) suggesting that changes in membrane lipid composition or in the properties of mitochondrial enzymes must underlie the increases in capacity (Guderley and Johnston 1996).

In response to the constraints of functioning at low and stable temperatures, Antarctic fish are reported to have increased mitochondrial volume densities (Dunn 1988; Johnston et al. 1988; Egginton and Sidell 1989; Londraville and Sidell 1990), although there appears to be no compensation of maximum rates of substrate oxidation in mitochondria from cold-adapted organisms when comparing mitochondria from oxidative muscles of confamilial species from different thermal habitats (Johnston et al. 1994; Johnston et al. 1998). Changes in membrane phospholipids of organisms living in distinct thermal environments are, however, particularly pronounced in the mitochondria (see Guderley 2004). Marked compensation of oxidative capabilities can occur during thermal acclimation (Guderley and Johnston 1996), and modifications in membrane phospholipids are thought to be the underlying cause (Guderley 2004). The majority of mitochondrial studies to date have investigated the effects of cold-acclimation rather than the effect of increased temperature, although reductions in volume density of mitochondria in muscle fibres have been reported in both crucian carp and striped bass following warm-acclimation (Egginton and Sidell 1989; Kilarski et al. 1996). The Arrhenius break temperature for mitochondrial function of the Antarctic nototheniid *Trematomus bernacchii* does not undergo thermal acclimation following two weeks of 4°C exposure (Weinstein and Somero 1998), and although albalone have been demonstrated to alter mitochondrial Arrhenius break temperatures in response to shifts in acclimation temperature of only a few degrees Celsius, the most stenothermal species demonstrate no change (Dahlhoff and Somero 1993). Acclimation was not observed, even in the more eurythermal species, at temperatures outside the normal physiological temperature range, even though the organisms survived these temperatures. An increase in cytochrome *c* oxidase activity has been demonstrated in the pectoral (red) muscle of *P. borchgrevinki* acclimated to 4°C for 5-6 weeks (Seebacher et al. in press), but the mechanisms behind the increase have yet to be determined.

The mechanical properties of muscle fibres are also thermally sensitive, and acclimatory changes can occur in the muscle contractile mechanism (Sidell 1980; Goldspink 1995). These changes have been demonstrated in both force production and maximum contraction speed of isolated muscle fibres (Johnston et al. 1985), and the increase in maximum velocity of contraction of slow, oxidative red muscle at higher acclimation temperatures results in an increase in the aerobic speed of swimming, at least in the short term (Johnston and Ball 1997; Taylor et al. 1997). Myofibrillar density is relatively independent of acclimation and changes are therefore likely to reflect

differences in the number of myosin cross-bridges per unit fibre cross-sectional area (Johnston and Maitland 1980; Kilarski et al. 1996). In many fishes, the relative proportion of red muscle in the myotome has also been shown to vary with acclimation temperature (Johnston and Lucking 1978; Sidell 1980; Sisson and Sidell 1987), with the amount of red muscle decreasing as acclimation temperature increases, presumably due to the higher power output of red muscle at higher temperatures (Johnston et al. 1985; Johnston and Ball 1997). Being a labriform swimmer, the red muscle of *P. borchgrevinki* is associated with the pectoral fins, rather than the myotome, and acclimatory changes in contraction velocity have yet to be investigated.

Acclimatory changes have been demonstrated in the activity and thermostability of myofibrillar ATP-ase in freshwater teleosts and carp (Johnston et al. 1975; Heap et al. 1985; Heap et al. 1986; Itoi et al. 2003), although muscle ATP-ase activity does not alter following warm-acclimation of *P. borchgrevinki* (Seebacher et al. in press). Changes in myofibrillar ATPase activity are generally reversible and reach a steady state after 4-5 weeks, but are inhibited in starved fish in which protein synthesis has been reduced to a very low level (Heap et al. 1985; Heap et al. 1986). The availability of fuels has a pronounced impact on the metabolic and contractile capacities of muscle in fish (Moon and Johnston 1980; Beardall and Johnston 1983; Sullivan and Somero 1983; Black and Love 1986; Houlihan et al. 1988; Lambert and Dutil 1997), although responses to starvation are reported to be more pronounced in white than in red fibres (Beardall and Johnston 1983; Beardall and Johnston 1985; Lowery and Somero 1990). The acclimatory capacity of fasted *P. borchgrevinki* investigated in this study is thus likely to represent the minimum potential for change.

In temperate-water eurythermal species, warm-acclimated fish are able to maintain higher swimming speeds than cold-acclimated animals at their respective acclimation temperatures (Fry and Hart 1948; Rome et al. 1984b; Sisson and Sidell 1987; Schurmann and Steffensen 1997). The same is true for Atlantic cod from the North Sea, with higher swimming speeds reported for 15°C-acclimated than for 5°C-acclimated fish (Schurmann and Steffensen 1997), probably due to the increased force generation per unit muscle cross-sectional area at warmer temperatures (Johnston 1989; Sidell and Moerland 1989; Johnson and Johnston 1991; Rome 1995; Johnson et al. 1996; Johnston and Ball 1997; Guderley 1998). The current study provided no evidence for this trend, with the critical swimming speeds of cold and warm-acclimated fish not significantly different at their respective acclimation temperatures. It is possible that the warm-

acclimation of swimming performance of *P. borchgrevinki* may be enhanced further by a longer acclimation period incorporating feeding, although further investigation is required.

The warm-acclimation of prolonged swimming performance of *P. borchgrevinki* provides no indication of whether the upper and lower thermal tolerance limits have been similarly adjusted. Lethal limits have long been known to be strongly affected by acclimation temperature (Loeb and Wasteneys 1912; Summer and Doudoroff 1938) and, in general, as acclimation temperature increases both upper and lower tolerance limits of a species increase (Beitinger and Bennett 2000). Estimates of the amount of time required by various fishes to adaptively change their lethal limits range from 1-20 days in temperate species, although this period may be extended at lower temperatures. As discussed in Chapter Nine, there is the possibility that the enhanced swimming performance of *P. borchgrevinki* represents a reallocation of metabolic activity away from investment activities, such as growth and reproduction (Donaldson 1990; Jobling et al. 1995; Hoffmann and Parsons 1997; Van der Kraak and Pankhurst 1997), towards activities that improve the immediate prospects for individual survival, such as locomotion (Schreck 1981; Wendelaar Bonga 1997).

In conclusion, and contrary to expectation, the thermal sensitivity of prolonged swimming ability of the supposedly stenothermal Antarctic notothenиid *P. borchgrevinki* is highly responsive to warm-acclimation. The increase in critical swimming speed of warm-acclimated fish at elevated temperatures supports the beneficial acclimation hypothesis which states that acclimation to a particular temperature gives an organism a performance advantage over another organism which has not had the opportunity to acclimate to that particular environment (Zamudio et al. 1995). The mechanisms by which prolonged swimming performance has been enhanced at higher temperatures remain to be identified, and further research is required at a sub-organismal level to identify which particular processes are responsible for the change. There is also scope for research into whether the observed warm-acclimation of aerobic swimming ability is associated with an increase in the upper and/or lower thermal limits of these fish.

Chapter Seven

The effect of an acute increase in temperature and warm-acclimation on the cardiac performance of *Pagothenia borchgrevinki*

INTRODUCTION

Inadequate local provision of ATP for cellular respiration is frequently implicated in the physiological limitation of aerobic swimming (Johnston 1982), although the role of the cardiovascular system may be equally as important (Farrell 1997). The cardiovascular system, in addition to its central role in oxygen transport, is essential for maintaining an adequate fuel supply and removing metabolic waste products from the working muscles (Hughes et al. 1988; Keen and Farrell 1994). For active fishes, especially when swimming, the oxygen consumption rate is essentially equivalent to the rate of oxygen delivery to the tissues (Gallaugher et al. 1995; Farrell 1997), and a build-up of waste products or lack of metabolites adversely affects the force production of muscle fibres (Taylor et al. 1996). Determination of maximum sustained swimming ability following coronary ligation in salmon led to the conclusion that maximum cardiac performance is necessary for maximum aerobic swimming performance (Daxboeck 1982; Farrell and Steffensen 1987). Cardiac performance is therefore likely to be one of the factors limiting performance at maximum levels (Kolok and Farrell 1994; Farrell 1997).

Fish, in general, increase cardiac output by up to three-fold during sustained exercise, and the relative contribution of stroke volume is usually greater than that of heart rate (Farrell 1991). Tuna form one exception to this generalisation (Brill and Bushnell 1991), and it has been suggested that Antarctic fish, with the high cholinergic

tonus dampening resting heart rate, may be another (Axelsson et al. 1992). A recent study on the Antarctic notothenid *Pagothenia borchgrevinki* has, however, reported similar proportional increases in both heart rate and stroke volume during a 5 minute swimming trial (Axelsson et al. 1994).

Relatively little is known about the influence of thermal acclimation on the cardiovascular physiology of teleosts (Barron et al. 1987), although a tight linkage has been identified between critical swimming speed, maximum cardiac output and maximum cardiac power output during temperature acclimation of rainbow trout (Keen and Farrell 1994). Thermal acclimation appears to be capable of effecting changes in heart rate, stroke volume and cardiac output in fish (Farrell 1984). The important issue with regard to aerobic swimming is the maintenance of cardiac scope which represents the operational flexibility of the cardiovascular system. The thermal sensitivity of cardiac scope is clearly dependent upon the relative thermal responses of resting and maximum cardiac performance. Marked warm-acclimation of prolonged swimming ability has been demonstrated in *P. borchgrevinki* (Chapter Six), but how this relates to changes in cardiac performance has not yet been investigated.

The effect of an acute increase in temperature on the heart rate of *P. borchgrevinki* has been studied, and thermal independence of heart rate has been observed (Franklin et al. 2001), even as high as 10°C (Forster et al. 1998). The results of Chapter Three of this thesis, however, bring into question this thermal insensitivity of heart rate in fish acclimated to close to their environmental temperature. In the benthic notothenid *Trematomus bernacchii*, thermal insensitivity has been reported up to 3°C, although a sharp rise was observed above this temperature (Axelsson et al. 1992). The cardiac output of these fish was maintained up to 5°C, through a decrease in stroke volume associated with the increase in heart rate (Axelsson et al. 1992).

The main objective of this investigation was to quantify and compare the cardiac responses of cold and warm-acclimated *P. borchgrevinki* to an acute increase in temperature. It was intended to determine the means by which cardiac output was modulated at different temperatures, by comparing the relative changes in heart rate and stroke volume, and to relate the acclimatory and acute thermal responses of cardiac performance to the acute and acclimatory responses of prolonged swimming performance examined in Chapter Six.

MATERIALS AND METHODS

Pagothenia borchgrevinki (mass 90.0 ± 13.2 g (mean ± SD), range 65.0 – 109.4 g, n = 16) were caught in McMurdo Sound, Antarctica in late November 2003, as described in Chapter One. The fish were randomly divided into two groups of eight. Both groups were kept in the Wet Laboratory of Scott Base for a 4-5 week acclimation period. One group of fish (the cold-acclimated group) was kept in a 65 L tank in the flow-through aquarium system at a water temperature of -1.0 ± 0.3°C, while the second group (the warm-acclimated group) was placed in a 100 L tank isolated from the flow-through system. The seawater temperature in this tank was maintained at 4.0 ± 0.5°C, using a heat exchanger linked to an adjacent freshwater tank which contained a thermostatically-controlled aquarium heater. A large proportion of the seawater in the static tank was able to be replaced daily without altering the temperature by more than 0.5°C. During the acclimation period, a 24-hour daylight regime was maintained and the fish were not fed.

After 4-5 weeks, fish were randomly dip-netted from the tanks and anaesthetised in a 0.1 g L^{-1} solution of MS222 (ethyl m-aminobenzoate methanesulphonate) dissolved in -1°C seawater. On average, it took 10 - 15 minutes to achieve mild anaesthesia. The fish was then placed in a surgical sling, ventral side up, and the gills continuously irrigated with a 0.05 g L^{-1} MS222 solution (aerated by an air-bubbler apparatus). A Doppler flow probe was installed around the ventral aorta allowing cardiac output and heart rate to be measured from the flow trace, and stroke volume to be calculated. The surgery involved making a small incision ventral and anterior to the heart, and exposing the ventral aorta posterior to the point where division into the afferent branchial arteries occurs. Care was taken to avoid the hypobranchial artery that runs alongside the ventral aorta at this point. A cuff-type single crystal Doppler flow probe was placed around the vessel, without puncturing the pericardium, and the incision stitched using silk suture thread. The lead from the probe was secured to the skin using silk sutures. Following surgery the fish were replaced in smaller opaque plastic tanks at their respective acclimation temperatures and left for 24 hours to recover from the effects of anaesthesia and surgery. The following day, the flow probe was connected to a 545C-4 Directional Pulsed Doppler Flow Meter (University of Iowa) feeding information into an ADInstruments Powerlab Data Acquisition System. The flow signals were recorded by a portable laptop computer running Chart for Windows Version 4 recording software. The

water in the tank was heated over a period of about five minutes using a submersible aquarium heater and mixing pump, and upon reaching the appropriate temperature resting flow data was recorded from the fish. The fish were then chased with a net for 5 minutes and active flow data recorded. The water temperature was then returned to the acclimation level by addition of cold water, and the fish given 4 hours to recover before exposure to the next temperature. All fish were tested at -1, 2, 4, 6, and 8°C, in random order.

At the end of the experiment, a careful *in situ* calibration of the Doppler flow probe was performed on each fish to obtain absolute values for blood flow in the ventral aorta. The fish were killed by over-anaesthesia and placed ventral side up in an operating sling. After exposing the heart the bulbus arteriosis was cannulated and an infusion pump (Gilson minipulse 3) was used to perfuse fish blood through the ventral aorta at a range of flow rates. The linear relationship between flow rate in mL min^{-1} and the Doppler signal in volts was used to transform the Doppler signals to absolute blood flow.

DATA ANALYSIS AND STATISTICAL METHODS

Stroke Volume

Stroke volume (mL kg^{-1}) was calculated from cardiac output (mL min^{-1} kg^{-1}) divided by heart rate (beats min^{-1}).

Scope

Factorial scopes for change in cardiac output, heart rate and stroke volume with exercise were calculated at each temperature from:

$$\text{Scope} = \text{Active rate} / \text{Resting rate}.$$

Q_{10}

Q_{10} values were calculated using the Van't Hoff equation (Hoar 1975):

$$Q_{10} = (R_2 / R_1)^{10/(T_2-T_1)}$$

where R_1 and R_2 are the rates at temperatures T_1 and T_2, respectively.

Statistical Analysis

Within acclimation groups, data were analysed by repeated-measures one-way analysis of variance (ANOVA). Where a significant difference was indicated, Tukey-Kramer post-hoc tests were used to compare the temperature levels. Differences between the acclimated groups were determined by t-test with Welch's correction for unequal variances. Statistical significance was taken at the level of $p<0.05$. All analyses were carried out using GraphPad Prism version 4.00 software and data are presented as mean ± SEM, unless otherwise stated.

RESULTS

Cardiac Output

The resting cardiac output of -1°C-acclimated fish at their acclimation temperature was 22.2 ± 2.9 mL min^{-1} kg^{-1}. Resting cardiac output increased as the temperature was increased to reach a maximum of 41.4 ± 4.9 mL min^{-1} kg^{-1} at 6°C, and then decreased slightly from 6 to 8°C, although none of the values were significantly different from cardiac output at the acclimation temperature. Exercise resulted in an increase in cardiac output at all temperatures, but the difference was not significant at 6°C. The active cardiac output was independent of temperature from -1 to 8°C, with the slight decline at 8°C being non-significant (Table 7.1, Fig. 7.1).

The resting cardiac output of warm-acclimated fish at 4°C (24.7 ± 1.8 mL min^{-1} kg^{-1}) was not significantly different from that of -1°C-acclimated fish at their respective acclimation temperature. Resting cardiac output of the 4°C-acclimated fish was independent of temperature from -1 to 8°C, and the only significant difference in resting cardiac output between the cold and warm-acclimated fish was a lower value at 6°C in the 4°C-acclimated group. Cardiac output of the warm-acclimated fish increased significantly with exercise at all temperatures, with a value at the 4°C acclimation temperature (52.7 ± 5.3 mL min^{-1} kg^{-1}) which was not significantly different from that of the cold-acclimated fish at their -1°C acclimation temperature. The active cardiac output was independent of temperature from -1 to 8°C, although there was a slight decrease at

-1°C to a minimum value of 43.3 ± 4.3 mL min^{-1} kg^{-1}. There was no significant difference in active cardiac output between the cold and warm-acclimated fish at any temperature (Table 7.1, Fig. 7.2).

Table 7.1. Resting and active cardiac outputs of *P. borchgrevinki* acclimated to two different temperatures.

	-1°C-acclimated fish		4°C-acclimated fish	
Temperature (°C)	Resting Cardiac Output (mL min^{-1} kg^{-1})	Active Cardiac Output (mL min^{-1} kg^{-1})	Resting Cardiac Output (mL min^{-1} kg^{-1})	Active Cardiac Output (mL min^{-1} kg^{-1})
-1	**22.2 ± 2.9**	**59.5 ± 10.1**	28.9 ± 4.4	43.4 ± 4.3
2	33.9 ± 5.7	69.2 ± 8.4	29.1 ± 3.5	50.9 ± 4.6
4	33.0 ± 5.2	60.1 ± 7.0	**24.7 ± 1.8**	**52.7 ± 5.3**
6	41.4 ± 4.9	59.4 ± 7.5	26.0 ± 2.7*	53.3 ± 6.0
8	28.6 ± 3.0	48.3 ± 5.4	21.7 ± 1.5	49.8 ± 4.8

Figures in bold represent fish at their respective acclimation temperatures. N = 8 for each group.
* Significantly lower than -1°C-acclimated fish.

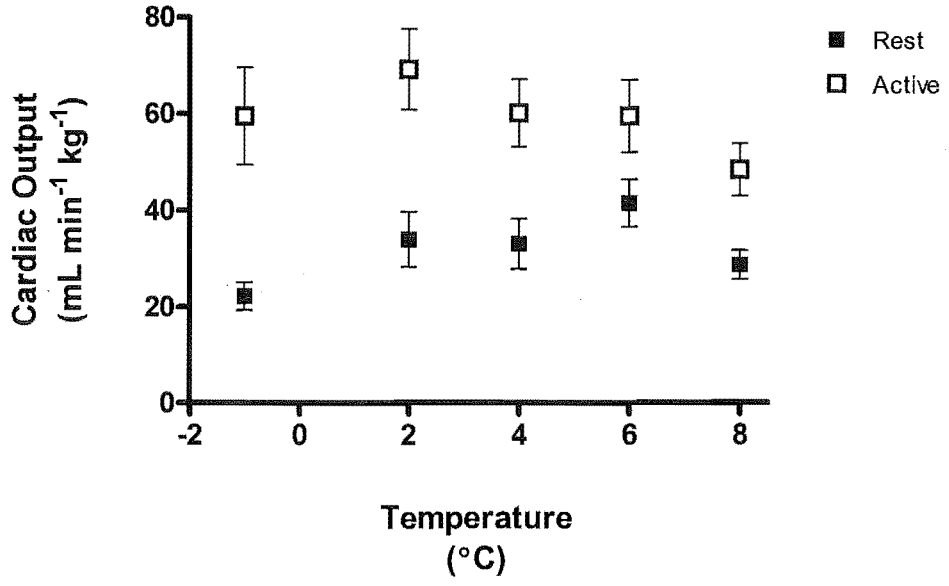


Fig. 7.1. Effect of an acute increase in temperature on resting and active cardiac outputs of -1°C-acclimated fish

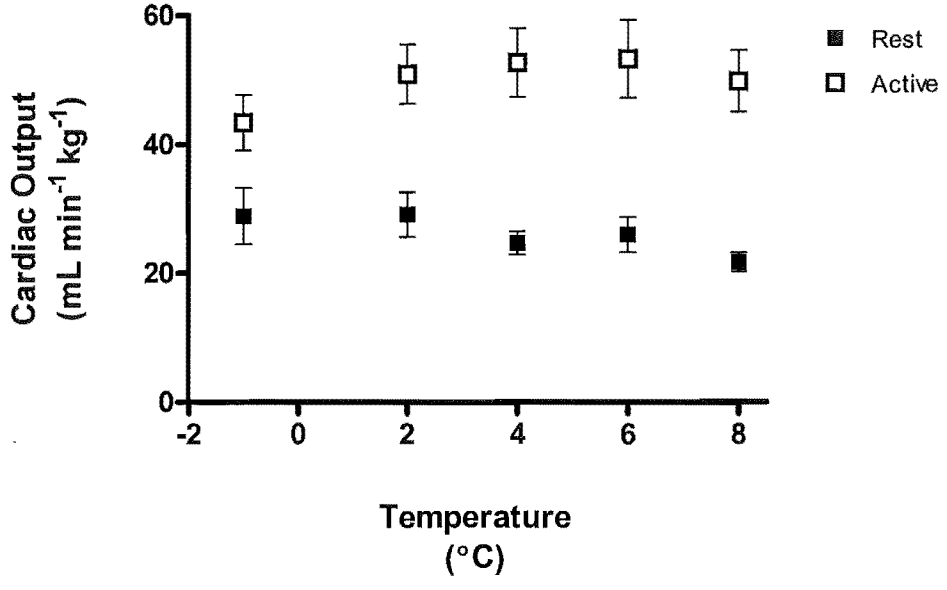


Fig. 7.2. Effect of an acute change in temperature on resting and active cardiac outputs of 4°C-acclimated fish

The maximum scope for change in cardiac output of -1°C-acclimated fish was 2.60 ± 0.26, measured at their acclimation temperature. The cardiac scope of these fish progressively decreased as the temperature was increased to reach a minimum value of 1.54 ± 0.12 at 8°C. In contrast, the cardiac scope of 4°C-acclimated fish attained a maximum value at 8°C (2.32 ± 0.21), and decreased with a decline in temperature to reach a minimum value of 1.43 ± 0.08 at -1°C (Fig. 7.3). There was no significant difference between the scopes for change in cardiac output of the cold and warm-acclimated fish at their respective acclimation temperatures.

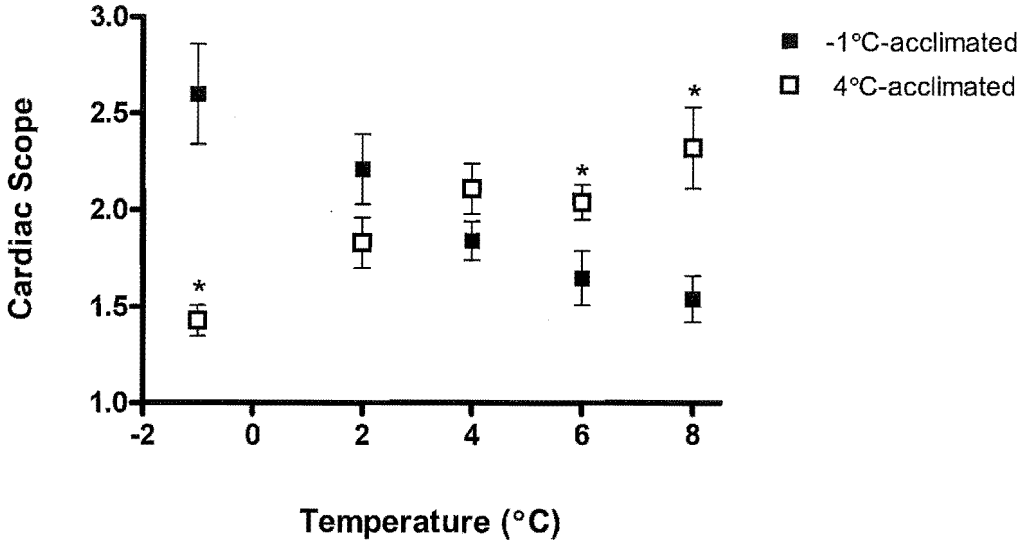


Fig. 7.3. Effect of an acute change in temperature on the scope for change in cardiac output of *P. borchgrevinki* acclimated to two different temperatures. * Significantly different from -1°C -acclimated fish.

Heart Rate

The resting heart rate of -1°C-acclimated fish at -1°C was 18.4 ± 0.9 beats min^{-1}. The resting heart rate progressively increased as the temperature was increased, with significant elevation of heart rate between each pair of consecutive experimental temperatures, with the exception of 4 to 6°C. The maximum resting heart rate of 40.1 ± 1.8 beats min^{-1} was attained at 8°C, and the temperature coefficient (Q_{10}) for the increase

in rate from -1 to 8°C was 2.38. Heart rate increased significantly from resting levels with exercise at -1 and 2°C, but there was no difference between the resting and active rates at temperatures from 4 to 8°C. The heart rate following exercise (active heart rate) was 27.6 ± 0.7 beats min^{-1} at the -1°C acclimation temperature and increased with temperature, although with a considerably lower temperature coefficient (Q_{10} = 1.60) than the resting rate. The maximum active heart rate of 42.2 ± 1.5 beats min^{-1} was attained at 8°C, although values from 2 to 6°C were not significantly different (Table 7.2, Fig. 7.4).

The resting heart rate of 4°C-acclimated fish at their acclimation temperature was 20.4 ± 2.6 beats min^{-1}, which was not significantly different from that of -1°C-acclimated fish at their respective acclimation temperature. In contrast to the cold-acclimated fish, however, resting heart rate of the warm-acclimated fish was independent of temperature from -1 to 8°C (Q_{10} = 0.89). There was a significant increase in heart rate with exercise at all temperatures, with a temperature coefficient (Q_{10}) for active heart rate from -1 to 8°C (1.64) which was similar to that of the -1°C-acclimated fish. The minimum active heart rate of 29.1 ± 0.7 beats min^{-1} was recorded at -1°C, with a maximum of 47.8 ± 1.4 beats min^{-1} at 6°C (Table 7.2, Fig. 7.5).

Table 7.2. Resting and active heart rates of *P. borchgrevinki* acclimated to two different temperatures.

	-1°C-acclimated fish		4°C-acclimated fish	
Temperature (°C)	Resting Heart Rate (beats min^{-1})	Active Heart Rate (beats min^{-1})	Resting Heart Rate (beats min^{-1})	Active Heart Rate (beats min^{-1})
-1	**18.4 ± 0.9**	**27.6 ± 0.7**	20.1 ± 1.5	29.1 ± 0.7
2	24.6 ± 1.9	34.9 ± 0.6	23.6 ± 3.1	37.1 ± 1.0
4	33.6 ± 1.4	36.2 ± 1.0	**20.4 ± 2.6***	**41.5 ± 1.3***
6	35.1 ± 2.2	39.0 ± 2.3	26.8 ± 3.5	47.8 ± 1.4*
8	40.1 ± 1.8	42.2 ± 1.5	18.1 ± 1.7*	45.3 ± 2.2

Figures in bold represent fish at their respective acclimation temperatures. N = 8 for each group.
* Significantly different from -1°C-acclimated fish.

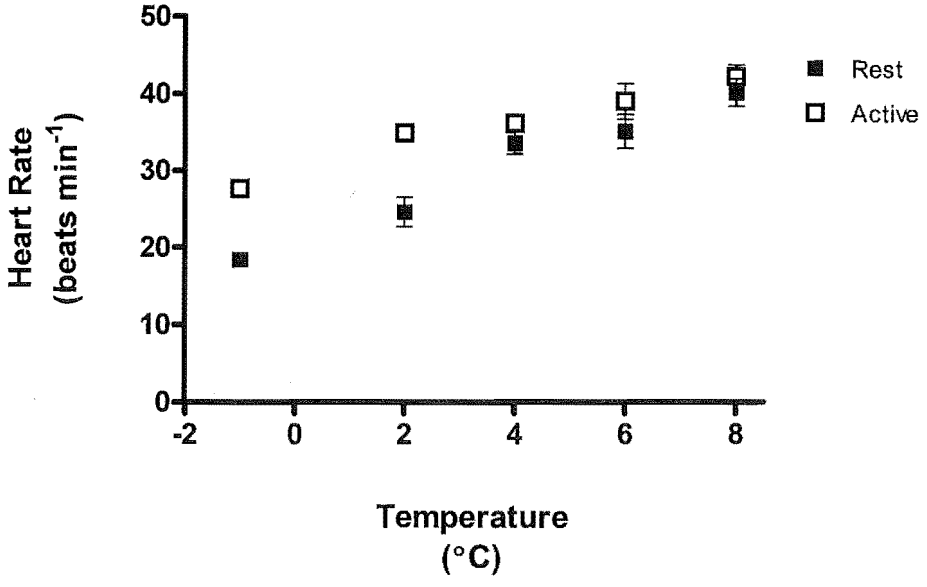


Fig. 7.4. Effect of an acute increase in temperature on resting and active heart rates of -1°C-acclimated fish. Note: where error bars are not visible they are obscured by the symbols.

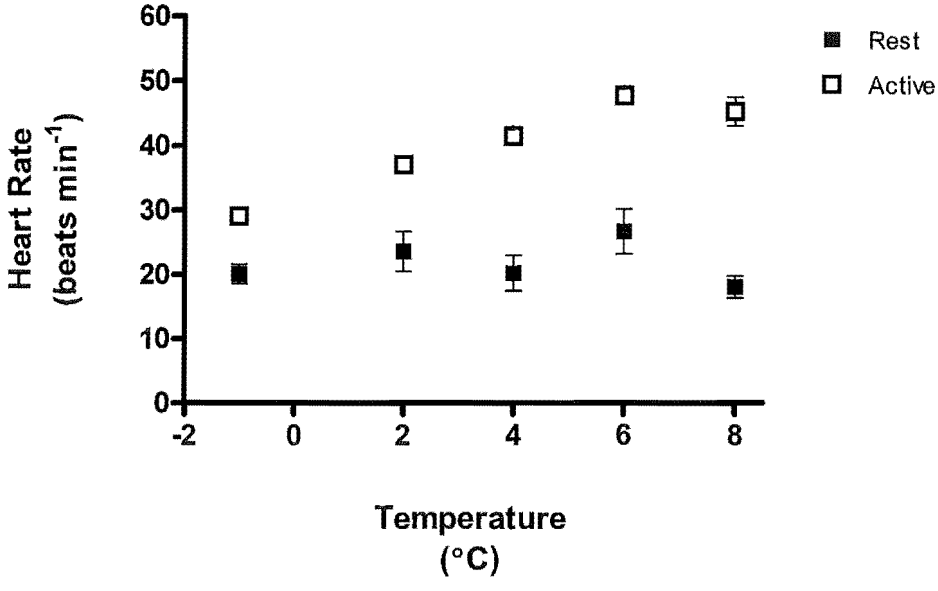


Fig. 7.5. Effect of an acute change in temperature on resting and active heart rates of 4°C-acclimated fish. Where error bars are not visible they are obscured by the symbols.

The scope for change in heart rate of -1°C-acclimated fish was greatest at their acclimation temperature, with a value of 1.53 ± 0.08. This scope for change decreased as the temperature increased, to reach a minimum value of 1.06 ± 0.07 at 8°C. In contrast, the scope for change in heart rate of the 4°C-acclimated fish was at a minimum (1.49 ± 0.09) at -1°C, and increased with increasing temperature to attain a maximum value of 2.59 ± 0.18 at 8°C. The scope for change in heart rate was significantly higher in the warm-acclimated fish at their 4°C acclimation temperature, than in the cold-acclimated fish at their respective acclimation temperature (Fig. 7.6).

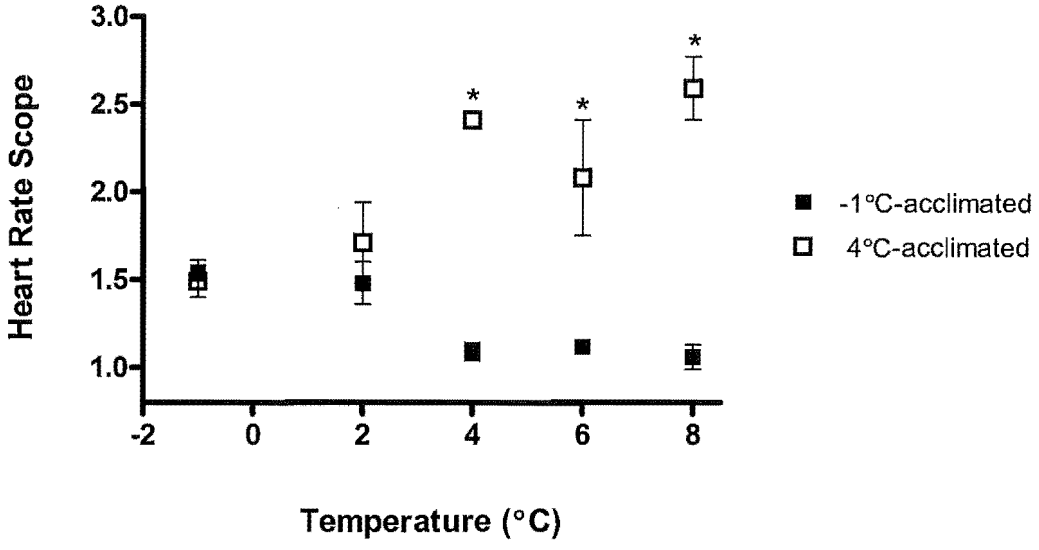


Fig. 7.6. Effect of an acute change in temperature on the scope for change in heart rate of *P. borchgrevinki* acclimated to two different temperatures. * Significantly different from -1°C-acclimated fish.

Stroke Volume

The resting stroke volume of -1°C-acclimated fish at their -1°C acclimation temperature was 1.22 ± 0.15 mL kg^{-1}. The resting stroke volume was independent of temperature from -1 to 8°C, although there was a slight, non-significant decrease to 0.87 ± 0.14 mL kg^{-1} at 8°C. While the stroke volume increased with exercise at all temperatures, the difference was only significant at -1 and 4°C. The active stroke volume at -1°C was 2.14 ± 0.34 mL kg^{-1}, and there was a progressive decrease with increasing temperature,

reaching a minimum of 1.25 ± 0.18 mL kg-1 at 8°C, although the difference between these values was not significant (Table 7.3, Fig. 7.7).

The resting stroke volume of 4°C-acclimated fish at their acclimation temperature was 1.45 ± 0.25 mL kg^{-1}. The resting stroke volume was independent of temperature from -1 to 8°C, although there were slight decreases to 1.15 ± 0.20 and 1.25 ± 0.12 mL kg^{-1} at temperatures of 6 and 8°C respectively. There was no significant change in stroke volume with exercise at any of the experimental temperatures. The active stroke volume at the 4°C acclimation temperature was 1.26 ± 0.11 mL kg^{-1} and active stroke volume decreased as temperature increased, with the maximum value of 1.49 ± 0.13 mL kg^{-1} attained at -1°C, and minimum value of 1.11 ± 0.10 mL kg^{-1} at 8°C, although this trend was not significant (Table 7.3, Fig. 7.8).

There were no significant differences in resting stroke volume between the two acclimation groups at any of the experimental temperatures, and the only difference in active stroke volume between the two groups was a lower value for the warm-acclimated fish at 2°C (Table 7.3).

Table 7.3. Resting and active stroke volume of *P. borchgrevinki* acclimated to two different temperatures.

	-1°C acclimated fish		4°C acclimated fish	
Temperature (°C)	Resting Stroke Volume (mL kg^{-1} $beat^{-1}$)	Active Stroke Volume (mL kg^{-1} $beat^{-1}$)	Resting Stroke Volume (mL kg^{-1} $beat^{-1}$)	Active Stroke Volume (mL kg^{-1} $beat^{-1}$)
-1	**1.22 ± 0.15**	**2.14 ± 0.34**	1.47 ± 0.21	1.49 ± 0.13
2	1.40 ± 0.23	2.05 ± 0.25	1.43 ± 0.24	1.37 ± 0.11*
4	1.02 ± 0.17	1.66 ± 0.19	**1.45 ± 0.25**	**1.26 ± 0.11**
6	1.17 ± 0.13	1.54 ± 0.23	1.15 ± 0.20	1.28 ± 0.13
8	0.87 ± 0.14	1.25 ± 0.18	1.25 ± 0.12	1.11 ± 0.10

Figures in bold represent fish at their respective acclimation temperatures. N = 8 for each group.
* Significantly different from -1°C-acclimated fish.

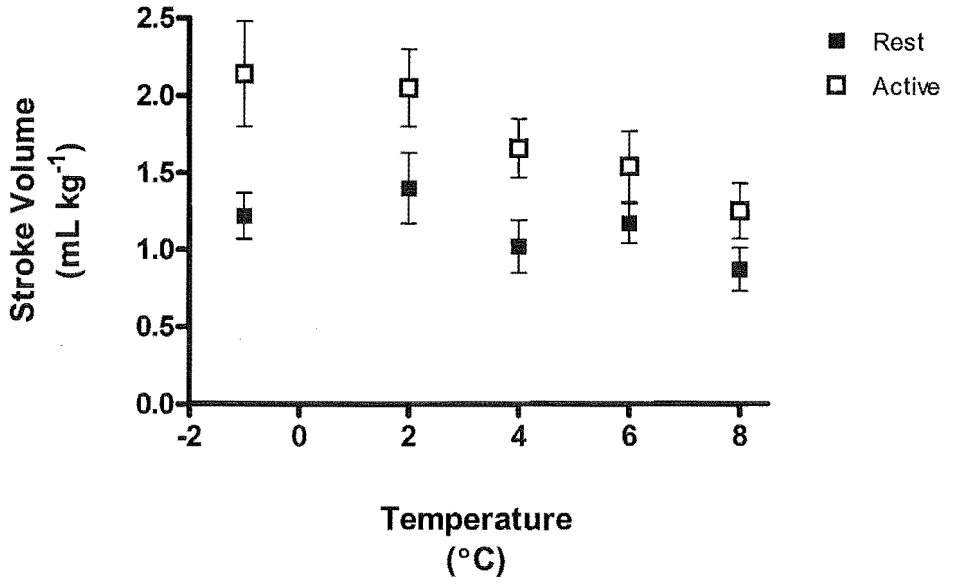


Fig. 7.7. Effect of an acute increase in temperature on resting and active cardiac stroke volumes of -1°C-acclimated fish

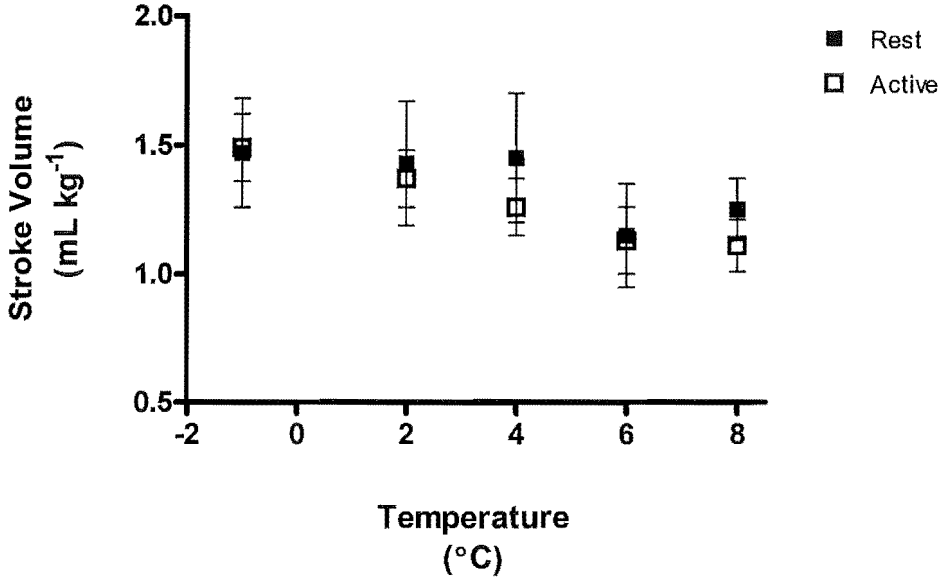


Fig. 7.8. Effect of an acute change in temperature on resting and active cardiac stroke volumes of 4°C-acclimated fish

The scope for change in stroke volume attained a maximum level of 1.89 ± 0.15 at the acclimation temperature of the -1°C-acclimated fish. There was a slight decrease in the scope for change in stroke volume with increasing temperature, with minimum values of 1.48 ± 0.11 and 1.48 ± 0.15 at 6 and 8°C, respectively. In 4°C-acclimated fish, the scope for change in stroke volume was lower than in fish acclimated to -1°C, with a maximum of 1.16 at 2°C and minimum of 0.94 at 8°C, and no apparent trend with increasing temperature (Fig. 7.9). The scope for change in stroke volume of the 4°C-acclimated fish was also significantly lower at their 4°C acclimation temperature than that of the cold-acclimated fish at their respective acclimation temperature.

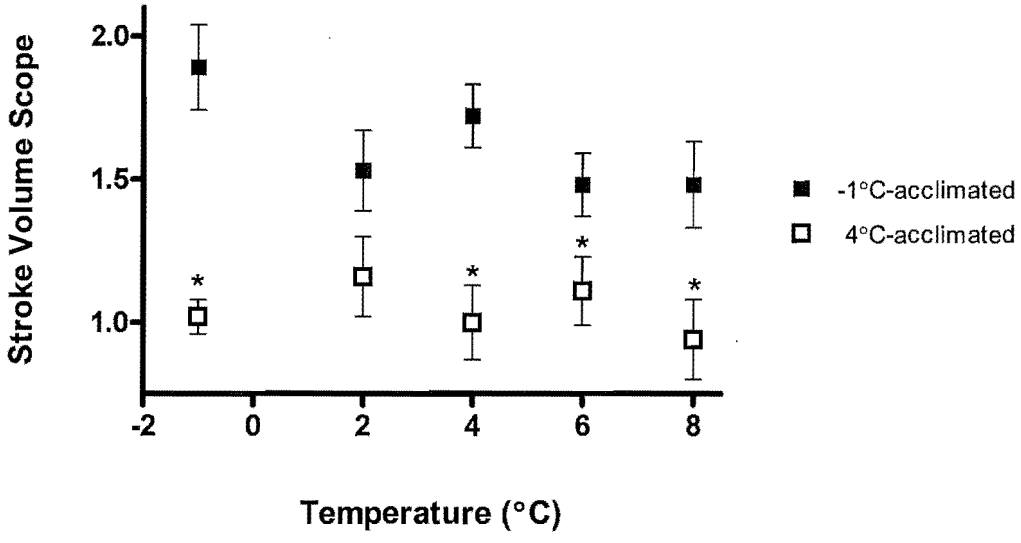


Fig. 7.9. Effect of an acute change in temperature on the scope for change in stroke volume of *P. borchgrevinki* acclimated to two different temperatures. * Significantly different from -1°C-acclimated fish.

DISCUSSION

The resting cardiac output of *P. borchgrevinki* at -1°C (22.2 ± 2.9 mL min^{-1} kg^{-1}) compares well with the value of 29.6 ± 6.8 mL min^{-1} kg^{-1} previously reported for this species at 0°C (Axelsson et al. 1992). The resting cardiac output of the cold-acclimated fish progressively increased as the temperature was increased from -1 to 6°C, although the change was not statistically significant due to the high level of inter-individual variation. The resting cardiac output of warm-acclimated fish at their 4°C acclimation temperature (24.7 ± 1.8 mL min^{-1} kg^{-1}) was considerably lower than that of cold-acclimated fish at the same temperature, and very similar to that of the cold-acclimated fish at their -1°C acclimation temperature, indicating near-perfect thermal compensation as defined by Precht (1973). There is a strong relationship between resting cardiac output and the swimming performance of fish. Fishes that display high levels of activity generally have higher cardiac outputs than more sluggish species, with a 15-fold range between hagfish at one extreme and tuna at the other (Satchell 1991; Farrell and Jones 1992). The cardiac output of a variety of relatively inactive temperate fishes ranges from 10-20 mL min^{-1} kg^{-1} at 10°C (Kolok et al. 1993), compared with 132 mL min^{-1} kg^{-1} for the highly active tunas (Bushnell and Brill 1991). Resting cardiac outputs of 19.5 to 21.3 mL min^{-1} kg^{-1} for sub-Antarctic nototheniids (Agnisola et al. 1997) and 17 to 29 mL min^{-1} kg^{-1} for Atlantic cod (Jones et al. 1974; Petersson and Nilsson 1980; Axelsson and Nilsson 1986; Axelsson 1988) have been determined at their habitat temperatures, while resting cardiac output of the more sedentary, benthic notothenid *Trematomus bernacchii* is reported as 17.6 mL min^{-1} kg^{-1} at 0°C (Axelsson et al. 1992).

Exercise resulted in an increase in cardiac output of both warm and cold-acclimated fish at all temperatures, although the increase in cardiac output of the -1°C-acclimated group at 6°C was not statistically significant. Exercise leads to an increase in the rate of oxygen consumption (Chapter Four), and it would be expected that there would be an increase in cardiac output in order to increase blood flow through the gills and transport a greater quantity of oxygen. Cardiac output has been demonstrated to increase during prolonged exercise in most fish (Taylor et al. 1993), although short-finned eels appear to be an exception (Davie and Forster 1980). The active cardiac output (cardiac output immediately after exercise) of the -1°C-acclimated fish at their acclimation temperature (59.5 ± 10.1 mL min^{-1} kg^{-1}) was not significantly different from

that of the 4°C-acclimated group at their respective acclimation temperature (52.7 ± 5.3 mL min^{-1} kg^{-1}) and these values compare well with the 49.7 - 52.6 mL min^{-1} kg^{-1} active cardiac outputs of rainbow trout at 8 - 11°C (Kiceniuk and Jones 1977; Keen and Farrell 1994). It is not known whether swimming fish attain the highest possible cardiac outputs, but the finding that maximum cardiac outputs of *in situ* heart preparations of both sea raven and rainbow trout are similar to *in vivo* values measured near critical swimming speeds in the respective species (Farrell and Jones 1992) suggests that they do. In this study, the animals were chased with a net and therefore did not reach critical swimming speed. As a result, the cardiac outputs measured may underestimate maximum cardiac performance, but with the primary objective being to compare cardiac parameters between fish acclimated to different temperatures, it is the relative rather than absolute values which are of greatest importance.

As stated in the introduction, it is the scope for change in cardiac output which represents the operational flexibility of the cardiovascular system and thus is of primary importance in aerobic swimming. In order to maintain cardiac scope across a broad temperature range, routine and maximal cardiac outputs must vary with temperature with similar thermal coefficients (Q_{10}). This was not the case in cold-acclimated *P. borchgrevinki*, with a Q_{10} for resting cardiac output of 2.4 from -1 to 6°C, and a Q_{10} of 1.0 for active cardiac output over the same temperature range. This thermal sensitivity of cardiac output in *P. borchgrevinki* is typical of the majority of fish. Resting cardiac output generally increasing exponentially up to the lethal limit of a species with Q_{10} values of about 2.5 (Cech et al. 1976; Barron et al. 1987; Kolok et al. 1993; Farrell 1997), while maximum cardiac output typically increases to reach a plateau at the preferred temperature, and then declines just prior to the upper lethal limit (Farrell 1997), with Q_{10} values typically between 1.2 and 1.4 (Graham and Farrell 1989; Keen and Farrell 1994; Kolok and Farrell 1994). This results in a decrease in cardiac scope as temperature is increased toward the upper lethal limit, as observed in *P. borchgrevinki*. Above 6°C, both resting and active cardiac outputs of *P. borchgrevinki* declined which is similar to the 7°C temperature at which cardiac output levels off in the Antarctic teleost *Pachycara brachycephalum* (Mark et al. 2002), and agrees well with the previously identified upper lethal limit for this species (Somero and De Vries 1967).

The thermal sensitivity of active cardiac output of 4°C-acclimated fish (Q_{10} -1 to 8°C = 1.16) was similar to that of -1°C-acclimated fish, but the resting cardiac output over the same thermal range was relatively independent of temperature (Q_{10} = 0.73),

enabling cardiac scope to be maintained, and even increased, at higher temperatures. While the maximum cardiac scope of -1°C-acclimated fish was attained at their acclimation temperature, with a steady decline in the magnitude of scope as temperature was increased, the cardiac scope of 4°C-acclimated fish exhibited the reverse trend, increasing from a minimum value at -1°C to a maximum at 8°C. The maximum cardiac scopes of both cold and warm-acclimated *P. borchgrevinki* were slightly lower than the maximum cardiac scope of 3.0 calculated as the average value for a variety of fishes (Farrell 1997), but it is possible that the values in the current study were sub-maximal as discussed above. The few reports of resting and maximum cardiac output in fish all agree that, although both resting and maximum levels of cardiac output can increase with temperature, cardiac scope generally decreases at warm temperatures (Farrell and Jones 1992; Farrell 1997).

In comparing the critical swimming speed of *P. borchgrevinki* (Chapter Six) with the relative magnitude of cardiac scope, it is apparent that the two parameters vary in tandem during acute and acclimatory thermal changes, in support of the theory that cardiac performance is one of the factors limiting aerobic swimming ability. The marked similarity in the pattern of enhancement of both cardiac scope and prolonged swimming ability at high temperatures following warm-acclimation is in agreement with the findings of Keen and Farrell (1994), who reported almost identical compensatory changes, both in direction and magnitude, in the maximal swimming and cardiac performances of rainbow trout during thermal acclimation. The authors used these similar responses to indicate that cardiac performance is adequate to supply the needs of working tissues, but not to exceed utilisation by those tissues, in line with the concept of symmorphosis (Taylor and Weibel 1981). Near the lethal thermal limit, an increasing percentage of the SMR is required for ventilation and circulation, contributing to a loss of aerobic scope (Mark et al. 2002). The venous oxygen tension then drops, which results in a decline in oxygen supply to the heart (Pörtner et al. 2001) and the resulting decrease in oxygen supply to the working muscles is known to rapidly decrease tension development (Farrell 1991). The greatest cardiac scope should therefore occur at the temperature that coincides with the maximum aerobic swimming speed (Taylor et al. 1997), and the results of the current study provide support for this statement. Maximum cardiac scope and peak critical swimming speed of cold-acclimated fish coincided at their -1°C acclimation temperature and, although maximum critical swimming speed and cardiac scope of the 4°C-acclimated group were attained at 2 and 8°C respectively, there

was a plateau in both parameters at high performance levels from 2-8°C. There was also a rapid decline in critical swimming speed of the -1°C-acclimated fish, in tandem with the decrease in cardiac scope, at temperatures above 2°C. Further evidence for a cardiovascular limit on swimming performance comes from the findings of Kolok (1992) who reported that individual variation in heart enzymatic activity was correlated with individual variation in critical swimming speed, and is supported by Farrell et al. (1990) who found that rainbow trout significantly increased both heart enzymatic activities and critical swimming speed in response to training.

One possible source of error in experiments of this type is in the calibration of the flow probes. In the past, cardiac outputs have been measured by the Fick method, relating the difference in oxygen content of arterial and venous blood to the overall oxygen consumption rate. It has, however, been shown that some 20-40% of oxygen used by the gills is taken directly from the water by the epithelial cells (Johansen and Pettersson 1981) and with the added complications of cutaneous gas exchange and blood shunts within the gills Fick estimates are often in error (Daxboeck et al. 1982; Meltcalfe and Butler 1982). While Doppler flow probes provide reliable information on zero flow and the relative changes in flow, they are difficult to calibrate. In order to improve the level of accuracy within this experiment, cuffs were individually made and carefully sized to the dimensions of the ventral aorta (diameter approximately 1 mm). Each probe was calibrated *in situ* at the end of the experiment, over the range utilised during the recording, and great care was taken during the calibrations as even slight changes in the positioning of a probe could have a significant effect on the results.

The state of the subject animal is another possible source of variation as stress, spontaneous activity, and blood loss have all been shown to elevate resting cardiac output (Farrell and Jones 1992). The surgical procedure used in this study required a small incision and very little blood loss. The fish were given 24 hours to recover from the stress of anaesthesia and surgery and all fish rested quietly on the base of the tank both during recovery and while obtaining resting values, with spontaneous activity restricted to the occasional movement of pectoral fins.

There may be a relationship between body mass and cardiac output, possibly complicated by temperature, although this has yet to be established (Farrell and Jones 1992). The fish used for this study were all of similar size, with no significant differences in total length (mass was not determined) between the two acclimation groups, thus minimising the effect of any relationship.

The resting heart rate of -1°C-acclimated *P. borchgrevinki* exhibited a typical thermal response, increasing as the temperature increased with a Q_{10} of 2.38 (-1 to 8°C). This is in contrast to previous studies on this species which have reported chronotropic inhibition from -1 to 10°C (Forster et al. 1998), and between -1 and 3°C (Franklin et al. 2001), but compares well with the results reported in Chapter Three of this thesis. The relatively sedentary Antarctic nototheniid *T. bernacchii* has also been reported to maintain heart rate to 2.5°C, with a rapid increase above this level (Q_{10} > 4 from 2.5 to 5°C) (Axelsson et al. 1992). The majority of fish exhibit Q_{10} values for resting heart rate of between 1.3 and 3.0, depending on the temperature range and acclimation temperature (Kiceniuk and Jones 1977; Wood et al. 1979; Henry and Houston 1984; Taylor et al. 1993; Morita and Tsukuda 1994; Farrell et al. 1996; Tiitu and Vornanen 2002). This thermal dependence of heart rate is thought to be due to shortening of the atrio-ventricular delay as a direct effect of the increase in temperature (Peyraud-Waitzenegger et al. 1980). The resting heart rate of 4°C-acclimated fish, however, was thermally insensitive (Q_{10} -1 to 8°C = 0.89), a similar response to that reported by the earlier studies on this species. The previous studies involved fish held in captivity and acclimated to 0°C, and it is possible that acclimation to a temperature less than 2°C above their habitat temperature has a significant effect on the thermal sensitivity of the cardiovascular system of these fish.

The effect of an acute increase in temperature on active heart rate was similar in both cold and warm-acclimated fish, with Q_{10} values (-1 to 8°C) of 1.60 and 1.64, respectively. It therefore appears as though there is greater potential for phenotypic plasticity in resting than in active heart rates during warm-acclimation. Warm-acclimation has been shown to increase the maximum rate of cardiac contraction in several fishes (Bailey and Driedzic 1990; Bailey et al. 1991), although the current study provided no evidence for this. The maximum heart rate attained by warm-acclimated fish was only slightly, and not significantly, higher than that of cold-acclimated fish (47.8 ± 1.4 beats min^{-1} at 6°C, and 42.2 ± 1.5 beats min^{-1} at 8°C, respectively).

The differing thermal sensitivities of resting heart rate contributed to a considerable difference in the effect of temperature on the scope for change in heart rate at the two acclimation temperatures. With resting heart rate increasing at a relatively greater rate with temperature than active heart rate in the -1°C-acclimated fish, maximum scope was attained at -1°C and the scope declined as temperature was increased. The opposite trend was observed in the 4°C-acclimated fish; active heart rates rose as the

temperature was elevated, but resting values remained unchanged and the scope for change in heart rate therefore increased with temperature. The maximum scopes for change in the heart rate of -1 and 4°C-acclimated *P. borchgrevinki* were 50% and 150%, respectively. The scope for change in heart rate of this species has previously been reported as 33% (Axelsson et al. 1994) and 100% (Axelsson et al. 1992) at 0°C. In other species the trend is variable, with an increase of over 100% in brown trout (Butler et al. 1992), but no effect of sustainable aerobic exercise on heart rate of rainbow trout (Taylor et al. 1993; Taylor et al. 1996). Both cardiac output and stroke volume increase considerably in response to an increase in perfusion pressure, but heart rate is unaffected (Farrell 1984) indicating that the increase in heart rate during swimming is not likely to be an intrinsic response to the increase in venous return. The increase in heart rate during swimming could be mediated in several ways including via mechanical stretch of the pacemaker cells, by a reduction in cholinergic tone, or by an increase in adrenergic stimulation (neural or humoral) (Farrell 1991). Experiments with teleost hearts do not support the idea that mechanical stretch of the pacemaker has a significant influence on heart rate (Farrell 1991). With very little change observed following blockade of adrenergic nerve activity (Axelsson and Nilsson 1986) and no significant increase in circulating catecholamine levels of fish during moderate exercise (Ristori and Laurent 1985; Hughes et al. 1988; Egginton 1997; Egginton and Davison 1998), there seems to be general agreement that in virtually all fishes adrenergic compounds have much weaker chronotropic effects, in contrast to the marked inotropic effects (Randall 1970; Stevens et al. 1972). It therefore seems likely that vagal release may play the most important role in the increase of heart rate during exercise (Satchell 1991).

The resting heart rate of -1°C-acclimated fish at their acclimation temperature was 18.4 ± 0.9 beats min^{-1}. This compares well with previous reports of heart rate in this species which vary from 11.3 ± 2.9 to 20.6 ± 0.9 beats min^{-1} at acclimation temperatures from 0 to -1.2°C (Axelsson et al. 1992; Axelsson et al. 1994; Forster et al. 1998; Franklin et al. 2001). The resting heart rate of 4°C-acclimated fish at 4°C (20.3 ± 2.8 beats min^{-1}) was not significantly different from that of the -1°C-acclimated fish at their respective acclimation temperature, again representing near-perfect thermal compensation. The "chronic" Q_{10} (-1 to 4°C), calculated from the heart rates of fish at their respective acclimation temperatures was 1.23, which is slightly lower than values from goldfish (Q_{10} 10 and 25°C = 1.54) (Tsukuda et al. 1985), sole (Q_{10} 10 and 24°C = 1.54) (Sureau et al. 1989), and rainbow trout (Q_{10} 8 and 18°C = 1.52) (Keen and Farrell 1994) following a

period of thermal acclimation. In contrast, the resting heart rate of -1°C-acclimated fish acutely exposed to 4°C was 33.6 ± 1.4 beats min^{-1}, resulting in an "acute" Q_{10} (-1 to 4°C) of 3.33. This is higher than the average Q_{10} of 2.0 calculated for heart rate after an acute temperature change in other fishes (Priede 1974; Butler and Taylor 1975; Cech et al. 1976; Graham and Farrell 1985), probably due to the fact that 4°C is close to the upper thermal limit of cold-acclimated *P. borchgrevinki*. For a given increase in temperature, the heart rate following warm-acclimation is generally lower than that for an acute change to the same temperature (Farrell 1996), and lower heart rates have also been demonstrated in eurythermal fish compared with stenothermal fish at equivalent temperatures (Farrell 1997). The marked difference between the chronic and acute Q_{10} values indicates that substantial modifications have been made in the regulation of beat frequency with thermal acclimation. Although the mechanism(s) responsible for these modifications have yet to be fully detailed, they may include alterations in the sensitivity to tonic adrenergic (Peyraud-Waitzenegger et al. 1980; Graham and Farrell 1989; Keen et al. 1993) and cholinergic stimulation (Tsukuda et al. 1985; Bowler and Tirri 1990), or in the intrinsic pacemaker rate (Lillywhite et al. 1999). Warm-acclimation is reported to result in an increase in the temperature at which the pacemaker fails in goldfish (Tsukuda et al. 1985; Morita and Tsukuda 1994). Cholinergic control of the heart is thought to be more important at lower temperatures, with the adrenergic influence increased at higher temperatures (Nilsson et al. 1996). Antarctic notothenioids typically have a large resting cholinergic tonus on the heart (Axelsson et al. 1992; Axelsson et al. 1994; Davison et al. 1997; Franklin et al. 2001), although the marked cholinergic tonus reported from captive fish may not be present in free-living fish (Davison et al. 1995).

The acclimatory resetting of heart rate to a lower frequency would negate any advantage offered by a higher heart rate in terms of increased blood circulation at higher temperatures but, as there is a maximum heart rate (Farrell 1997), failure to reset heart rate results in a reduction of scope at higher temperatures, as observed in -1°C-acclimated *P. borchgrevinki*. It has been suggested that the degree of acclimatory change in heart rate may, at least partially, be offset by acclimatory changes in haemoglobin concentration in order to maintain oxygen transport capacity (Houston 1980). Oxygen delivery is equal to the product of cardiac output and the arteriovenous difference in oxygen content, and the haematological response that has the greatest effect on the arteriovenous difference is a quantitative change in the blood haemoglobin concentration (Farrell 1997), usually due to alteration of haematocrit (Houston 1980). An 8% increase

in haematocrit has previously been observed in rainbow trout following warm-acclimation (Gallaugher et al. 1992), and a significant increase in both haematocrit and haemoglobin concentration has previously been reported in *P. borchgrevinki* acclimated to 4.5°C for 8 – 13 days (Tetens et al. 1984). There were, however, no significant differences in either haematocrit or haemoglobin concentration between -1 and 4°C-acclimated *P. borchgrevinki* in this study (Chapter Two).

In relating the thermal response of heart rate during exercise to the changes in cardiac output, it is apparent that it is not a lack of increase in heart rate during exercise which limits cardiac output, in fact active cardiac output does not keep pace with changes in heart rate, as heart rate continues to increase with temperature right to the upper lethal limit (Morita and Tsukuda 1994). Studies on *in situ* perfused rainbow trout hearts, acclimated to 5 or 15°C, show an impressive degree of compensation for the thermal sensitivity of heart rate (Q_{10} = 2.2), resulting in relative thermal insensitivity (Q_{10} = 1.3) of maximum cardiac output (Graham and Farrell 1989), and a similar relationship between the two parameters was apparent in this study, with active heart rate increasing as the temperature was elevated, while active cardiac output remained stable or declined. If cardiac output varied directly with heart rate then the excessive flow and pressure at high temperatures could compromise gill function (Taylor et al. 1997) and therefore the compensation for the effects of temperature on heart rate contributes to broadening the range of thermal tolerance. An increase in heart rate but decrease in stroke volume has been shown to maintain cardiac output to 5°C in *T. bernacchii* (Axelsson et al. 1992), and Taylor et al. (1993, 1996) reported a steady increase in both resting and exercised heart rates of rainbow trout with increasing temperature, but a peak of exercised cardiac output in the middle of the thermal range coinciding with maximum stroke volume. Temperature-induced increases in heart rate cannot therefore be used for predicting changes in maximum cardiac output above the preferred temperature (Farrell 1997).

The resting stroke volume of -1°C-acclimated *P. borchgrevinki* was 1.22 ± 0.15 mL kg^{-1}. This is considerably lower than the previously reported value for this species which, at 2.16 ± 0.53 mL kg^{-1} (Axelsson et al. 1992), is more comparable with the active stroke volume of the current study. Resting stroke volume of 4°C-acclimated fish at 4°C (1.45 ± 0.25 mL kg^{-1}) was slightly higher than that of -1°C fish at their respective acclimation temperature, but still low in comparison with the previous study and similar to the previously reported 1.56 mL kg^{-1} cardiac stroke volume of *T. bernacchii* (Axelsson et al. 1992). Stroke volumes in the current investigation were calculated from the

measured values of cardiac output and heart rate, rather than measured directly. This results in cumulative errors which may be responsible for the wide variation in values. The nutritional status of fish has been demonstrated to influence heart mass (Goolish and Adelman 1987) and the 4-5 week fast may therefore have had an influence on the results. The resting stroke volumes determined in both this and the previous studies are still relatively high in comparison with those of temperate teleosts which vary from 0.21-0.51 mL kg^{-1} (Kiceniuk and Jones 1977; Davie and Forster 1980; Axelsson and Nilsson 1986; Axelsson 1988; Axelsson et al. 1989). The majority of the temperate-water species were an order of magnitude larger than the fish used in the current study, but resting stroke volumes of sub-Antarctic fishes of similar mass to *P. borchgrevinki*, also fall between 0.30 and 0.45 mL kg^{-1} at 10°C (Agnisola et al. 1997). The size of the ventricle is clearly one of the factors affecting stroke volume, but although the relative ventricular size of *P. borchgrevinki* (0.156% body weight) is twice as large as that of the most sedentary trematomids (Axelsson et al. 1992), it is within the 0.1-0.2% range typical for inactive to moderately active teleosts (Eastman 1993) thus offering no simple explanation for the greater stroke volume.

The resting stroke volumes of both cold and warm-acclimated *P. borchgrevinki* were relatively independent of temperature (Q_{10} -1 to 8°C = 0.70 and 0.84, respectively), as was the active stroke volume of warm-acclimated fish (Q_{10} -1 to 8°C = 0.72). Active stroke volume of the -1°C-acclimated fish, however, decreased slightly with increasing temperature (Q_{10} -1 to 8°C = 0.55), although the trend was not significant due to the high level of inter-individual variation. Decreases in stroke volume at warmer temperatures have been reported for various species of fish (Graham and Farrell 1985; Graham and Farrell 1989; Kolok et al. 1993; Keen and Farrell 1994; Kolok and Farrell 1994), although changes in stroke volume with temperature in contrast to the changes in heart rate, appear to be strongly species-specific. In flounder, stroke volume increased by 64% from 5 to 16°C (Cech et al. 1976), while in largescale sucker stroke volume decreased by almost 30% over the same temperature range (Kolok et al. 1993). A decrease in stroke volume with increasing temperature has also been reported in trout (Farrell et al. 1996), although both maximum and routine stroke volumes of *in situ* perfused trout hearts are reported to be independent of temperature (Overgaard et al. 2004).

Although increased temperature has been demonstrated to increase the force generated by attached actinomyosin cross-bridges, the calcium sensitivity of the cardiac muscle (Driedzic and Gesser 1994), and the isometric force of carp ventricular strips

paced at constant frequency (Matikainen and Vornanen 1992), spontaneously beating atrial and ventricular strips decrease maximum isometric tension with increasing temperature (Ask 1983; Driedzic and Gesser 1985; Matikainen and Vornanen 1992) and the maximum stroke volume of rainbow trout hearts decreases at high heart rates without any temperature increase (Farrell et al. 1989). Stroke volume is set by the size of the ventricle, the degree of ventricular filling, and the force of contraction which determines the degree of cardiac emptying, it has therefore been suggested that the increase in heart rate at higher temperatures may significantly constrain cardiac filling time and decrease contractility, resulting in a reduction in maximum stroke volume (Farrell 1997). The cardiac muscle of vertebrates is sensitive to filling pressure, as described by the Frank-Starling mechanism, such that stroke volume is adjusted relative to venous return (Lillywhite et al. 1999). Decreased cardiac filling therefore decreases the end-diastolic volume, resulting in a lower stroke volume. This negative relationship between active stroke volume and active heart rate has been demonstrated by both Graham and Farrell (1989), and Keen and Farrell (1994), and was apparent in the results of this study. Maximum active stroke volume of the cold-acclimated fish (2.14 mL kg^{-1}) occurred at the minimum active heart rate (27.6 beats min^{-1}) while the minimum active stroke volume (1.25 mL kg^{-1}) occurred at the maximum heart rate (42.2 beats min^{-1}). A similar trend, although of smaller magnitude, was observed in the warm-acclimated fish, with maximum active stroke volume (1.49 mL kg^{-1}) at the minimum heart rate of (29.1 beats min^{-1}), and minimum stroke volume (1.11 mL kg^{-1}) at a heart rate only 2.5 beats min^{-1} from the maximum rate (45.3 beats min^{-1}). If a decrease in contractility at high heart rates leads to the observed decrease in cardiac performance at warmer temperatures, then it may be that some aspect of excitation-contraction coupling is rate-limiting.

Although the evidence has not yet been provided, the possibility exists that during thermal acclimation of fish hearts modifications in sarcolemmal function may occur, affecting Ca^{2+} entry and influencing the contractile force and time-course of contraction (Axelsson et al. 1998). Cardiac myosin isoforms have been reported in teleosts (Karasinski 1988), and a temperature-induced switch has been documented (Vornanen 1994) which may be important in Antarctic nototheniids which possess proteins which are denatured at relatively low temperatures (Johnston et al. 1975). Warm-acclimation has been shown to increase the ability to maintain isometric tension at higher heart rates (Bailey and Driedzic 1990; Bailey et al. 1991), although there was no significant

evidence provided for this in the current study with slight decreases in resting and active stroke volumes of both cold and warm-acclimated fish with increasing temperature.

The stroke volume of -1°C-acclimated fish increased in response to exercise at all temperatures, although the differences were only significant at -1 and 4°C. In contrast, stroke volume of the 4°C-acclimated fish was unresponsive to exercise at all temperatures. The results from the warm-acclimated fish compare well with the findings of Axelsson et al. (1992) who reported a 75% increase in cardiac output during sustained swimming of *P. borchgrevinki* at 0°C which was due to doubling of heart rate with no change in stroke volume. In contrast, a later study at the same temperature found a 78% increase in cardiac output, due to increases of 36 and 33% in stroke volume and heart rate, respectively (Axelsson et al. 1994). The 160% increase in cardiac output of -1°C-acclimated *P. borchgrevinki* in response to exercise at their acclimation temperature in the current study, was achieved through increases of 89% and 53% in stroke volume and heart rate, respectively. Typically, the relative contribution of stroke volume to the increase of cardiac output in fish is equal to or greater than that of heart rate (Randall 1970; Farrell and Jones 1992). For example, during prolonged swimming the percentage changes in stroke volume versus heart rate are respectively 200% versus 50% in rainbow trout (Kiceniuk and Jones 1977), 55-63% versus 7-15% in dogfish (Piiper et al. 1977), and 26% versus 18% in Atlantic cod (Axelsson and Nilsson 1986). There is, however, an important evolutionary trend away from varying stroke volume towards varying heart rate as the primary means of increasing cardiac output (Lillywhite et al. 1999), with the highly active tunas relying more on frequency modulation (Farrell 1991; Blank et al. 2002). It has been suggested that Antarctic fishes could be another exception to the generalisation because, with heart rate under such a large inhibitory cholinergic tone, 2 to 3-fold increases in heart rate are possible (Axelsson et al. 1992). Another observation which supports the use of heart rate to modulate cardiac output in Antarctic fish is that recovery from hypoxia in *T. bernacchii* is associated with tachycardia, but no increase in stroke volume above resting levels (Axelsson et al. 1994). The results of the current study indicate modulation of cardiac output primarily through changes in stroke volume in cold-acclimated *P. borchgrevinki*, particularly at higher temperatures. Increasing heart rate has been shown to be inefficient for elevating cardiac output in rainbow trout when changes are particularly severe, such as following acute elevation of water temperature (Brodeur et al. 2001), and the same may apply to the Antarctic species. In contrast to the cold-acclimated fish, 4°C-acclimated *P. borchgrevinki* increased cardiac output by 111%

during exercise at their acclimation temperature due to a 141% increase in heart rate with no change in stroke volume. Evidence is accumulating which indicates there may have been underestimation of the contribution of heart rate in earlier work in which the fish underwent invasive surgery (Thorarensen et al. 1996; Webber et al. 1998; Altimiras and Larsen 2000; Brodeur et al. 2001). There is also recent evidence to suggest that modulation of heart rate may be more prevalent than originally thought. Largemouth bass appear to be primarily frequency modulators (Cooke et al. 2003; Eggington et al. unpubl. data) and two studies have reported dependence of cardiac output on heart rate in trout (Altimiras et al. 2002; Overgaard et al. 2004).

In the current study there appeared to be a general trend, with the cold-acclimated fish placing greater reliance on stroke volume to modulate cardiac output, and the warm-acclimated fish achieving increases in cardiac output almost entirely through changes in heart rate. This is in support of the work of Kolok et al. (1993) who reported that largescale sucker relied more on an increase in stroke volume to maintain scope for cardiac output at 5 and 10°C, whereas at 16°C the fish modulated cardiac output through an increase in heart rate. The haemoglobin-less Antarctic icefish are also reported to modulate heart rate, but not stroke volume in response to increasing temperature (Tota et al. 1991). Further investigation is required to determine whether this trend is widespread.

In conclusion, the results of this study indicate that cardiac performance of -1°C-acclimated *P. borchgrevinki* is highly thermally sensitive, with a rapid decline in cardiac scope above the acclimation temperature. Fish acclimated to 4°C for 4-5 weeks, however, demonstrate the reverse trend with cardiac scope increasing with an increase in temperature, and declining as temperature is reduced below the acclimation temperature. The similar pattern of decline in both prolonged swimming performance and cardiac performance of -1°C-acclimated *P. borchgrevinki* with increasing temperature and the almost identical compensatory changes, both in direction and magnitude, of swimming and cardiac performances during thermal acclimation add support to the theory that cardiac performance is one of the factors limiting aerobic swimming performance. The apparent change in the mechanism of modulating cardiac output at different acclimation temperatures, with a greater dependence on heart rate at higher temperatures, requires further investigation.

Chapter Eight

The effect of an acute increase in temperature on the hypoxia tolerance of *Pagothenia borchgrevinki*

INTRODUCTION

During their evolution over the past several million years, fish have been exposed to large fluctuations in environmental oxygen levels, and to marked variations in oxygen demand caused by changes in environmental temperature (Nikinmaa 2002). The majority of aquatic environments still experience frequent fluctuations in oxygen saturation and, as a result, most teleost fishes are adapted to cope with such changes (Hughes 1973; Rantin and Johansen 1984). The hypoxia tolerance of fishes in temperate environments is reported to be well correlated with the minimum environmental oxygen concentration (Smale and Rabeni 1995). In contrast, the inverse relationship between oxygen solubility and temperature results in high and stable ambient oxygen tension in McMurdo Sound, with levels varying throughout the year from 74-105% of air saturation (Littlepage 1965). Summer levels increase above air saturation, due to the prolonged photoperiod and high productivity of phytoplankton (Holeton 1970; Knox 1994). The diffusion gradients from water to blood are therefore always favourable (Eastman 1993), and Antarctic notothenioids are unlikely to ever experience hypoxic conditions.

Antarctic fish differ from temperate and tropical species in having a reduced haematocrit and low haemoglobin content (di Prisco et al. 1998), features which counterbalance the increase in blood viscosity brought about by the subzero seawater temperature (Wells et al. 1990), but which may also compromise their ability to respond to hypoxic challenges. The majority of Antarctic notothenioids also do not exhibit the hypoxic bradycardia observed in temperate-water fishes (Axelsson et al. 1992). This

bradycardia is thought to benefit gas exchange in the gills (Randall and Shelton 1963), heart, and peripheral tissues (Farrell 1982) due to a longer residence time of the blood. The bradycardia may also promote cardiac filling (Farrell 1984), which augments stroke volume leading to recruitment of the secondary gill lamellae and an increase in the functional surface area of the gills available for gas exchange (Farrell 1980; Soivio and Tuurala 1981). It has been suggested that the bradycardia is the result of increased vagal inhibition (Randall 1966), and with the normoxic level of cholinergic tone near maximal in Antarctic fishes, further increase may not be possible (Axelsson et al. 1992). The Antarctic nototheniid *Pagothenia borchgrevinki* has, however, been shown to alter the oxygen-affinity of haemoglobin in response to hypoxia at its environmental temperature, in a manner very similar to that of teleosts which naturally inhabit oxy-labile environments (Wells et al. 1989). Arctic polar fish also possess the mechanisms to respond to hypoxia, although they tend to be less hypoxia tolerant than fishes from temperate waters (Schurmann and Steffensen 1992; Steffensen et al. 1994). It has thus been suggested that: "the ability to make short-term adaptive changes in the oxygen delivery system in response to hypoxic exposure may be typical for vertebrates in general, rather than a feature only seen in those organisms which encounter environmental hypoxia on a regular basis" (Wells et al. 1989).

The greatest challenges to physiological systems occur when stressors are compounded, and a system already compromised by one variable is challenged by another (Barton et al. 1986; Sigismondi and Weber 1988; Reid et al. 1997). In nature, animals must deal with multiple challenges imposed by their environment, in the form of either biotic or abiotic changes (Guderley 2004), and the potentially lethal conditions of hypoxia and hyperthermia vary together with a number of secondary environmental factors (Love and Rees 2002), due mainly to the fact that the dissolved oxygen content of water is decreased by an elevation in temperature (Ali 1980). A variety of physiological parameters of poikilothermic fish are directly and indirectly impacted by changes in environmental temperature, including metabolism (Targett 1978), growth, cardiac output, ventilation, and excretory processes (Reid et al. 1997), and it is thus likely that hypoxia tolerance would also be affected. Prior exposure to a stressor should compromise gas exchange capacity and cause fish to die at higher residual oxygen concentrations than unstressed fish (McLeay et al. 1984). Also, with the increased energy demands of respiratory processes and elevated metabolic rates at higher temperatures (Hughes et al. 1983), oxygen concentrations should be depleted faster and the fish should die sooner.

The majority of fish demonstrate a reduction in hypoxia tolerance at elevated temperatures (Fry and Hart 1948; Downing and Merkens 1957; Fernandes and Rantin 1989; Schurmann and Steffensen 1997; Stecyk and Farrell 2002), although the trend is by no means universal (Ultsch et al. 1978; Ott et al. 1980), and vice versa; if the oxygen tension of water is significantly below air-saturation, thermal tolerance is lowered considerably (Alabaster and Welcomme 1962). The low metabolic rate of Antarctic fish and the high dissolved oxygen content of the water may permit hypoxia tolerance at environmental temperatures, but the effect of increased temperature and the resulting increase in metabolic rate (Wilson et al. 2002) on hypoxia tolerance of these fishes has not been investigated.

The objective of this study was to determine the effect of exposure to an acute increase in temperature on the hypoxia tolerance of *Pagothenia borchgrevinki*. It was hypothesized that an increase in temperature, within the thermal tolerance range of the fish, should decrease hypoxia tolerance through an increase in resting metabolic demands.

MATERIALS AND METHODS

Pagothenia borchgrevinki (mass 87.6 ± 14.5 g (mean ± SD), range 59.1 – 117.9 g, length 222.8 ± 13.2 mm (mean ± SD), range 200 – 256 mm (values obtained after the experiment), n = 20) were caught and transported to Scott Base, as described in Chapter One. The fish were held in a flow-through aquarium system (water temperature -1.0 ± 0.3°C) for a minimum of three days prior to the experiments, to allow recovery from the stress of capture. The fish were unfed and a natural photoperiod (24-hour daylight) was maintained. Following the recovery period, the fish were randomly subjected to a progressive hypoxic challenge at either -1°C or 3°C. The respirometry chambers used for the investigation were the cylindrical chambers described in Chapter Four, with volumes of 1410-1470 mL. The fish were placed in the chambers 24 hours prior to commencement of the experiment and the chambers immersed in a 45 L tank of seawater at -1 or 3°C. The -1°C tank, as part of the flow-through aquarium system, had a continuous flow of air-saturated fresh seawater, while the 3°C tank was isolated from the flow-through system and heated to 3.0 ± 0.3°C using a heat exchanger linked to an

adjacent freshwater tank which contained a thermostatically controlled heater. Oxygen tensions were maintained in the static tank by the use of an air-bubbler apparatus, and a large proportion of the seawater was replaced between experiments. During the 24-hour settling period, the water was circulated through both respirometry chambers using an aquarium pump. At the commencement of measurements the respirometry chambers were sealed by placement of rubber bungs in the lids, taking care not to disturb the fish which were shielded from adjacent human activities by the opaque walls of the tanks. Immediately after sealing the chambers a 1 mL sample was withdrawn by syringe, and the fluid volume replaced with fully saturated seawater by suction from a second syringe. The sample was injected into the measuring chamber of a Strathkelvin cell (MC 100) containing an IL 1302 oxygen electrode which was connected to a Strathkelvin oxygen meter (model 781). The oxygen tension (P_{O_2}) was recorded after 2 minutes, by which time the reading had stabilised. The oxygen tension of seawater within the respirometry chamber decreased as a function of the oxygen consumption rate, with the oxygen tension of the water measured at 30 minute intervals. No attempt was made to remove carbon dioxide from the system and therefore as oxygen was depleted, the carbon dioxide concentration rose. The oxygen meters were calibrated at the beginning and end of each experimental run using air-saturated seawater (at the same temperature and salinity as that used in the subject respirometer) and the respirometry chambers were thoroughly cleaned between each fish. The level of background oxygen consumption in blank respirometers was found to be negligible over the duration of the experiment at both temperatures. An oxygen-depleted, control respirometer revealed no leaks in the system.

The ventilation frequency of the fish was assessed immediately prior to taking each water sample by counting the number of opercular movements over the duration of a minute. Subjective assessment of the relative depth of ventilation was made by observing the magnitude of movement of the operculum cover. The experiment was terminated when a fish either lost equilibrium or ceased ventilation. A final measurement of the oxygen tension within the chamber was made and defined as the lethal P_{O_2}. The fish were then killed immediately by a sharp blow to the head and blood samples obtained by acute cardiac puncture. Approximately 0.5 mL samples were drawn into pre-heparinized (ammonium heparinate, Sigma Ltd.) hypodermic syringes using 25-gauge needles, with the entire sampling procedure completed within 30 seconds. In order to determine percentage haematocrit, an aliquot of whole blood was drawn into a capillary

tube, sealed at one end with haematocrit clay, and centrifuged at 20 000g for 90 seconds. The tubes were measured immediately upon removal from the centrifuge. A 5 μL sample of whole blood was frozen on liquid nitrogen in an Eppendorf tube for determination of haemoglobin concentration in Christchurch. The remaining blood was centrifuged (3000g for two minutes) and the plasma drawn off by pipette. The plasma (frozen in liquid nitrogen in an Eppendorf tube) was transported to Christchurch for assessment of osmolarity and glucose concentration. The fish were then blotted to remove any excess water, weighed and measured (from nose to tip of tail). All blood samples were kept in a liquid nitrogen dewar for up to two weeks at Scott Base before being transferred into a chilly bin of dry ice and transported by LC130 Hercules (8-hour flight) to New Zealand. At Canterbury University the samples were stored at -80°C for up to a month before analysis.

DATA ANALYSIS AND STATISTICAL METHODS

Haematology

Blood haemoglobin concentration, mean corpuscular haemoglobin content, plasma glucose concentration and osmolarity were determined using the methods detailed in Chapter Two.

Oxygen Consumption

After correction for meter drift, the rate of oxygen consumption in units of mg O_2 kg^{-1} h^{-1} was calculated from:

$$V_{O_2} = \frac{\Delta P_{O_2} \times C \times V \times 31.999}{t \times M}$$

where:

ΔP_{O_2} = change in oxygen partial pressure over the measurement period (mm Hg)

C = oxygen capacitance of seawater at a given temperature (μmol L^{-1} mm Hg^{-1})

V = volume of water in the respirometer (L)

31.999 = molecular weight of O_2

t = duration of measurement (h)

M = mass of the fish (g)

In order to determine the volume of water in the chambers, the fish were assumed to be neutrally buoyant. With buoyancy (% body weight in -1.9°C seawater) of *P. borchgrevinki* being equal to 2.75 (Eastman 1993), neutral buoyancy is a close approximation. The oxygen capacitance values used were 2.28 (-1°C) and 2.13 (3°C) μmol L^{-1} $mmHg^{-1}$.

The oxygen consumption values were corrected to 100 g mass using the following formula:

$$V_{O_2}(100) = V_{O_2} \times (M/100)^{(1-A)}$$

where:

$V_{O_2}(100)$ = O_2 consumption for a 100 g animal

V_{O_2} = O_2 consumption for an animal with mass M

M = mass of the animal (g)

A = mass exponent describing the relationship between metabolic rate and body mass (the mass exponent used was 0.80, see Chapter Four for justification).

The "normoxic" oxygen consumption rate of the fish at -1°C was determined from the difference between the second and third readings (water oxygen tension ~115-135 mm Hg), as V_{O_2} of the closely related *Trematomus bernacchii* has previously been shown to be stable over the water P_{O_2} range from 100-130 mm Hg (Davison 2001). Due to the fact that oxygen tension of the water was decreasing at a more rapid rate at 3°C, the "normoxic" oxygen consumption rate was determined between the first two oxygen tension readings (water oxygen tension ~90-110 mm Hg).

Critical Oxygen Tension

The critical oxygen tension (critical P_{O_2}) was defined as the ambient oxygen tension below which the rate of oxygen consumption sharply declined. The critical P_{O_2} was

identified for each fish individually, and a mean value calculated at each temperature. Ambient oxygen tension values at the beginning and end of each time period were averaged to give a mean water P_{O_2} for the interval.

Lethal Oxygen Tension

The definition of lethal oxygen tension (lethal P_{O_2}) adopted in this investigation was the ambient oxygen tension at which an animal lost equilibrium and/or ceased ventilation, as the loss of equilibrium is considered to be a precursor to death (see Plante et al. 1998).

Statistical Analysis

Data from the two hypoxic groups were compared using unpaired t-tests. Haematology of the hypoxic fish was compared with control data (from Chapter Two) using one-way analysis of variance (ANOVA). Where a treatment effect was indicated, inter-group differences were determined using the Tukey-Kramer post-hoc test. Statistical significance was taken at the level of $p<0.05$, and analyses were carried out using GraphPad Prism version 4.00 software. Data are presented as mean ± SEM, and all resting oxygen consumption rates have been corrected to 100 g mass.

RESULTS

There were no significant differences in mass, length or condition factor between the groups of fish subjected to progressive hypoxia at -1 and 3°C. The mean length and mass values of both hypoxic groups were, however, significantly greater than those of control fish (data from Chapter Two). The condition factor of the fish exposed to progressive hypoxia at 3°C was also significantly lower than that of control fish (Table 8.1).
One of the fish exposed to hypoxia at 3°C was removed from the data set due to the fact that it had transparent blood with a haematocrit of only 4%, and pale, blanched gills when it ceased ventilation at the relatively high ambient P_{O_2} of 53.8 mm Hg.

Haematology

The haematocrit of *P. borchgrevinki* was elevated above the control level by progressive hypoxia at -1°C, with a value of 28.1 ± 1.2% at the lethal P_{O_2}. Progressive hypoxia at 3°C resulted in a significantly greater elevation of haematocrit than that resulting from hypoxic stress alone, with a haematocrit of 32.2 ± 1.3% measured at the lethal P_{O_2}. Progressive hypoxia also had the effect of increasing blood haemoglobin concentrations above control levels at both -1 and 3°C, although levels at the higher temperature were not significantly different from those due to hypoxia alone. The mean corpuscular haemoglobin content (MCHC) of fish subjected to hypoxia at 3°C (176.6 ± 10.0 g L^{-1}), was significantly lower than values from both fish subjected to hypoxia at -1°C (209.0 ± 9.8 g L^{-1}) and control fish. The MCHC of hypoxic fish at -1°C was slightly lower than that of the control fish, although the difference was not significant. (Table 8.1, Fig. 8.1).

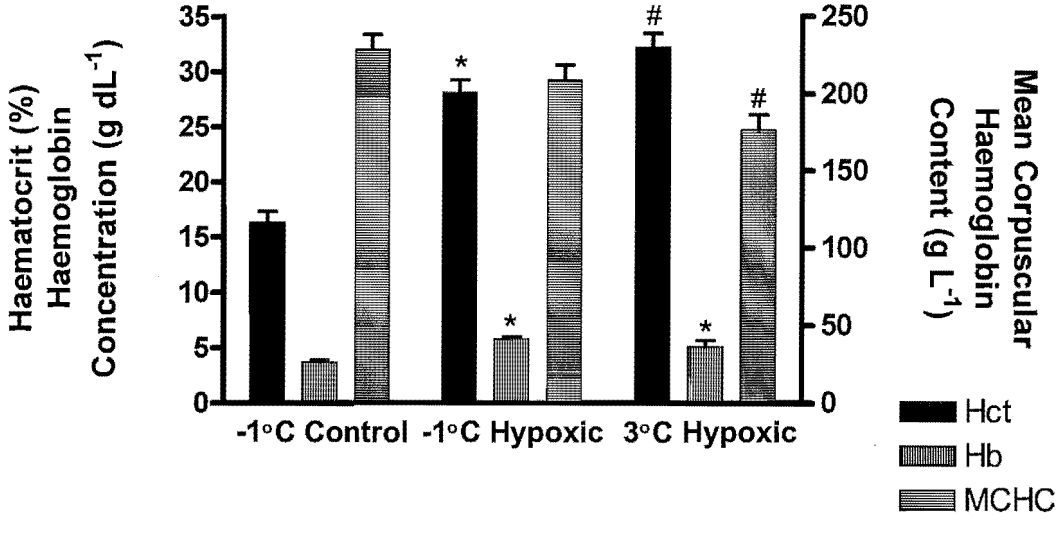


Fig. 8.1. Haematological parameters of *P. borchgrevinki* exposed to hypoxia at two different temperatures. Blood sampling was carried out at the lethal P_{O_2}. * Significantly different from control value. # Significantly different from both control and -1°C hypoxic values.

Plasma glucose levels were unchanged from control levels following a progressive hypoxic challenge at -1°C. Progressive hypoxia at 3°C, however, resulted in an increase in plasma glucose concentration to 7.4 ± 0.7 mmol L^{-1}, which was significantly higher than that of both hypoxic fish at -1°C, and control fish (Table 8.1).

Plasma osmolarity was unaffected by progressive hypoxia at -1°C, although the plasma osmolarity of fish exposed to progressive hypoxia at 3°C (611.7 ± 9.8 mOsm L^{-1}) was higher than values from both -1°C hypoxic and control fish. The difference was, however, only significant with regard to the control fish (Table 8.1).

Table 8.1. Effect of hypoxia on haematological parameters of *P. borchgrevinki* at two different temperatures.

Temperature (°C)	-1 (Control)	-1 (Hypoxic)	3 (Hypoxic)
Mass (g)	67.2 ± 5.7	87.7 ± 4.6*	87.5 ± 4.8*
Total Length (mm)	197.3 ± 4.8	220.7 ± 3.5*	224.8 ± 4.9*
Condition Factor	0.86 ± 0.03	0.81 ± 0.02	0.77 ± 0.02*
Haematocrit (%)	16.3 ± 1.0	28.1 ± 1.2*	32.2 ± 1.3#
Haemoglobin (g dL^{-1})	3.7 ± 0.2	5.8 ± 0.2*	5.1 ± 0.6*
MCHC (g L^{-1})	228.6 ± 9.8	209.0 ± 9.8	176.6 ± 10.0#
Glucose (mmol L^{-1})	4.6 ± 0.7	4.9 ± 0.5	7.4 ± 0.7#
Osmolarity (mOsm L^{-1})	574.0 ± 8.9	593.3 ± 9.3	611.7 ± 9.8*

Control values were obtained from Chapter Two of this thesis, N = 8. N = 10 for hypoxic fish at -1°C, N = 9 at 3°C. * Significantly different from control value. # Significantly different from both control and -1°C values. Values are means ± SEM.

Ventilation

The resting "normoxic" ventilation frequency of *P. borchgrevinki* was significantly higher at 3°C (32.0 ± 1.5 min^{-1} at an ambient P_{O_2} of 127.8 mm Hg), than at -1°C (16.1 ± 1.2 min^{-1} at an ambient P_{O_2} of 148.7 mm Hg) (Table 8.2).

At -1°C, the ventilation frequency increased with decreasing water P_{O_2} down to an ambient oxygen tension of about 60 mm Hg. Below this level, the ventilation

frequency of the fish decreased as the ambient oxygen tension declined. The mean ambient oxygen tension at which the fish either lost equilibrium or ceased ventilation (lethal P_{O_2}) was 20.4 ± 0.8 mm Hg (Fig. 8.2). The ventilatory movements of the fish were very shallow and barely discernable at high ambient oxygen tensions, but became noticeably deeper as water P_{O_2} decreased below about 80 mm Hg. Below approximately 40 mm Hg the opercular movements deepened considerably and the timing became irregular.

At 3°C, the ventilation frequency did not demonstrate the marked increase with declining in oxygen tension observed at -1°C. The ventilation frequency at the higher temperature was maintained at a relatively stable level down to an ambient P_{O_2} of approximately 70 mm Hg, below which the ventilation frequency decreased with decreasing water oxygen tension. The lethal P_{O_2} at 3°C (31.2 ± 3.3 mm Hg) was significantly higher than at -1°C (Fig. 8.3). The opercular movements of the fish were deeper at all ambient oxygen tensions than those observed at -1°C, and below an ambient P_{O_2} of about 50 mm Hg the movements became more laboured and erratic.

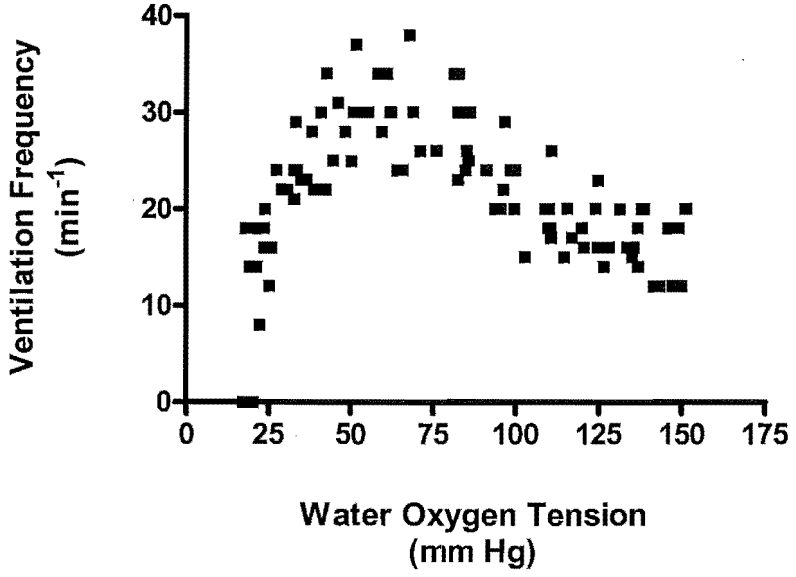


Fig. 8.2. Ventilation frequency of *P. borchgrevinki* during progressive hypoxia at -1°C. The data points represent individual values from 10 fish.

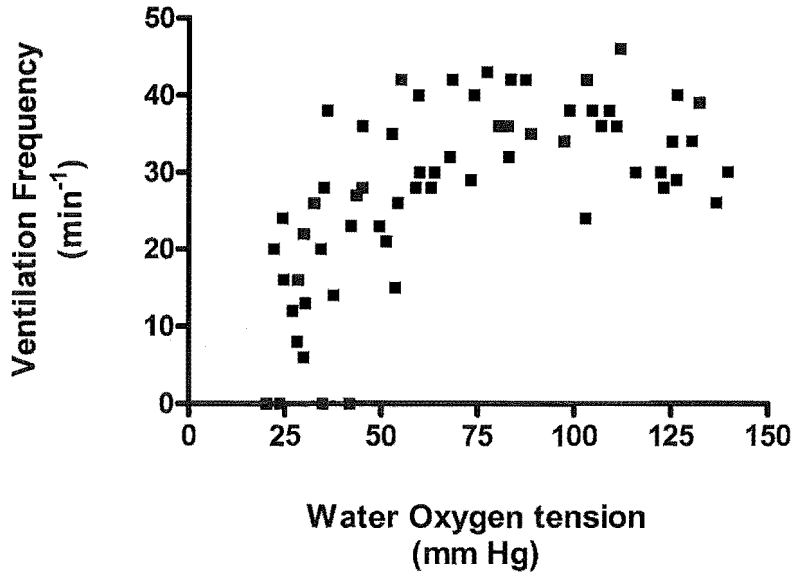


Fig. 8.3. Ventilation frequency of *P. borchgrevinki* during progressive hypoxia at 3°C. Data points represent values from 9 individual fish.

Oxygen Consumption

The resting "normoxic" oxygen consumption rate of *P. borchgrevinki* was significantly higher at 3°C, than at -1°C. Resting oxygen consumption rate of the fish at -1°C (water P_{O_2} = 148.7 mm Hg) was 27.6 ± 1.7 mg O_2 kg^{-1} h^{-1}, while the rate at 3°C (water P_{O_2} = 127.8 mm Hg) was 51.3 ± 4.4 mg O_2 kg^{-1} h^{-1} (Table 8.2).

The oxygen consumption rate of fish at -1°C increased initially as the ambient oxygen tension decreased from saturation levels to approximately 75 mm Hg. The rate of oxygen consumption then slowly declined with a further decrease in water P_{O_2} (Fig. 8.4).

The resting oxygen consumption of the fish at 3°C did not exhibit the initial increase observed at -1°C, but instead declined steadily as the ambient oxygen tension decreased below 90 mm Hg. The decline in oxygen consumption rate was more rapid at 3°C than at -1°C (Fig. 8.5).

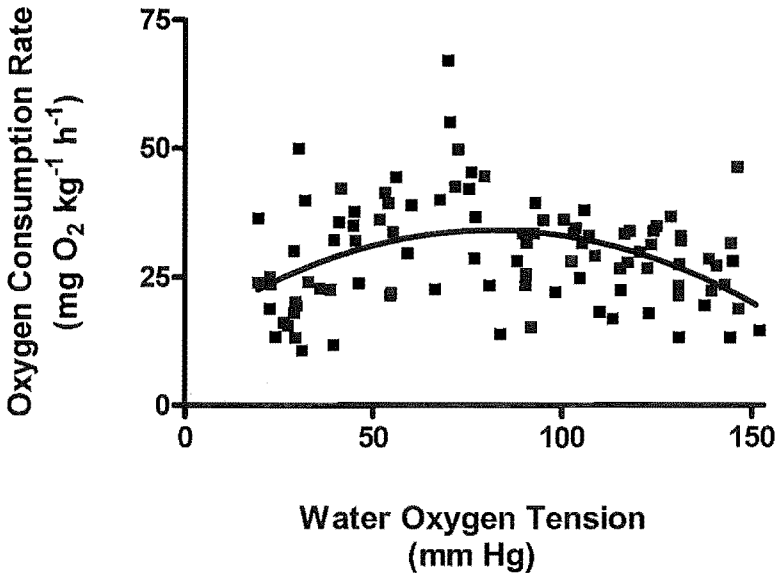


Fig. 8.4. Resting oxygen consumption rate of *P. borchgrevinki* during progressive hypoxia at -1°C. Data points represent values from 10 individual fish. The rate of decrease in ambient oxygen tension varied as a function of the oxygen consumption rate of individual fish. The equation of the "best-fit" second order polynomial for the data is:

$y = 14.31 + 0.487x - 0.003x^2$ ($r^2 = 0.15$).

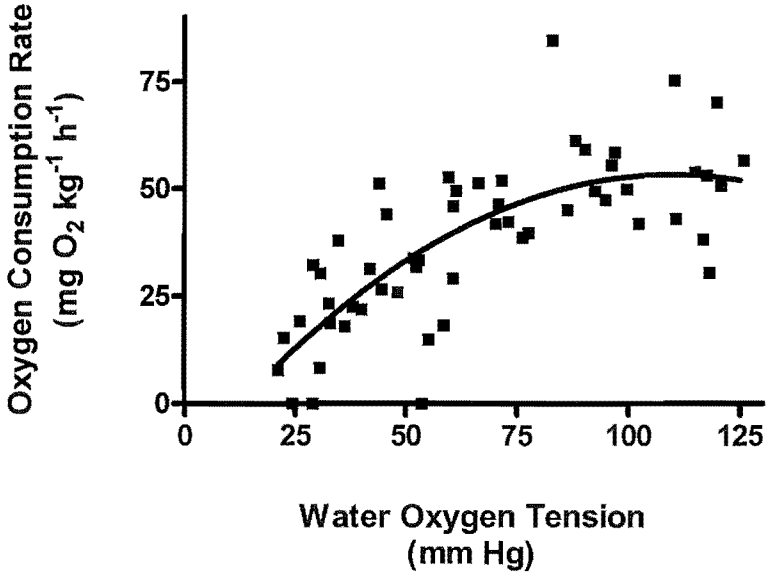


Fig. 8.5. Resting oxygen consumption rate of *P. borchgrevinki* in response to progressive hypoxia at 3°C. Data points represent values from 9 individual fish. The rate of decrease in ambient oxygen tension varied as a function of the oxygen consumption rate of individual fish. The equation of the "best-fit" second order polynomial for the data is:

$y = -14.44 + 1.236x - 0.006x^2$ ($r^2 = 0.57$).

The critical oxygen tension of the fish was significantly higher at 3°C than at -1°C, with mean values of 66.1 ± 2.4 mm Hg and 49.8 ± 2.2 mm Hg, respectively (Table 8.2). The critical oxygen tension was determined individually for each fish from a graph of oxygen consumption rate versus water oxygen tension, as the point below which resting oxygen consumption rate exhibited a sharp decline. As illustrated in Fig. 8.6, the critical P_{O_2} was more easily identified from the data of individual fish, than from graphs of the complete data sets (Fig. 8.4 and 8.5).

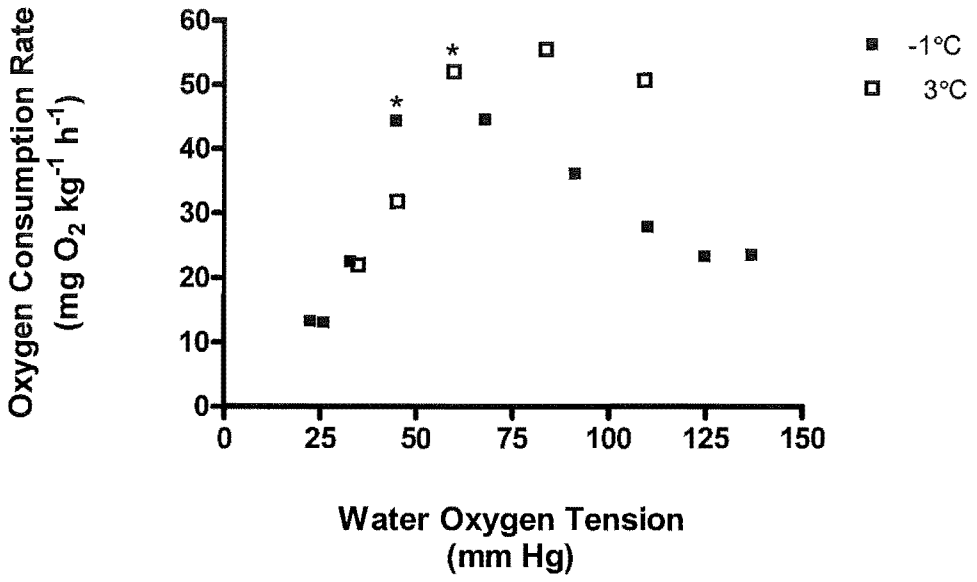


Fig 8.6. Typical oxygen consumption rates of two individual fish during progressive hypoxia at different temperatures.
* The points identified as representing critical oxygen tension.

Table 8.2. Metabolic and ventilatory parameters of *P. borchgrevinki* exposed to progressive hypoxia at two temperatures.

Temperature (°C)	-1	3
Initial Water P_{O_2} (mm Hg)	148.7 ± 2.5	127.8 ± 2.2*
Initial V_{O_2} (mg O_2 kg^{-1} h^{-1})	27.6 ± 1.7	51.3 ± 4.4*
Initial V_f (min^{-1})	16.1 ± 1.2	32.0 ± 1.5*
Critical Oxygen Tension (mm Hg)	49.8 ± 2.2	66.1 ± 2.4*
Lethal P_{O_2} (mm Hg)	20.4 ± 0.8	31.2 ± 3.3*

* Significantly different from value at -1°C. N = 10 at -1°C, N = 9 at 3°C.
P_{O_2} = oxygen tension, V_{O_2} = oxygen consumption rate, V_f = ventilation frequency.

The rate at which fish depleted oxygen from the water within the respirometry chambers was dependent on the metabolic rate, and therefore more rapid at 3°C than -1°C, as identified by a significantly steeper regression line at the higher temperature. At both temperatures the fish did not exhaust the oxygen supply to zero, but ceased aerobic respiration in the presence of measurable oxygen. As illustrated in Fig. 8.7, the minimum ambient oxygen tension at which the fish could still extract oxygen from the water was slightly higher at 3°C than at -1°C. Due to the difference in oxygen consumption rates at the two temperatures, fish at 3°C reached the lethal oxygen tension after 2.00 - 3.25 hours, while the fish at -1°C reached the lethal P_{O_2} after 3.67 - 5.75 hours.

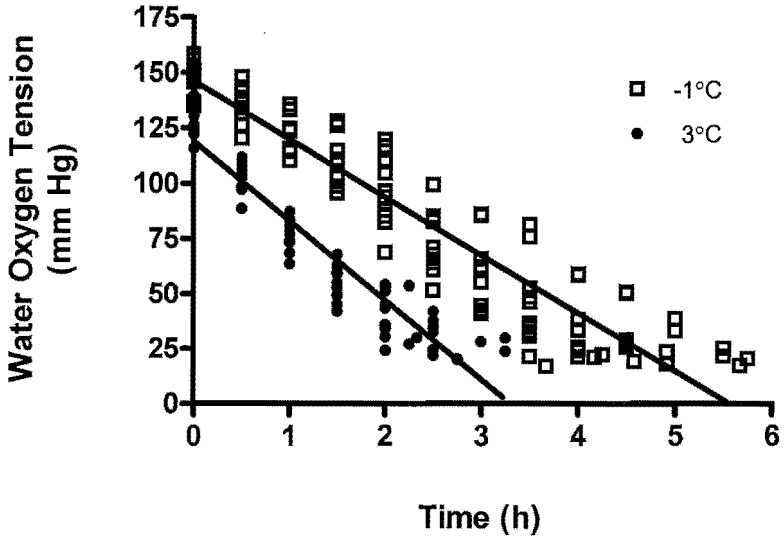


Fig. 8.7. Oxygen tension of water within the respirometry chamber as a function of time at two different temperatures. The equations for the regression lines at -1 and 3°C, respectively, are:
$y = -26.3x + 146.2$ ($r^2 = 0.89$), and $y = -36.0x + 119.0$ ($r^2 = 0.90$).

DISCUSSION

The results of this study indicate that *Pagothenia borchgrevinki* exhibits a marked haematological response to hypoxia, as has been previously observed in response to other stressors, such as exercise and increased temperature (Wells et al. 1984; Davison et al. 1988; Wells et al. 1989; Franklin et al. 1991; Davison et al. 1992; Davison et al. 1993; Franklin et al. 1993). The haematocrit following the hypoxic challenge at -1°C (28.1 ± 1.2%), compares well with the value of 27.8 ± 2.7% reported from the same species exposed to an oxygen tension of 60 mm Hg for 11-14 days, at a temperature of -1.5°C (Wells et al. 1989). The 72% increase in haematocrit resulting from exposure to progressive hypoxia at -1°C is, however, only a moderate increase when compared to the 110% increase following acute exposure to 10°C (Franklin et al. 1991), and the 110-136% increase resulting from exhaustive exercise (Davison et al. 1988; Franklin et al. 1993) in this species. The 98% increase in haematocrit of *P. borchgrevinki* in response to the combination of progressive hypoxia and acutely increased temperature was significantly higher than the response to hypoxia alone, and closer in magnitude to the maximum response. The haematological responses of the more sedentary, benthic nototheniid *Trematomus bernacchii* are reported to be less marked than those of the more active *P. borchgrevinki*, with a 66% increase in response to acute 10°C exposure, and a 31% increase following exhaustive exercise (Davison et al. 1994). The 67% increase in haematocrit of *T. bernacchii* in response to progressive hypoxia (Davison et al. 1994), however, is of similar magnitude to the 72% increase observed in *P. borchgrevinki* in the current study, indicating that the proposed lifestyle effect on the degree of stress-induced elevation of haematocrit (Egginton and Davison 1998) may not apply to hypoxic stress.

In other fishes, the effect of acute hypoxia on haematocrit appears to vary between species, and between different studies carried out on the same species. There are reports of both increased levels (Tetens and Lykkeboe 1981; Yamamoto et al. 1985; Boutilier et al. 1988; Peterson 1990; Wells and Weber 1990; Val et al. 1992), and of no detectable change (Bushnell et al. 1984; Murad et al. 1990; Perry and Reid 1994; Perry and Gilmour 1996). The marked stress-induced increase in haematocrit of Antarctic fish is considerably greater than changes observed in temperate and tropical fishes, and it has been suggested that, with low mean corpuscular haemoglobin contents in the Antarctic species (Wells et al. 1980), a greater increase in haematocrit may be required to achieve a

particular magnitude of increase in haemoglobin concentration (Franklin et al. 1993). The stress-related changes in haematocrit of Antarctic fish have been attributed to an increased requirement for oxygen transport to the tissues (Wells et al. 1984), and it would therefore be expected that hypoxia should elicit an increase. Antarctic fish maintain lower resting normoxic haematocrits than the majority of temperate species, presumably to lower blood viscosity at the subzero environmental temperatures (Macdonald and Wells 1991), and *T. bernacchii* are capable of normal function and sustained swimming for 10 minutes with haematocrit reduced to less than 2% (Wells et al. 1990), indicating that haemoglobin is less critical in a cold stable environment. The haemoglobin concentration of the blood of *P. borchgrevinki* increased in response to hypoxia, although there was no additional increase resulting from the elevated temperature. The 57% increase in haemoglobin concentration resulting from progressive hypoxia compares well with the 66% increase reported from this species following 11-14 days at an ambient oxygen tension of 60 mm Hg (Wells et al. 1989). Progressive hypoxic stress has also been demonstrated to elevate the blood haemoglobin concentration of *T. bernacchii*, although to a lesser extent (Davison et al. 1994). Nototheniid species tend to have lower baseline haemoglobin concentrations than temperate marine teleosts (Everson and Ralph 1968; Wells et al. 1990), and the results of the current study support the theory that that the primary role of haemoglobin may be to protect against environmental hypoxia (Holeton 1970; Holeton 1972; Holeton 1974). Increased haemoglobin concentration has been measured during hypoxia in a number of fish species (Wood and Johansen 1972; Swift and Lloyd 1974; Greaney and Powers 1978; Soivio et al. 1980), although not in all (Wood et al. 1975; Lykkeboe and Weber 1978; Jensen and Weber 1982; Hicks and McMahon 2002).

The large increases in haematocrit of *P. borchgrevinki* in response to hypoxia could be due to recruitment of erythrocytes from the spleen (Yamamoto 1987; Yamamoto and Itazawa 1989; Wells and Weber 1990), erythrocyte swelling (Nikinmaa 1983; Wells and Weber 1990), or due to haemoconcentration resulting from movement of water out of the plasma, as discussed in Chapter Two. Amitotic division of mature erythrocytes within the circulation has even been demonstrated in response to hypoxia in flounder (Soldatov 1996), although flounder do not have the large splenic reservoir of erythrocytes typical of Antarctic fish. Polycythaemia, resulting from the release of erythrocytes from the spleen, is the major cause of stress-induced elevation of haematocrit in notothenioids (Wells et al. 1989; Franklin et al. 1993). The spleen of *P.*

borchgrevinki is known to contract under the control of the autonomic nervous system, releasing its reservoir of erythrocytes into circulation and providing an extremely rapid response (Nilsson et al. 1996), and the spleens of hypoxic fish have been described as pale in colour and lighter in weight than those of normoxic fish (Wells et al. 1989). The elevation of haematocrit in response to acute hypoxia has also been attributed to splenic contraction in rainbow trout (Wells and Weber 1990) and yellowtail (Yamamoto et al. 1983). The stimulus for splenic contraction in *P. borchgrevinki*, however, appears to be neural and cholinergic rather than via circulating catecholamines as in the majority of teleosts (Kita and Itazawa 1990).

The oxygen transport capability of an organism is modulated, not only by quantitative changes in haemoglobin concentration, but also by qualitative changes in the intrinsic properties of the haemoglobin molecule (Jensen et al. 1998; Hochachka and Somero 2002). The responses of Antarctic notothenioids to stress appear to depend mainly on increases in haematocrit to increase oxygen carrying capacity, and, unlike temperate fish, are not thought to include major *in vivo* increases in oxygen affinity (Macdonald et al. 1988). Active pelagic fishes living in well-oxygenated waters tend to have oxygen equilibria favouring unloading of oxygen at the tissues, whereas sluggish fishes, and those in low-oxygen conditions tend to have equilibria favouring oxygen uptake at the gills (Grigg 1967; Raymond and De Vries 1976; Powers 1980; Wood 1980; Wells and Jokumsen 1982; Tetens et al. 1984; Macdonald et al. 1987; Hochachka and Somero 2002). Notothenioid haemoglobins have relatively low affinities for oxygen (Montgomery and Wells 1993), with the fish relying on the high environmental oxygen concentration to saturate their low affinity haemoglobins (Tetens et al. 1984). The low oxygen affinity, Bohr factor, and high oxygen carrying capacity of *P. borchgrevinki* blood (Grigg 1967; Wells and Jokumsen 1982; Tetens et al. 1984; Eastman 1993; Montgomery and Wells 1993) appears to be adaptive to its cryopelagic mode of life in well-oxygenated waters. The haemoglobin of *P. borchgrevinki* has been demonstrated to exhibit a Root effect (di Prisco et al. 1988), although in the absence of a target organ (choroid rete or swim bladder) (Herbert et al. 2003) this effect is non-adaptive and may not occur in whole blood (Montgomery and Wells 1993). The oxygen affinity of blood has been proposed as a factor limiting oxygen uptake at low ambient levels of oxygen (Grigg 1969a), and has been shown to be responsive to short-term changes in oxygen requirements in fishes (Grigg 1969b; Jensen and Weber 1985). The blood physiology of *P. borchgrevinki* was originally thought to suggest a poor capacity to regulate oxygen

affinity (Tetens et al. 1984), appropriate for a fish which may never experience hypoxia in its natural environment, although it has since been demonstrated that the oxygen affinity of *P. borchgrevinki* blood does increase following two weeks of exposure to an ambient P_{O_2} of 60 mm Hg (Wells et al. 1989). These authors reported an increase in the oxygen-carrying capacity of the blood of about 40%, which is similar to the response of temperate species to hypoxia. There has been no research carried out on the blood oxygen affinity of *P. borchgrevinki* exposed to hypoxia at higher temperatures, although the oxygen affinity decreases in response to increased temperature (Wells and Jokumsen 1982). With the slower reduction in ambient oxygen tension at -1°C in the current study, it is possible that fish at the lower temperature had a greater opportunity to acclimate haemoglobin-oxygen affinity to the hypoxic conditions than the 3°C group, thus improving their hypoxia tolerance.

During acclimation to hypoxic conditions in fish, there is generally a reduction in the erythrocytic nucleoside triphosphate (NTP) / haemoglobin ratio, resulting from decreases in levels of either adenosine triphosphate (ATP) (Tetens and Lykkeboe 1981), or guanosine triphosphate (GTP) (Wood and Johansen 1972; Weber and Lykkeboe 1978; Jensen and Weber 1982; Jensen and Weber 1985). This reduction has been reported within a few hours from the onset of hypoxia (Jensen and Weber 1985; Tetens and Lykkeboe 1985; Weber and Jensen 1988), although changes in some species occur over weeks (Greaney and Powers 1978; Soivio et al. 1980), and contributes towards an increase in the haemoglobin-oxygen affinity, both directly through allosteric interactions and indirectly via the Bohr effect (Wood and Johansen 1973). The majority of fish selectively reduce GTP in preference to ATP, as the effect of the former is reported to be greater on the oxygen affinity of fish haemoglobin than that of the latter (Weber and Lykkeboe 1978; Jensen and Weber 1982), although some hypoxia-tolerant species do not alter NTP levels at all, possibly due to the high normoxic oxygen binding and carrying capacities of their blood (Pichavant et al. 2003). Adenosine triphospate, however, forms the majority of the NTP pool in erythrocytes of trout, plaice, dogfish (Tetens and Christensen 1987; Jensen et al. 1998) and the erythrocytes of *P. borchgrevinki* are reported to contain predominantly ATP (Tetens et al. 1984; Lowe and Wells 1997). High erythrocyte concentrations of GTP are thought to be selected for in species which encounter large fluctuations in oxygen tension in their natural environment, such as carp, tench, eel and goldfish (Weber and Jensen 1988). A reduction in ATP levels has been shown to modulate oxygen affinity in response to hypoxia in *P. borchgrevinki* (Wells et

al. 1989), with one theory being that the storage environment within the spleen may be relatively hypoxic, resulting in the erythrocytes released from this reservoir having low levels of ATP (see Wells et al. 1989).

The most fundamental adaptation to reduced oxygen supply is variation in the haemoglobin structure, a primary determinant of haemoglobin function. Most vertebrates, including temperate fishes, have multiple forms of the haemoglobin molecule that often exhibit differences in oxygen-binding properties in response to different environmental and intrinsic factors (D'Avino and di Prisco 1988; di Prisco et al. 1988). Such polymorphism is essential in fish which change habitats during development or shift between different environments (Jensen et al. 1998), and Atlantic cod with different haemoglobin alleles have been found to exhibit different thermal preferences when exposed to hypoxia (Petersen and Steffensen 2003). The majority of notothenioids have one major and one minor haemoglobin which are functionally indistinguishable from one another (di Prisco et al. 1991; di Prisco et al. 1998), and it has been suggested that the relatively constant physicochemical conditions in the Southern Ocean may have reduced the need for multiple haemoglobins (Wells et al. 1980). *P. borchgrevinki*, however, possesses up to five functionally distinct haemoglobins (di Prisco et al. 1991; di Prisco and Giardina 1996), which may translate into a greater ability to acclimate to environmental change. Two other Antarctic nototheniids, *T. newnesi* and *Pleuragramma antarcticum*, both relatively active, pelagic species, exhibit haemoglobin multiplicity (di Prisco et al. 1991) indicating a probable link with lifestyle (di Prisco and Tamburrini 1992).

The lack of change in mean corpuscular haemoglobin content (MCHC) of *P. borchgrevinki* exposed to hypoxia at -1°C indicates that the increase in haematocrit was primarily due to an increase in erythrocyte numbers, in agreement with previous hypoxia studies involving this species (Wells et al. 1989; Franklin et al. 1993), and the closely-related *T. bernacchii* (Davison et al. 1994). These findings are, however, in contrast to reports of erythrocyte swelling as a hypoxic response in the phylogenetically ancient lampreys (Nikinmaa 2001), and in a variety of temperate-water teleost fishes (Lykkeboe and Weber 1978; Soivio and Nikinmaa 1981; Tetens and Lykkeboe 1981; Jensen and Weber 1982).

At 3°C, the decrease in mean corpuscular haemoglobin content of *P. borchgrevinki* during hypoxic exposure indicates a significant level of erythrocyte swelling. It has, however, been suggested that there may be a difference in volume

between erythrocytes in circulation and those released from storage in the spleen (Egginton and Davison 1998), which would complicate assessment of the relative contribution of cell swelling. *P. borchgrevinki* demonstrates some of the largest stress-related changes in erythrocyte volume of an Antarctic species (Egginton and Davison 1998), although only in response to severe stressors, such as acute exposure to 8-10°C (Franklin et al. 1991; Ryan 1995; Forster et al. 1998), and exhaustive exercise (Franklin et al. 1993; Lowe and Wells 1997). In temperate-water fishes, erythrocyte swelling is associated with an increase in the level of circulating catecholamines (De Vries and Ellory 1981; Nikinmaa 1982; Ling and Wells 1985; Fuchs and Albers 1988; Salama and Nikinmaa 1988), and this release of catecholamines into the bloodstream has been demonstrated in response to hypoxia (Butler et al. 1978; Tetens and Christensen 1987; Boutilier et al. 1988; Reid and Perry 1991; Perry and Reid 1994). The catecholamines stimulate β_1-adrenergic receptors on the erythrocyte membrane, resulting in the activation of cell surface Na^+/H^+ antiporters (Nikinmaa and Huestis 1984; Cossins and Richardson 1985) and an increase in intracellular Na^+ and Cl^- levels. Water moves in by osmosis and, along with swelling of the erythrocyte, NTP concentrations are diluted increasing the haemoglobin-oxygen affinity (Walsh et al. 1998), as discussed above. Antarctic nototheniids do not tend to release catecholamines into the blood stream in response to the majority of stressors (Davison et al. 1995), although the plasma catecholamine levels of severely heat-stressed *P. borchgrevinki* (Forster et al. 1998) are comparable to the highest levels recorded from hypoxic temperate-water fish (Thomas and Perry 1992; Franklin et al. 1993). Only a stressor of sufficiently severe nature will elicit release of catecholamines from the chromaffin tissue (Randall and Perry 1992), and it may be that Antarctic nototheniids have a higher threshold for catecholamine release than the temperate water species, possibly as an energy-saving mechanism. Treatment of temperature-stressed *P. borchgrevinki* erythrocytes with the β-adrenergic antagonist sotalol inhibits swelling, suggesting that the antiporter does respond to endogenous catecholamines (Forster et al. 1998). This has led the authors to conclude that, at least under extreme circumstances, the Na^+/H^+ exchanger may be activated *in vivo* in *P. borchgrevinki*, and the results of the current study indicate that hypoxic stress at elevated temperature may be a stressor of sufficient severity.

The plasma glucose concentration of *P. borchgrevinki* was unaffected by exposure to progressive hypoxic stress at -1°C. The results obtained from Chapter Two indicate that elevation of glucose levels in response to moderate stress may take at least

24 hours, possibly due to the relatively slow stress-induced increase in cortisol levels of this species (Ryan 1995). The fish exposed to progressive hypoxic stress at 3°C, however, increased plasma glucose levels by 61% at the critical oxygen tension. Increased plasma glucose levels have been reported as a response to severe hypoxia in a variety of fishes (Van Raaij et al. 1996; Zhou et al. 2000; Ishibashi et al. 2002; MacCormack et al. 2003), although the magnitude of the response does vary between species (Van Raaij et al. 1996). The hypoxia-induced hyperglycaemia is thought to be the result of hepatic glycolysis stimulated by circulating catecholamines in the majority of species (Mommsen et al. 1988; Sheridan 1988; Zhou et al. 2000), although it has also been suggested that glycolysis itself may increase the acidity of the blood thereby resulting in the liberation of catecholamines from the chromaffin tissue (Satchell 1991). Antarctic notothenioid fishes exhibit low activity levels of enzymes involved in the glycolytic pathway and a low anaerobic capability (Dunn and Johnston 1986; Johnston 1987; Davison et al. 1988) and the source of the hyperglycaemia has yet to be determined. With erythrocyte swelling also occurring in these hyperglycaemic fish, it is likely that an increase in circulating catecholamines is at least partially responsible for stimulating the increase in glucose levels.

Plasma osmolarity did not differ significantly from control levels in hypoxic *P. borchgrevinki* at -1°C, but at 3°C hypoxic fish were unable to maintain electrolyte balance and therefore plasma osmolarity increased by 7%. Functions of the gills, in addition to respiratory gas exchange, include ionoregulation and the associated functions of excretion, acid-base regulation and water balance (Taylor et al. 1997). As oxygen demand increases in line with an increase in temperature any adjustments likely to increase trans-epithelial oxygen flux (such as increased ventilation rate and/or depth) could compromise both ionoregulation and water balance, by increasing the functional permeability of the gills. Plasma osmolarity of this species was unchanged following acute exposure to 3°C under normoxic conditions, although a significant increase was observed after 12 hours at 6°C (Chapter Two). A 7.5% increase in osmolarity has been reported in *P. borchgrevinki* 30 minutes after a 10 minute exposure to 10°C (Franklin et al. 1991), although another study found no change in response to the same stressor (Forster et al. 1998). Marine teleosts, in general, become hyperosmotic in the face of severe stress due to increased branchial permeability resulting in water loss (Egginton 1997). The increase in osmolarity measured in the current study during the hypoxic challenge at 3°C could have been the result of osmotic efflux of water from the plasma

(haemoconcentration), increased diffusion at higher temperatures with osmoregulatory mechanisms unable to excrete the greater number of ions, or permeability changes resulting from activation of the primary stress response (release of corticosteroids and/or catecholamines into the blood stream). Taking into account the lack of osmotic response to an increase in temperature alone, it is unlikely that increased diffusion at the higher temperature levels was a significant factor, although it is possible that haemoconcentration may have contributed to both the increased osmolarity and large increase in haematocrit of these fish. As discussed above, the occurrence of both erythrocyte swelling and hyperglycaemia in hypoxic fish at 3°C indicates that this stressor may have been sufficiently severe to raise plasma catecholamine levels. Catecholamines have been shown to be released into the bloodstream at higher arterial oxygen tensions in trout at elevated temperatures (Perry and Reid 1994). Cortisol also has a direct influence on the responsiveness of red blood cells to catecholamines by increasing numbers of internalised β-adrenoceptors, particularly under conditions of chronic stress (Perry and Reid 1993). Upon exposure of fish red blood cells to hypoxia, these additional receptors are rapidly recruited to the surface and therefore cortisol may pre-adapt the teleost erythrocyte to receive additional physiological inputs (Perry and Reid 1993). Elevation of plasma cortisol levels in response to stress is delayed in *P. borchgrevinki* (Ryan 1995), but may still have had an effect over the 2-3 hour time-frame. As discussed in Chapter Two, elevation of plasma osmolarity is a component of the acute stress response in these Antarctic fish, with a decrease in plasma osmolarity following prolonged exposure (acclimation) to 4°C (Gonzalez-Cabrera et al. 1995; Guynn et al. 2002).

During the current investigation, the oxygen tension of venous blood from the heart of one hypoxic fish at -1°C was measured. The P_{O_2} of the blood was 2.8 mm Hg at the lethal water oxygen tension of 20.3 mm Hg, compared with venous oxygen tensions of 38 mm Hg (Tetens et al. 1984) to 43 mm Hg in normoxic *P. borchgrevinki* (Axelsson and Davison, unpubl. data.). The values relate well to the theory that an oxygen gradient of 20-30 mm Hg from water to blood is required in order to provide an adequate level of uptake (Saunders 1962). Cech et al. (1979) demonstrated a close correlation between the extent of reduction in inspired oxygen tension and the fall in arterial oxygen tension at a variety of temperatures, although more recent studies have failed to identify a correlation (Hughes et al. 1983; Glass et al. 1990). The exact nature of the breathing stimulus in fish exposed to hypoxia has not yet been conclusively identified, although the majority of

data appear to favour the hypothesis that blood oxygen content or delivery rate, rather than oxygen tension, is the decisive factor (Randall 1982; Smith and Jones 1982; Perry and Wood 1989; Randall 1990). A secondary pH or CO_2 drive has also been suggested (Perry and Wood 1989), along with a possible effect of non-arterial chemoceptors (Glass et al. 1990). Circulating catecholamines are also thought to stimulate ventilation under conditions in which they are released into the circulation (Randall and Taylor 1991; Gilmour 1998), although this view is not universally held (Perry et al. 1992).

The resting "normoxic" ventilation frequency of *P. borchgrevinki* at -1°C (16.1 ± 1.2 min^{-1}) is within the range of values reported in the literature (Tetens et al. 1984; Wells 1987; Forster et al. 1998; Davison 2001; Wilson et al. 2002). The ventilation rate from the current study is slightly, although not significantly, lower than the value reported in Chapter Four at 0°C (19.1 ± 1.7 min^{-1}), which may be a result of the 1°C difference in acclimation temperature between the studies. The resting ventilation frequency of *P. borchgrevinki* at 3°C was 32.0 ± 1.5 min^{-1}, which is double the rate of fish at -1°C, and significantly higher than the value obtained for the same species at 3°C in Chapter Four (23.7 ± 2.9 min^{-1}). The fish in the former chapter had been held in captivity at 0°C for at least three months prior to the experiments, while fish in the current study were caught from McMurdo Sound 3-4 days prior to the experiments and held at -1°C. This highlights the difference in thermal sensitivity between "fresh" and "captive" fish. There were significant differences in the ambient oxygen tensions at the two temperatures although it can be seen from Fig. 8.2 that the ventilation frequency of fish at -1°C was about 20 min^{-1} at a water P_{O_2} of 128 mm Hg, still considerably lower than that of fish at 3°C.

The most rapid response of oxygen-regulating species to hypoxia is to increase ventilation rate and/or volume (Saunders 1962; Holeton and Randall 1967; Kerstens et al. 1979; Lomholt and Johansen 1979; Hochachka 1980), and a close inverse relationship has been demonstrated between the ambient oxygen tension and the amount of water inspired by fish (see Holeton 1980). An increase in ventilation results in an increase in the volume of oxygen available at the respiratory surface, thereby enhancing diffusion (Hochachka 1980) and, provided that the tension of oxygen is adequate to saturate the blood, equilibrium can be maintained (Randall and Shelton 1963). In the current study, there was a marked increase in ventilation frequency of *P. borchgrevinki* in response to progressive hypoxia at -1°C, but the response was severely attenuated by an acute increase in temperature to 3°C. The gradual increase in ventilation frequency at -1°C as

ambient oxygen tension declined from about 150-60 mm Hg, compares well with the previous observation in this species of an increase in ventilation frequency in response to moderate hypoxia at its environmental temperature (Macdonald et al. 1988). The mean ambient oxygen tension at which the fish lost equilibrium and/or ceased ventilation (lethal P_{O_2}) at this temperature (20.4 ± 0.8 mm Hg) is slightly higher than the value of 14.6 ± 0.8 mm Hg determined using a similar protocol at -0.5°C (Davison et al. 1990), and slightly lower than the value of around 30 mm Hg reported for the benthic Antarctic notothenid *T. bernacchii* at 0.5°C (Davison 2001). Arctic charr at 2°C are reported to lose equilibrium at oxygen tensions of 22-28 mm Hg (Holeton 1973). The energy required by the respiratory apparatus makes up a sizeable portion of the total energy expenditure in teleosts (Johansen et al. 1967), with Hughes and Shelton (1962) estimating that ventilation utilises some 20-30% of standard metabolic rate. An increase in respiratory activity would therefore place a greater metabolic demand on an animal, and at some oxygen tension the amount of energy used to increase ventilation volume will be greater than the benefits derived (Saunders 1962; Hochachka 1980; Stecyk and Farrell 2002). Common carp, an anoxia tolerant species, exhibit a similar pattern of change in ventilatory frequency to that of *P. borchgrevinki*, with an initial increase in frequency as water P_{O_2} decreases, followed by a rapid decrease (Stecyk and Farrell 2002). The ventilation frequency of both freshwater and marine fishes in response to hypoxia appears to be variable and species-specific, with some exhibiting an initial increase as P_{O_2} begins to fall and others a constant rate, but the majority demonstrating a decrease at low ambient P_{O_2} levels (Marvin and Heath 1968; Crocker and Cech 2002; Stecyk and Farrell 2002). Fernandes and Rantin (1989), however, have shown that in one species of cichlid fish the ventilation frequency remains constant down to an ambient oxygen tension of 50 mm Hg, and then increases.

Changes in ventilatory stroke volume were not quantitatively assessed during this investigation, although observations were made of the extent of opercular movements. In normoxic fish at -1°C, the ventilatory movements were barely discernable. The deepening of opercular movements with decreasing ambient oxygen compares well with results from *T. bernacchii*, in which ventilation deepened as the water P_{O_2} decreased below about 70 mm Hg at 0.5°C (Davison 2001). Respiratory water flow is obviously able to be altered by changes in either the frequency or amplitude of ventilatory movements, and the majority of fish elevate tidal volume with essentially no change in

frequency (Hughes 1973; Holeton 1980; Randall 1982; Smith and Jones 1982; Perry and Wood 1989). From a metabolic point of view, a greater increase in ventilatory stroke volume in relation to frequency is thought to be the most economical way of increasing gill ventilation (Fernandes and Rantin 1989; Perry and Wood 1989). It has previously been suggested that both Antarctic icefish (Hemmingson et al. 1969; Hemmingson and Douglas 1970; Holeton 1972), and the red-blooded notothenioids (Hemmingson et al. 1969; Wells 1987) conform to this general pattern of modulation. There is, however, considerable variety in the means of increasing gill ventilation among fishes, with hypoxia shown to increase ventilation rate in Amazonian catfishes and stargazer, increase depth of ventilation in trout, increase both frequency and depth in eel and tench (Randall and Shelton 1963; MacCormack et al. 2003), and increase ventilatory depth but decrease ventilatory frequency to result in a net increase in the ventilatory volume in the dragonet (Hughes and Ballintijn 1968). The current study indicates that the severity of the hypoxic challenge and ambient temperature may also exert effects on the relative contribution of ventilatory depth and rate. Near environmental temperature and at low-moderate levels of hypoxia, it appears as though changes in ventilatory frequency may make the greatest contribution to the increase in gill ventilation of *P. borchgrevinki*. At lower ambient oxygen tension levels, the observed decrease in ventilation frequency may be accompanied by an increase in stroke volume, although this remains to be quantitatively determined. Increases in ventilation rate and/or stroke volume cannot, however, be assumed to relate to a similar magnitude of increase in available oxygen, as the percentage utilisation of oxygen generally decreases as flow across the gills is increased (Saunders 1962; Holeton and Randall 1967; Hughes and Saunders 1970). This effect has not been demonstrated in flatfishes or carp (Lomholt and Johansen 1979; Steffensen et al. 1982), suggesting that fishes which are more hypoxia tolerant may possess a higher capacity for increasing gill ventilation while maintaining extraction at a constant level (Steffensen et al. 1982). The result may also depend upon the degree of hypoxic challenge, with gill oxygen extraction of cichlids remaining constant at moderate hypoxia (80 mm Hg), but decreasing in severe hypoxia (Fernandes and Rantin 1989).

In addition to the previously discussed alterations in blood oxygen carrying capacity, oxygen affinity, and gill ventilation, fish have the potential to enhance oxygen extraction ability through changes in gill surface area (Jensen et al. 1993; Chapman et al. 1999). Fish do not perfuse 100% of the secondary gill lamellae at rest, in fact trout perfuse no more than 60% (Booth 1979), and it is thought that the patterns of gill

perfusion may be actively regulated (Nilsson and Pettersson 1981), giving fish the opportunity to increase the number of lamellae perfused during hypoxia (Taylor et al. 1997) and allowing for an increase in oxygen uptake without a corresponding change in gill ventilation. The fact that *P. borchgrevinki* affected by a disease which reduces the gill surface area (X-cell disease), exhibit a corresponding increase in critical P_{O_2} (Davison et al. 1990), suggests that changes in gill surface area may be an important factor in the hypoxia tolerance of this species.

At 3°C, the effect of progressive hypoxia on the ventilatory frequency of *P. borchgrevinki* was very different from that observed at -1°C and the lethal P_{O_2} was significantly higher. The fish did not increase ventilation frequency with decreasing oxygen tension, but did maintain ventilation frequency at or slightly above normoxic levels down to an oxygen tension of about 70 mm Hg. It is possible that the greater rate of decline in ambient oxygen tension at the higher temperature may have reduced the ability of fish to acclimate, and therefore have contributed to the higher lethal oxygen tension. It is, however, more likely that the increase in lethal P_{O_2} was due to an increased rate of oxygen consumption, decreased solubility of oxygen in the water, and decrease in oxygen extraction efficiency (Fernandes and Rantin 1989) at 3°C. An increase in lethal P_{O_2} has previously been described as resulting from increased temperature in Atlantic cod (Schurmann and Steffensen 1992; Steffensen et al. 1994), although not by all investigators (Plante et al. 1998). The deeper opercular movements of fish at 3°C indicate that there may be a greater contribution of ventilatory stroke volume to gill ventilation at higher temperatures. Ventilation frequencies of up to 70 min^{-1} (Forster et al. 1987) have been observed in response to exercise at 0°C in this species, although the maximum ventilation frequency attained by *P. borchgrevinki* at critical swimming speed at 3°C was 46.0 ± 4.0 min^{-1} (Chapter Four), and in a previous study the maximum ventilation frequency of the same species was about 44 min^{-1} at 4°C (Wilson et al. 2002), both of which are similar in magnitude to the maximum rate achieved under hypoxic conditions at 3°C. There may therefore be an effect of temperature on either the maximum rate or method of modulation, although further investigation is required to identify any patterns. Increases in temperature are accepted to be a major factor influencing the ventilation of fishes, although both frequency and amplitude have been reported to increase, with no general trend having been identified (Nikinmaa 2002; Stecyk and Farrell 2002). With increasing ventilatory stroke volume proposed to be the most economical way of

increasing gill ventilation (Fernandes and Rantin 1989; Perry and Wood 1989), it may be that efficiency gains a greater importance under the added stress of increased temperature. Bluegill sunfish increase ventilation frequency with hypoxia at lower temperatures, but not at higher temperatures (Spitzer et al. 1969) while, in contrast, adjustment of stroke volume contributes more to increases in gill ventilation of carp at low temperatures, with domination of the frequency response at higher temperatures (Glass et al. 1990).

The resting "normoxic" oxygen consumption rate of *P. borchgrevinki* at -1°C (27.6 ± 1.7 mg O_2 kg^{-1} h^{-1}) falls at the lower end of values previously reported for this species (Wohlschlag 1964; Forster et al. 1987; Wells 1987; Wilson et al. 2002). As mentioned in Chapter Four, some of the early values are likely to have been elevated by handling stress. The slight, although non-significant difference between the value from this study and that obtained from 0°C-acclimated fish in Chapter Four (32.8 ± 1.8 mg O_2 kg^{-1} h^{-1}) may be due to the 1°C difference in acclimation temperature, and relates well to the slightly lower ventilation frequency in the current investigation. The resting "normoxic" oxygen consumption rate measured in this study at 3°C was significantly higher than the value of 36.4 ± 2.1 mg O_2 kg^{-1} h^{-1} measured at the same temperature in Chapter Four. The Q_{10} values for the change in ventilation frequency (Q_{10} = 4.7) and the change in resting oxygen consumption rate (Q_{10} = 5.6) were, however, of similar magnitude over the temperature range from -1 to 3°C. As mentioned in the discussion of ventilation rates above, the greater thermal sensitivity observed in the current experiment is likely to be due to the difference between freshly caught fish and those acclimated to a temperature almost 2°C above environmental levels for at least three months, which highlights the fact that differences in acclimation temperature of only 1-2°C may have marked effects on thermal sensitivity and tolerance of these fish.

Oxygen consumption rates are elevated for several hours following the handling stress of transfer into the respirometry chambers although, as detailed in Chapter Four, stable and minimal values for Antarctic notothenioids are reported to be reached after 2-12 hours (Morris and North 1984; Wells et al. 1984; Wells 1987; Johnston et al. 1991). The 24-hour settling period employed in the current study should therefore have been sufficient to minimise any handling effects. The fish used in this investigation had only been in captivity for three days, and would therefore have still been within the period of their SDA (Johnston and Battram 1993; Boyce and Clarke 1997; Ware 1999; Boyce et al.

2000; Brodeur et al. 2002), although the low oxygen consumption rates indicate that the additional energy cost associated with the processing of food was minimal.

Closed-box respirometry is not the perfect method for assessing the effect of progressive hypoxia on resting oxygen consumption, as carbon dioxide tensions increase as the oxygen is depleted. In the Antarctic field setting, it was, however, the most practical option. In Atlantic cod, of a similar size to the *P. borchgrevinki* involved in this study, CO_2 levels were found to increase by 4-5 mm Hg in 1.41 L respirometry chambers during progressive hypoxia (Steffensen et al. 1994), and in the natural environment, due to the inherent reciprocity of environmental oxygen and carbon dioxide changes, hypoxia will invariably be associated with a degree of hypercapnia (Nikinmaa and Salama 1998). Hypercapnia is reported to have no effect on the oxygen consumption rate of tench (see Jensen and Weber 1985), and is not a strong stimulator of respiration in fish (Dejours 1975). A build-up of metabolic wastes and carbon dioxide in respirometry chambers has, however, previously been shown to reduce the affinity of haemoglobin for oxygen in fish blood (Saunders 1962).

The increase in oxygen consumption rate (V_{O_2}) of *P. borchgrevinki* as ambient P_{O_2} decreased to about 75 mm Hg, is in line with the theory of Hughes and Shelton (1962) who stated that oxygen consumption of a resting animal should increase as the oxygen content of the medium is reduced, due to the greater effort involved in adequately ventilating the gills. Below 75 mm Hg, the rate of oxygen consumption was maintained at a relatively constant level down to the critical oxygen tension (49.8 ± 2.2 mm Hg), at which point the rate began to rapidly decline. Most, if not all, teleost fishes are designed to cope with fluctuations in the oxygen saturation of their environmental media and maintain a constant rate, despite decreases in ambient oxygen tension. They are thus referred to as oxygen regulators (Hughes 1973; Rantin and Johansen 1984). With decreasing ambient oxygen saturation, however, a level (critical oxygen tension) is reached at which the standard metabolic rate can no longer be maintained (Hemmingson and Douglas 1970). At this point, organisms move from being oxygen regulators to oxygen conformers, and the rate of oxygen consumption decreases linearly with decreasing oxygen saturation. The observed depression of respiratory metabolism at ambient oxygen tensions below the critical level is thought to be the result of an insufficient supply of oxygen to meet the resting demands (Cech et al. 1979). It is, however, important to bear in mind that the oxygen uptake of an animal summarizes a

whole range of metabolic and other activities of an animal. Different processes may well have differing critical oxygen tensions, with changes in oxygen consumption reflecting the extent to which some combination of these are affected by ambient oxygen (Hughes et al. 1983). Unfortunately, there is often no clear-cut point at which the change from regulation to conformation occurs, and the critical oxygen tension cannot always be sharply defined (Dejours 1975). In the current study, the critical oxygen tension was found to be more readily determined from individual fish data than from the entire data sets. Difficulties were encountered in relating the results of the current investigation to findings reported in the literature, in that there is considerable flexibility in the definition of the term 'critical oxygen tension'. In order to limit comparisons to those which are valid, only studies which employed a similar protocol to the current investigation have been included. The critical oxygen tension determined for *P. borchgrevinki* at -1°C in the current study, lies between previous values from this species which vary from 30 to 60 mm Hg at -0.5 to 0°C (Forster et al. 1987; Macdonald et al. 1988; Davison et al. 1990). Various species of the genus *Notothenia*, have been shown to lower environmental P_{O_2} to levels of around 20 mm Hg, with only a slight decline in the resting oxygen consumption rate (Hemmingson et al. 1969; Holeton 1970), and haemoglobin-less Antarctic icefish have been reported to regulate oxygen uptake down to ambient oxygen tensions of 30-50 mm Hg (Hemmingson et al. 1969; Hemmingson and Douglas 1970) at environmental temperatures. The low metabolic rates of these Antarctic species may contribute to the hypoxia tolerance at low temperatures. *T. pennellii* (formerly *T. centronotus*), an inactive benthic Antarctic nototheniid appears, however, to be an oxyconformer, with no identifiable critical oxygen tension (Macdonald et al. 1988). The metabolic responses of fish to hypoxia are as highly variable and species-specific as the ventilatory responses (Marvin and Heath 1968; Cech et al. 1990), although a critical P_{O_2}, below which oxygen consumption rate declines, has been observed in the majority of species (Steffensen et al. 1994; Claireaux and Lagardere 1999).

A few fish in the current study exhibited a sharp increase in V_{O_2} as ambient oxygen tension approached the lethal limit at -1°C, possibly due to agitation and escape responses. As a generalisation, hypoxia tends to decrease spontaneous activity in fish (Nilsson et al. 1993; Schurmann and Steffensen 1994), whereas increased temperature tends to increase it (Peterson and Anderson 1969; Stevens and Fry 1972), although this pattern does not appear to apply when the stressors are extremely severe. Wells (1987)

monitored the response of spinalectomized *P. borchgrevinki* to decreasing oxygen tension, and identified a critical oxygen tension of approximately 60 mm Hg, suggesting that the critical oxygen tension determined in the current study was not unduly elevated by the movements.

The effect of progressive hypoxia on the resting oxygen consumption rate of *P. borchgrevinki* at 3°C differed considerably from the response at -1°C. At the elevated temperature, there was a much less marked increase in oxygen consumption rate as the ambient P_{O_2} began to decrease, and a more prolonged period during which oxygen consumption rate of the fish conformed to the decline in ambient oxygen tensions. The critical oxygen tension at which the rate of oxygen consumption began to steeply decline (66.1 ± 2.4 mm Hg) was significantly higher than at -1°C. The ability of the gills to extract oxygen from water at low ambient oxygen tensions has also been shown to be impaired by chronic exposure to the soluble fraction of fuel oil (Davison et al. 1993), indicating that the increase in critical P_{O_2} observed at 3°C may be due to the increased stress load on the fish, rather than a temperature-specific response. This is supported by the finding that the critical oxygen tension increases with any factor which increases the oxygen demand of fish (Claireaux and Lagardere 1999). Given the increase in metabolic rate, decrease in oxygen solubility, and decrease in extraction efficiency (Fernandes and Rantin 1989), it would be expected that both critical and lethal oxygen tensions should increase with a rise in temperature. The higher ventilatory volume needed to maintain resting metabolic rate at the higher temperatures is thought to explain the observation that critical oxygen tension increases with temperature (Schurmann and Steffensen 1997), and to be the limiting factor under hypoxic stress (Hughes and Shelton 1962). An inverse relationship between critical oxygen tension and temperature has been demonstrated in the great majority of species (Fry and Hart 1948; Beamish 1964; Spitzer et al. 1969; Butler and Taylor 1975; Cech et al. 1979; Fernandes and Rantin 1989; Cech et al. 1990; Schurmann and Steffensen 1997), with a couple of exceptions (Ultsch et al. 1978; Ott et al. 1980). The stress of hypoxia has also been demonstrated to lead to a dramatic decrease in preferred temperature in a variety of species (Bryan et al. 1984; Schurmann et al. 1991; Wood 1991; Schurmann and Steffensen 1992; Petersen and Steffensen 2003).

With the exact value of the critical oxygen tension often difficult to determine, it has been suggested that the quadratic or second-degree polynomial could be used to

describe the relationship between oxygen uptake rate and ambient oxygen level (Magnum and Van Winkle 1973). This avoids having to force the complex relationship into a simple regulator/conformer dichotomy. The most informative quantity in the quadratic equation ($y = A + Bx + Cx^2$) is the coefficient *C*. When *C* has a negative value, the curve departs from a straight line in the direction taken by a regulatory response, and the greater the departure from zero, the greater the degree of regulation (Magnum and Van Winkle 1973). This method of analysis has been used previously to compare the degree of oxyregulation between Antarctic nototheniid species (Wells 1987). In the current study, the value of the coefficient *C* for the relationship between oxygen uptake and ambient P_{O_2} at 3°C was -0.006, with a value of -0.003 at -1°C. This suggests a greater degree of regulation at 3°C, which does not appear to be the case from observation of the graphs, or comparison of the critical P_{O_2} values. The apparently contradictory result may be due to the fact that the -1°C data was not well represented by a second order polynomial ($r^2 = 0.15$). Wells (1987) also found a discrepancy between the lowest ambient oxygen tension at which oxygen was able to be extracted, and the degree of oxyregulation indicated by the polynomial model, when comparing *T. pennellii* and *P. borchgrevinki*. The second-degree polynomial cannot therefore be assumed to provide a good representation of all oxygen regulation / conformation relationships in fish.

There are a variety of factors which can affect the critical oxygen tension of fish, including other energy-utilising processes. The energy requirements of digesting food have been found to contribute to an elevation of critical P_{O_2}, with regurgitation of food observed in recently fed cod near their critical oxygen tension, presumably in order to reduce immediate oxygen requirements (Claireaux et al. 2000). Decreased food consumption has also been observed under hypoxic conditions (Chabot and Dutil 1999; Zhou et al. 2000). There was no regurgitation observed in the current study, although, with the fish still within the period of their SDA, it is possible that the energy requirements of digestion may have had an effect on the critical oxygen tension.

A relationship has previously been identified between mass and critical oxygen tension of *P. borchgrevinki*, with larger fish exhibiting a higher critical P_{O_2} (Davison et al. 1990). This may be due to the lower mass-specific metabolism of large fish (Targett 1978), although size has been shown to have no effect on the hypoxia tolerance of Atlantic cod (Plante et al. 1998). A positive correlation has also been identified between

hypoxia tolerance and condition factor in killifish (Love and Rees 2002), possibly due to fish with a higher condition factor having better nutritional status, and therefore a greater quantity of endogenous substrates (glycogen) to fuel anaerobic metabolism (Hochachka 1980). In the current study, there was no significant relationship between lethal oxygen tension and condition factor or mass, within either of the hypoxic groups, although the detection of an effect may have been limited by the narrow size range within the groups. The condition factor of hypoxic fish at 3°C was lower than that of both control fish and hypoxic fish at -1°C. Without measurements taken prior to the experiment, it is not possible to determine whether the difference was present initially or was due to water loss from the severely stressed fish at 3°C.

Another possible complicating factor is that the P_{O_2} of water inspired by the fish may actually be lower than that measured in the chamber, due to mixing of inspired and expired water (Hughes et al. 1983). Measurements in carp, comparing inspired water with the chamber water, have indicated that expired water may re-enter the buccal cavity either by a kind of reverse flow through the gills, or as a result of the fish re-breathing some expired water (Hughes et al. 1983). It is therefore possible that the critical oxygen tensions may have been slightly over-estimated as a result of not measuring the oxygen content of water actually inspired by the fish. *P. borchgrevinki* tend to make occasional sweeping movements of the pectoral fins while at rest which may assist with mixing of water adjacent to the gills (Wells 1987).

At both temperatures, *P. borchgrevinki* were silvery white after being confined overnight in the respirometry chambers. At P_{O_2} values near the critical oxygen tension, however, the fish began to darken in colour and were dark grey by the time they lost equilibrium. This response has also been reported in several other nototheniid species subjected to high and low oxygen levels (Fanta et al. 1989), as well as in response to other stressors (Franklin et al. 1991; Davison et al. 1992; Ryan 1995). Melanocytes are normally under both hormonal and neural control but the mechanism stimulating them in response to stress has not yet been explored.

In conclusion, acute elevation of temperature does have a significant effect on the hypoxia tolerance of *Pagothenia borchgrevinki*. Because the integrated stress response comprises many non-specific elements, most notably the drain of metabolic energy, many effects of stressors are additive (Wendelaar Bonga 1997), and in this study both critical and lethal oxygen tensions were elevated by acute exposure to a temperature of

3°C. From the haematological responses, it is apparent that the combination of increased temperature and hypoxia is a far greater stressor than hypoxic challenge alone, which is in agreement with the theory that the greatest challenges to physiological systems occur when a system already compromised by one variable is challenged by another (Reid et al. 1997). *P. borchgrevinki*, at temperatures near their environmental level, tolerate hypoxia to low ambient oxygen levels, in support of the theory that hypoxia tolerance is a feature of all teleosts rather than restricted to those which experience hypoxic conditions in their natural environment. Further support for this general nature of hypoxia tolerance, is provided by the fact that both the relatively active *P. borchgrevinki* and the more sedentary *T. bernacchii* exhibit similar responses to hypoxia, in contrast to their differing responses to a variety of other stressors. This study details the effects of an acute temperature change on the hypoxia tolerance of *P. borchgrevinki*. Hypoxia tolerance is, however, reported to vary with thermal acclimatisation (Love and Rees 2002) and so it is possible that prolonged exposure to 3°C may lead to a reduction in the critical oxygen tension in line with the acclimatory changes in haematology, swimming performance and cardiac performance observed in this species.

Chapter Nine

General Conclusions

In contrast to the "general adaptation syndrome" proposed by Selye (1936) to encompass the universal and non-specific reactions of organisms to stress, the physiological responses of Antarctic nototheniids to increased temperature were found to vary qualitatively and quantitatively both between species and within species, depending upon the severity and duration of thermal change.

In the majority of fishes, the main primary stress response to any stressor of moderate to high severity is the release of catecholamines into the bloodstream (Randall and Perry 1992) and the majority of secondary stress responses are attributed to the effects of these circulating hormones (Wendelaar Bonga 1997). The release of catecholamines is controlled predominantly by nervous stimulation of the chromaffin tissue (Nilsson 1994), with the oxygen content of the blood also having an indirect controlling effect (Perry and Reid 1994). Although plasma catecholamine concentrations were not quantified in the current study, it is possible to make some hypotheses about their presence/absence from the secondary stress responses. For example, erythrocyte swelling is commonly associated with and attributed to increased levels of circulating catecholamines in most fish (De Vries and Ellory 1981; Nikinmaa 1982; Ling and Wells 1985; Salama and Nikinmaa 1988; Nikinmaa and Tufts 1989; Randall and Perry 1992; Thomas and Perry 1992), including *P. borchgrevinki* (Forster et al. 1998). The absence of changes in the mean corpuscular haemoglobin content (MCHC) of *P. borchgrevinki* in response to hypoxia at near environmental temperature, and of both *P. borchgrevinki* and *T. bernacchii* during acute exposure to 3 or 6°C, but a decrease in MCHC in the blood of *P. borchgrevinki* following hypoxia at 3°C, relates well to previous findings which indicate that plasma catecholamine concentrations of sedentary notothenioids do not increase in response to moderate levels of stress (Davison et al. 1995; Egginton 1997),

but that catecholamine levels of both *T. bernacchii* and *P. borchgrevinki* can be increased by extremely severe stressors, such as acute 10°C exposure (Forster et al. 1998). With acute exposure to 6°C, a temperature very near the upper lethal limit of -1.86°C-acclimated Antarctic nototheniids (Somero and De Vries 1967), eliciting no change in mean corpuscular haemoglobin content, the threshold for the release of catecholamines appears to be very high in these fish. Whether this represents an energy saving mechanism at the low temperatures or is the result of a move towards other, possibly cholinergic, mechanisms of physiological control (Egginton 1996) is unknown. Other sedentary species exhibit relatively high thresholds for catecholamine release (Vijayan and Moon 1994), and it is possible that this may be a trait retained from the benthic notothenioid ancestor. Catecholamines are released in higher quantities by *P. borchgrevinki* during severe stress (Forster et al. 1998) and with erythrocyte swelling detected in this species in response to repeated burst swimming, in contrast to erythrocyte shrinkage of *T. bernacchii* subjected to the same protocol (Lowe and Wells 1997), the evolutionary move away from the benthic habitat may be associated with a slight lowering of the threshold for catecholamine release.

During acute exposure to elevated temperatures (3 and 6°C) there was a rapid increase in the haematocrit of *P. borchgrevinki*, accompanied by a smaller, and not statistically significant, increase in haemoglobin concentration. In the absence of erythrocyte swelling, these increases and the elevation of haematocrit following progressive hypoxia at -1°C are likely to be mainly due to polycythaemia. The release of erythrocytes from the spleen is reported to be the major cause of the stress-induced elevation of haematocrit in Antarctic nototheniids (Wells et al. 1989; Franklin et al. 1993), with the stimulus being neural and cholinergic, rather than the result of circulating catecholamines as in the majority of teleosts (Kita and Itazawa 1990; Nilsson et al. 1996). There was, however, no change in haematocrit of *T. bernacchii* in response to the same thermal stressors. *T. bernacchii* can increase haematocrit in response to acute 10°C exposure (Davison et al. 1994), and enforced continuous swimming (Macdonald and Wells 1991), but the threshold for a stress-induced rise in haematocrit appears to be higher in *T. bernacchii*, possibly another energy-conservation measure related to the benthic lifestyle.

In contrast to the relatively greater increases in haematocrit of *P. borchgrevinki* in response to temperature, exercise and handling stress (Davison et al. 1988; Franklin et al. 1991; Franklin et al. 1993; Davison et al. 1994), progressive hypoxia elicited a similar

percentage increase in *P. borchgrevinki* to that reported from *T. bernacchii* (Davison et al. 1994). This corroborates the idea that the physiological response to low ambient oxygen tension is a generalised feature of all teleosts, rather than one restricted to those which frequently encounter hypoxic conditions in their natural environment (Wells et al. 1989). The demonstration in this study and others (Forster et al. 1987; Macdonald et al. 1988; Davison et al. 1990) that *P. borchgrevinki* can tolerate hypoxia to low ambient oxygen tensions at temperatures within the thermal range encountered in the wild, adds further support to the theory. Rapid changes in temperature, however, augment the response to other stressors (Barton and Iwama 1991; Pickering 1992; Wendelaar Bonga 1997), and this was demonstrated by the decrease in hypoxia tolerance of *P. borchgrevinki* acutely exposed to 3°C.

A rapid increase in plasma glucose concentrations is another secondary stress response attributed to an increase in the levels of circulating catecholamines in most fishes (Vijayan and Moon 1992; Pottinger et al. 2000). The lack of an immediate change in glucose levels during acute exposure to 3 and 6°C is thus another indication that these stressors were below the threshold for catecholamine release of the Antarctic nototheniids. The delayed increase in plasma glucose concentration of both Antarctic nototheniids in response to thermal stress raises the possibility of an influence of cortisol. While adrenaline is released into the circulation very quickly, the release of cortisol is much slower and its effects are likely to be more prolonged (Gamperl et al. 1994; Waring et al. 1996). In *P. borchgrevinki*, the release of cortisol is even slower than in temperate-water teleosts, probably due to a less sensitive HPI axis (Ryan 1995). Cortisol levels during 5°C exposure were elevated above baseline levels after 3 hours and remained elevated for at least 48 hours which relates well to the time-frame of the hyperglycaemia. With the lipid metabolic pathways of Antarctic fish being highly cold-adapted (Crockett and Sidell 1990; Sidell and Hazel 2002), and with lipid acting as the primary fuel for energy metabolism of these animals (Crockett and Sidell 1990; Sidell 1991; Sidell et al. 1995), the delayed increase in plasma glucose concentrations may indicate an initial reliance on lipid metabolism to meet the additional energy demands associated with stress. Antarctic nototheniids, particularly those with the highest activity levels (Hubold 1985; Sidell et al. 1995), store substantial quantities of lipid (Eastman and De Vries 1982; Friedrich and Hagen 1994), and with high activities of triacylglycerol in the lipid storage tissues (Sidell and Hazel 2002) it appears that the esterified storage lipids can be made available to the pathways of energy metabolism. How one compound fulfils both

buoyancy (Eastman and De Vries 1982) and metabolic requirements is not yet understood. Increased levels of plasma free fatty acids (FFAs) have been measured in other fishes in response to stress (van der Boon et al. 1991; Wang et al. 1994; Waring et al. 1996), and data from a variety of sedentary species indicate that plasma FFA levels may be a more sensitive indicator of stress than glucose concentrations in organisms with relatively low-energy lifestyles (White and Fletcher 1989; Waring et al. 1992; Pottinger et al. 1994; Waring et al. 1996). There has been no research into the contribution of plasma FFAs to the elevated metabolic demands of stress in Antarctic nototheniids, but with the importance of lipid metabolism to these animals it is conceivable that lipids may also be involved in the stress response.

Acute exposure of the Antarctic nototheniids to 6°C resulted in a brief elevation of plasma osmolarity. In the majority of marine fish, stress-related osmotic dysfunction is attributed to the effect of adrenaline in increasing branchial permeability, therefore causing water to be lost and ions to be gained (Pic et al. 1974; Pic et al. 1975; Adedire and Oduleye 1984). The delayed response of the Antarctic nototheniids and the absence of other secondary stress responses associated with catecholamine release indicates that the response is more likely to be the result of an inability of regulatory mechanisms to cope with the increasing rates of diffusion at the higher temperature, or due to increases in the concentrations of corticoid hormones, with cortisol in particular known to exert an effect on the regulation of hydromineral balance (Wendelaar Bonga 1997). As in the case of plasma glucose levels, the latency prior to detection of an osmotic change in *P. borchgrevinki* at 6°C relates well to the delayed rise in cortisol levels measured in this species (Ryan 1995). Osmoregulatory capacity was restored within 24 hours at 6°C, and with no change in plasma osmolarity measured during either exposure to 3°C or following progressive hypoxia at -1°C, these results support the theory that, due to the reduced osmotic gradient between the plasma and seawater compared with temperate-water fishes, the ionic or osmotic dysfunction of Antarctic fish should be minor (Franklin et al. 1991). The hypo-osmotic shift in plasma of *P. borchgrevinki* following warm-acclimation, comparable to that of other Antarctic nototheniids (Gonzalez-Cabrera et al. 1995; Guynn et al. 2002), indicates that the initial and transient hyper-osmotic effect was likely to be the result of a stress-related break-down in osmoregulatory capacity. The trend for an inverse relationship between environmental temperature and serum osmolarity appears to be restricted to organisms with environmental temperatures approaching 0°C (Burton 1986; Guynn et al. 2002), suggesting that the high osmolarity

at low temperatures is a specialised physiological mechanism for life in near-freezing water, as hypothesized by Gonzalez-Cabrera et al. (1995).

From the results of this and previous studies (Davison et al. 1995; Forster et al. 1998), it is clear that circulating catecholamines are only involved in the stress response of Antarctic nototheniids at extremely high stress levels. The prolonged elevation of circulating hormone levels following their release (Forster et al. 1998) indicates that these fishes do not have well-developed mechanisms to deal with these substances. Neural cholinergic control mechanisms have been implicated in several stress responses, including changes in heart rate, total vascular resistance and splenic contraction (Davison et al. 1995), but it is also possible that corticosteroids, in particular cortisol, may play a major controlling role in the stress response of these fishes. With regard to the identification of a good physiological indicator of the stress response in Antarctic nototheniids, it is apparent that no single haematological parameter reflects the magnitude of the thermal stressor in both *P. borchgrevinki* and *T. bernacchii*. Elevation of haematocrit was a good indicator of stress levels in *P. borchgrevinki*, with the increase in haematocrit being both rapid and related to the severity of the stressor, but was of no use in *T. bernacchii*. The concentration of plasma fatty acids, however, is another possibility which has yet to be investigated.

In addition to inter-specific differences in the haematological response to acute thermal stress, there were significant differences between baseline haematology values of the two nototheniids, with haematocrit, haemoglobin concentration, and plasma glucose concentration all higher in *P. borchgrevinki*. With a limited effect of phylogeny, being both members of the same taxonomic family, the differences are attributable primarily to the different activity levels of the two species. The resting oxygen consumption rates of the two nototheniids, however, were very similar, in contradiction of the theory that animals with higher levels of activity or cost of locomotion should exhibit higher resting metabolic rates than more sluggish species in order to attain high rates of metabolism during exercise (Pörtner et al. 1998). This similarity of resting oxygen consumption rates did not prevent *P. borchgrevinki* from attaining considerably higher maximum oxygen consumption rates and aerobic scopes at both 0 and 3°C.

The similarity of resting metabolic rates of the two Antarctic nototheniids suggests that they may be set at the lowest level at which life can be sustained in the sub-zero waters, which appears to be approximately 50% higher than the 20 mg O_2 kg^{-1} h^{-1} theoretically-determined minimum metabolic rate required to sustain life in free-living

temperate-water fish of 10-100 g (Brett and Groves 1979). As discussed in Chapter 5, this elevation of metabolic rates may be due to additional costs incurred by cold-adaptation to the Antarctic environment (Wells 1987). These include the energy requirements of synthesis of glycopeptide antifreezes in the liver (Cheng and De Vries 1991), tubular secretion in the aglomerular kidneys (De Vries and Eastman 1981), and the maintenance of high mitochondrial volume densities in the swimming muscles (Johnston 1987; Dunn 1988; Archer and Johnston 1991; O'Brien et al. 2003). It is likely, however, that there has been some evolutionary compensation for these higher costs, including a reduction in proton leakage across the inner mitochondrial membrane (Pörtner et al. 1998; Hochachka and Somero 2002), maintenance of relatively high osmolarity body fluids (Dobbs and De Vries 1975; O'Grady and DeVries 1982), and a reduction in muscle fibre number (Johnston 2003; Johnston et al. 2003) of the Antarctic species. With 20-40% of routine energy expenditure associated with the maintenance of ionic gradients across membranes (Jobling 1994), the lower surface-to-volume ratio of fewer, larger fibres would decrease the number of energy-utilising pumps required to maintain equilibria (Hochachka 1986; Johnston 2003).

The resting oxygen consumption rates of the temperate-water notothenioids *B. variegatus* and *N. angustata* are close to the theoretically-determined minimum metabolic rate when extrapolated down to 0°C, supporting a 30-50% elevation of rates in the Antarctic species. This difference in rates between temperate-water and Antarctic notothenioids is similar to differences reported in the majority of other comparisons between closely-related and ecologically-similar temperate and Antarctic species (Johnston et al. 1991; Johnston and Battram 1993; Bushnell et al. 1994; Steffensen et al. 1994; van Dijk et al. 1999), but is considerably lower than the 200+% elevation of rates of Antarctic species predicted by the theory of metabolic cold adaptation (Wohlschlag 1960; Wohlschlag 1964). As previously hypothesized (Macdonald et al. 1987; Clarke 1991; Montgomery and Wells 1993), cold adaptation appears to be the consequence of increased energy requirements, rather than metabolic adaptation to the low temperature itself.

Resting oxygen consumption rates of both Antarctic and temperate-water species exhibited a similar pattern of thermal sensitivity, with a plateau of relative thermal independence surrounding the acclimation temperature and a considerable increase in thermal sensitivity towards the extremes of the thermal range. The maximum oxygen consumption rates of the Antarctic species followed a different pattern, increasing as the

temperature increased from 0 to 3°C and then decreasing above this level. This resulted in a maximum scope for aerobic activity at 3°C and a decline in whole-animal aerobic scope from 3 to 6°C. The decrease at temperatures approaching the upper thermal limit is thought to mark the decline of oxygen delivery capacities below the rising energy demands of cardiovascular and other aerobic tissues (Jones 1971; Mark et al. 2002). While ventilation and blood circulation are increased to augment oxygen supply, the ventilatory and circulatory systems consume most of the delivered oxygen themselves, thus leading to a vicious circle of an ever-increasing oxygen deficiency (Frederich and Pörtner 2000; Mark et al. 2002; Pörtner 2002). The relative degree of influence of the circulatory and ventilatory systems on the decline in aerobic scope has not yet been identified (van Dijk et al. 1999), although with maintenance of the scope for change in ventilatory frequency of 0°C-acclimated *P. borchgrevinki* to at least 6°C, it appears as though the marked decrease in cardiac scope of the cold-acclimated fish may be the major contributor to the decline in aerobic scope at higher temperatures. Fish acclimated to closer to their environmental temperature (Chapter Eight) demonstrated an increase in resting ventilation rate with temperature and it is therefore possible that the scope for change in ventilation frequency may decrease with increasing temperature in these fish. The effect of increasing temperature on the ventilatory stroke volume of this species has also yet to be determined, and thermal insensitivity of ventilation frequency may not necessarily reflect thermal sensitivity of total gill ventilation. The relative contributions of the circulatory and ventilatory systems to the decline in aerobic scope of *P. borchgrevinki* remain to be determined.

The decline in cardiac scope of -1°C-acclimated *P. borchgrevinki* at higher temperatures was due mainly to a greater rate of increase in resting than active heart rate. The resting heart rate increased with a Q_{10} of 2.38 from -1 to 8°C, in contrast to previous findings (Forster et al. 1998; Franklin et al. 2001). The increase in rate from -1 to 6°C was due to an increase in adrenergic tone on the heart (45%) masking a smaller increase in cholinergic tone (37%), in contrast to the thermal insensitivity of heart rate from -1 to 3°C reported in one of the previous studies which was the result of a 57% increase in cholinergic tone masking a 21% increase in adrenergic tone (Franklin et al. 2001). With the study of Franklin et al. (2001) employing a gradual temperature change, it is possible that sudden thermal changes impair the ability of the cholinergic system to maintain control. In contrast to the thermal sensitivity of heart rate, ventral aortic pressure of *P. borchgrevinki* was regulated from -1 to 8°C. The cardiac output of this species was found

to double with an increase in temperature from -1 to 6°C (although the result was not statistically significant) indicating that regulation of ventral aortic pressure is likely to be through a decrease in total vascular resistance. With low total vascular resistances in the Antarctic notothenioids, it has been suggested that the potential for any change in resistance may be limited (Axelsson et al. 1992) and further research is required to determine the mechanisms behind this regulation of pressure.

A 4-5 week period of warm-acclimation resulted in resting heart rate of *P. borchgrevinki* gaining considerable thermal independence. The resetting of resting heart rates of warm-acclimated fish to lower baseline rates enabled scope for change in heart rate to be maintained at higher temperatures, and thereby contributed to cardiac scope attaining high values at temperatures as high as 8°C. The means by which cardiac output was modulated to cope with the increased demands of exercise differed between cold and warm-acclimated fish. While cold-acclimated fish increased cardiac output primarily through changes in stroke volume, particularly at higher temperatures, warm-acclimated fish were almost completely reliant on frequency modulation at all temperatures. Generally, the relative contribution of stroke volume to the increase of cardiac output in fish is equal to or greater than that of heart rate (Randall 1970; Farrell and Jones 1992), although the highly active tunas have greater reliance on frequency modulation (Farrell 1991; Blank et al. 2002), and recent evidence has identified a few more species which depend primarily on modulation of heart rate (Altimiras et al. 2002; Cooke et al. 2003; Overgaard et al. 2004). The move away from varying stroke volume towards varying heart rate has been described as an evolutionary trend (Lillywhite et al. 1999), although a trend toward increasing cardiac output through changes in heart rate at higher temperatures has been reported in other species (Tota et al. 1991; Kolok et al. 1993). Whether this thermal trend is widespread has yet to be determined.

Without significant adaptation, the recovery rates of Antarctic fishes from various forms of stress are expected to be considerably longer than in temperate-water species, due to the rate-depressing effects of low temperature. Heart rate, ventilation frequency and oxygen consumption rate of Antarctic nototheniids, however, returned rapidly to baseline levels after both exercise and acute thermal stress, in line with previous studies which have indicated considerable rate compensation (Wells et al. 1984; Forster et al. 1987; Davison et al. 1988; Saint-Paul et al. 1988; Egginton et al. 1991; Franklin et al. 1991; Egginton 1994; Egginton 1997). One unusual feature of the recovery from an acute increase in temperature was that following return of the fish to -1°C ventral aortic

pressure increased, and this hypertensive effect was maintained for over an hour after exposure to 6°C. Ventral aortic hypertension has not previously been observed in *P. borchgrevinki* during recovery from other stressors such as forced swimming or hypoxia, and it has been hypothesized that the regulation of ventral aortic pressure during both stress and recovery may be due to a reduced tolerance for high pressures in the low pressure cardiovascular systems of these fishes (Axelsson et al. 1992). Whether the hypertensive effect measured in this study was the result of an increase in cardiac stroke volume or due to increases in branchial or systemic vascular resistance, and which effector(s) mediated the response (catecholamines, the autonomic nervous system, or other cardio-active substances such as Angiotensin II) has yet to be determined.

Both circulation and respiration are pulsatile in fish and cardio-respiratory synchronisation can therefore have a positive effect on gas exchange efficiency (Malte 1992). Cardio-respiratory synchrony is low in some species, e.g. trout (Borch et al. 1993) but Egginton et al. (unpubl. data) found that opercular and cardiac contractions of both *P. borchgrevinki* and *T. bernacchii* shared similar rates, demonstrating central coupling close to the hypothesized optimal 1:1 synchrony. In the current study, evidence for an element of heart rate / ventilation coupling was provided by the heart rates of -1°C-acclimated *P. borchgrevinki* (Chapter 7) being of similar magnitude to the ventilation rates of fish measured 3 days post-capture at -1°C (Chapter 8) at both -1 and 3-4°C. The heart rates of 4°C-acclimated *P. borchgrevinki* (Chapter 7) were similar to ventilation rates of fish which had been kept in Christchurch at 0°C for two months (Chapter 4) at the various experimental temperatures, indicating that a temperature only ~2°C above the environmental level may elicit acclimatory responses in these fish. Cardio-respiratory synchrony is abolished by atropine, suggesting that it is under vagal control (Taylor 1985). It would therefore be interesting to determine whether the same synchrony exists in -1°C-acclimated fish at 6-10°C, temperatures at which the cholinergic control of heart rate is progressively being lost.

Acclimation to 0°C appears to have an effect on the level of cholinergic tonus on the heart of *P. borchgrevinki*. In this study, the resting heart rate at -1°C was found to be the result of a 43.8% inhibitory cholinergic tone, similar to the 44.6% measured previously in this species kept for 3-4 days at -1.2°C in the aquarium system at Scott Base (Franklin et al. 2001). Cholinergic tones of 50.0-54.5% have, however, been determined on the hearts of *P. borchgrevinki* transported to New Zealand and held in captivity at -0.5 to 0°C for two months prior to experiments (Axelsson et al. 1992;

Axelsson et al. 1994). The increase in cholinergic tone on the heart of fish held captive for long periods may be the result of the different acclimation temperatures, with Q_{10} values for changes in cholinergic tone in response to acute temperature increases varying from 1.56 (-1 to 6°C, this study) to 2.93 (-1 to 3°C, Franklin et al. 2001), or may be due to a strengthening of cholinergic control as the metabolism of an animal slows down over long periods in captivity (Axelsson et al. 1992; Davison et al. 1995). The excitatory adrenergic tonus on the heart measured in the current study was also closer to the 35.5% value from fish held for 3-4 days at -1.2°C (Franklin et al. 2001), than to the 3.2% excitatory input recorded from fish held for 2 months at 0°C (Axelsson et al. 1992). Thermal sensitivity of resting oxygen consumption rates also varied with the 2°C change in acclimation temperature, with 0°C-acclimated *P. borchgrevinki* and *T. bernacchii* exhibiting thermal independence from 0-3°C, while the resting oxygen consumption rate of *P. borchgrevinki* measured three days post-capture from McMurdo Sound increased significantly from -1 to 3°C. A similar trend was apparent in ventilation frequency, although whether the thermal insensitivity of ventilation rate of 0°C-acclimated fish represents thermal insensitivity of total gill ventilation or a greater dependence on ventilatory stroke volume remains to be determined. Ventilatory stroke volume is proposed to be the most economical way of increasing gill ventilation (Fernandes and Rantin 1989; Perry and Wood 1989), and it is possible that efficiency may gain greater importance at higher temperatures. With the cost of ventilation forming a considerable proportion of resting metabolic rate (Hughes and Shelton 1962; Johansen et al. 1967), the two parameters are likely to be closely linked.

Increased ventral aortic pressure and mean corpuscular haemoglobin content, and decreased heart rate and plasma chloride concentrations have previously been measured in *P. borchgrevinki* kept in Christchurch (acclimated to ~0°C) for 3 weeks, compared with those kept in aquaria at Scott Base (acclimated to less than -1°C) for 2-4 days (Davison et al. 1995), and lower blood pH and plasma chloride levels and higher blood carbon dioxide tension and lactate have been measured in *N. coriiceps* maintained in captivity in the United Kingdom than in specimens sampled in the South Orkney Islands (Egginton 1997). Taking into account the results of this study, the lower plasma chloride levels of *P. borchgrevinki* at the slightly higher acclimation temperature in Christchurch may have been due to the hypo-osmoregulation associated with warm-acclimation in these fishes. While elevated cortisol levels have been reported from both *T. bernacchii* and *P. borchgrevinki* after ~1 week of captivity in one study (Lowe and Wells 1997), low

levels were measured in *P. borchgrevinki* after ~3 days in captivity in another investigation (Ryan 1995) at temperatures of -1.3 to -1.9°C. In the current study, there were no differences in haematocrit, haemoglobin concentration, mean corpuscular haemoglobin concentration, plasma glucose level or osmolarity between *P. borchgrevinki* sampled 72 hours post-capture at -1°C, and fish maintained in a flow-through aquarium at Scott Base, Antarctica at -1.0 ± 0.3°C for five weeks. This suggests that the majority of changes detected in captive fish outside Antarctica are not due to confinement stress, but are more likely to be the result of the warmer seawater. Changes in the nutrient composition of food and/or chemistry of the seawater may also have an influence. The acclimatory changes occurring in these fish following prolonged exposure to 0°C are also likely to be responsible for the observation that while freshly caught *P. borchgrevinki* do not survive more than a few days at 6°C, fish acclimated to 0°C for several months tolerate exposure to 6°C for at least 48 hours, including exhaustive swimming, with no apparent adverse effects. The differences in thermal sensitivities of Antarctic nototheniids acclimated to temperatures less than 2°C apart must therefore be kept in mind when interpreting the results of thermal investigations carried out on these fish.

The marked effect of warm-acclimation on the prolonged swimming ability of *P. borchgrevinki* is an even greater indication of the considerable thermal plasticity of these, supposedly stenothermal fish. While cold-acclimated fish exhibited extreme temperature-dependence of prolonged swimming ability, with performance only maintained to 2-3°C, in agreement with the findings of a previous investigation (Wilson et al. 2002), warm-acclimated fish maintained performance to 8°C. The thermal performance breadth for prolonged swimming thus increased from 3°C in -1°C-acclimated fish, to 9°C in 4°C-acclimated fish, in support of the theory that that the ability to function at high temperature confers a significant degree of eurythermy upon a species (Coppes and Somero 1990; Bennett et al. 1992).

The limitation of aerobic swimming ability is thought to occur at a high level of biological organisation and then translate into changes at the cellular level (Pörtner 2002). Jones et al. (1970) have determined that, in theory, the ventilation-perfusion ratio should increase with temperature, and therefore the cardiac pump should be the limiting factor at lower temperatures and the branchial pump should be limiting at higher temperatures, although Farrell (1997) proposed that a decline in cardiac performance was the primary limitation at high temperatures. The parallel changes, both in direction and

magnitude, of maximum swimming performance and maximum cardiac performance during warm-acclimation of *P. borchgrevinki* add support to the cardiovascular limitation of aerobic performance, and are in agreement with conclusions drawn by Keen and Farrell (1994) during work on trout. Good oxygen availability and a stable cold-stenothermal environment support low-energy lifestyles in Antarctic fish, not least via the reduction of the energy cost of cardiovascular and ventilatory work (Mark et al. 2002). Adjustment of ventilation and circulation capacities to low levels, as predicted by symmorphosis (Taylor and Weibel 1981), should therefore result in stenothermality, although the current study has demonstrated that the Antarctic nototheniids are not as stenothermal as previously thought.

The biochemical mechanisms behind the acclimatory increase in aerobic swimming performance have yet to be defined. Adenosine triphosphate (ATP), generated by the stepwise degradation of carbohydrate and lipid, is an essential prerequisite for muscle contraction, and therefore oxidative capacity and mitochondrial function seem to be likely targets of thermal compensation (Guderley and St Pierre 1996; Guderley 2004). The oxidative capacities of isolated mitochondria have been shown to change during both thermal acclimation (Dahlhoff and Somero 1993; Guderley and Johnston 1996) and seasonal acclimatisation of fish (St Pierre et al. 1998; Guderley and St Pierre 1999), although the acclimation of stenothermal species may be restricted (Dahlhoff and Somero 1993; Hardewig et al. 1999). Recent evidence indicates that there is a significant increase in cytochrome *c* oxidase (CCO) activity in the pectoral (red) muscle of *P. borchgrevinki* following 4-5 weeks of acclimation to 4°C (Seebacher et al. in press), although whether this is achieved through increases in enzyme numbers, changes in the properties of the enzymes, or changes in the operating environment of the enzymes has yet to be determined. There is, however, reported to be no change in either the Arrhenius break temperature, or the temperature at which the acceptor control ratio (an index of efficiency of coupling of electron transport to synthesis of ATP) declines in *T. bernacchii* after 2 weeks of acclimation to 4°C (Weinstein and Somero 1998). With both breaks occurring at around 18°C, there may be no stimulus for these values to alter in response to a 6°C change in temperature. Very little is yet known about the time-frame of acclimatory processes in Antarctic fish and it is also possible that two weeks is not long enough for these changes to take place.

Antarctic fish have CCO and citrate synthase activities per gram of wet muscle mass which are higher at 1°C than temperate species (Crockett and Sidell 1990), while

mitochondrial cristae densities (Johnston et al. 1998; O'Brien and Sidell 2000) and maximum rates of substrate oxidation by individual mitochondria do not appear to be cold-adapted (Johnston 1987; Johnston et al. 1994; Johnston et al. 1998; Weinstein and Somero 1998), indicating that the increased levels of enzyme activity are due to higher mitochondrial volume densities. Increases in the oxidative capacity of individual mitochondria with temperature can occur at the enzyme level with qualitative and quantitative changes in enzymes such as CCO and citrate synthase (Wodtke 1981b; Wodtke 1981a), or at the structural level with changes in membrane phospholipid composition and properties (Hazel 1972a; Hazel 1972b; Wodtke 1981b; Guderley et al. 1997) and/or cristae density (St Pierre et al. 1998). Given the influence of the membrane environment on the functioning of membrane proteins, changes in membrane composition are thought to be the prime mechanism by which the oxidative capacities of existing mitochondria could be modified (Guderley and St Pierre 2002), although this has yet to be investigated in the Antarctic nototheniids.

At high temperatures, the higher mitochondrial densities of Antarctic fishes are likely to result in greater energy losses (Hardewig et al. 1999; Pörtner et al. 1999; Pörtner et al. 2000), as mitochondrial proton leakage in the resting cell comprises a large fraction of the standard metabolic rate (Brand 1990; Pörtner et al. 1998). A reduction in mitochondrial capacity in the warm would thus reduce the oxygen demand of mitochondrial maintenance, increasing the ability of ventilation and circulation to maintain aerobic scope (Pörtner 2002). Whether this in fact occurs and the resting metabolic rates of these fish are reduced following warm-acclimation is unknown.

Another possible contributing factor to the enhancement of swimming performance at high temperatures following warm-acclimation is an improvement in muscle performance (Sidell 1980; Egginton and Ross 1992; Goldspink 1995). Thermal acclimation has been shown to alter both the force production and maximum contraction speed of isolated fibres (Johnston et al. 1985; Taylor et al. 1997) and acclimatory changes have been demonstrated in the activity and thermostability of myofibrillar ATP-ase in freshwater teleosts and carp (Johnston et al. 1975; Heap et al. 1985; Heap et al. 1986; Itoi et al. 2003). Muscle ATP-ase activity of *P. borchgrevinki*, however, is unaltered following a 4-5 week period of warm-acclimation (Seebacher et al. in press).

The marked enhancement of aerobic swimming performance and cardiac scope of warm-acclimated *P. borchgrevinki* at temperatures up to 8°C raises the possibility that the upper lethal limit of these fish may also have increased. It has long been known that

the lethal limits of organisms are strongly affected by acclimation temperature (Loeb and Wasteneys 1912; Summer and Doudoroff 1938) and, in general, as acclimation temperature increases both the upper and lower tolerance limits of a species increase (Beitinger and Bennett 2000). Adjustment of mitochondrial density and capacity is reported to be one of the main determinants of thermal limits (Pörtner et al. 1998; Pörtner et al. 2000), and there is thus scope for further research into the effects of acclimation on mitochondrial properties and thermal tolerance limits of *P. borchgrevinki*.

The warm-acclimation of osmoregulatory capacity, cardiac performance and aerobic swimming performance of *P. borchgrevinki* following prolonged exposure to a temperature defined as the upper viable limit of -1.86°C-acclimated fish (Guynn et al. 2002) does not necessarily translate into an ability of these animals to survive long-term at elevated temperatures. A central aspect of stress adaptation is the reallocation of metabolic activity away from investment activities, such as growth (Hoffmann and Parsons 1997) and reproduction (Donaldson 1990; Jobling et al. 1995; Van der Kraak and Pankhurst 1997), towards activities that restore homeostasis, such as respiration, locomotion, hydromineral regulation, and tissue repair (Schreck 1981; Wendelaar Bonga 1997). It is therefore possible that the increase in critical swimming speed of *P. borchgrevinki* at higher temperatures following warm-acclimation may represent a channelling of resources into locomotion, at the expense of processes required for long-term survival. While survival is often possible over a relatively wide range, locomotion, active feeding, growth and reproduction tend to take place within successively narrower ranges (Wootton 1992) and the survival of individuals in a particular environment does not therefore imply that they will reproduce (Donaldson 1990), as nicely summed up by Guderley (2004): "Reproductive success is the key element upon which selection will act, and individual survival – as favoured by attributes conferring greater endurance or sprinting capacity or thermal compensation – is only relevant insofar as it increases the opportunities for successful reproduction". In studies investigating the effects of pollutant exposure, it has been demonstrated that while acute exposure results in impairment of swimming performance, chronic exposure can lead to a compensatory return to normal swimming performance, but in the presence of inhibited growth and pathological changes in gills and other tissues (Webb and Brett 1973; McLeay and Brown 1979). The return of haematocrit and plasma glucose concentrations of *P. borchgrevinki* to baseline levels, along with the energy-intensive hypo-osmotic shift are positive indicators that the fish have successfully acclimated, but further research is

required to determine the effects of prolonged 4°C exposure on growth and reproduction. Measurement of resting metabolic rates of warm-acclimated *P. borchgrevinki* at 4°C will provide some indication of whether the basal costs of life at higher temperatures are able to be reduced during acclimation, thereby enabling maintenance of aerobic scope and freeing energy for growth and reproduction. An increase in the maximum metabolic rate at elevated temperatures has been described following warm-acclimation in fish (Beamish 1978; Schurmann and Steffensen 1997) and it is likely that long-term adaptation will increase the energy efficiency of most processes (Pörtner 2002).

The considerable differences in acute thermal responses of *P. borchgrevinki* and *T. bernacchii* challenge the validity of extrapolating the acclimatory response of *P. borchgrevinki* to other, even closely-related, species. *P. borchgrevinki* has a lower resting cholinergic tone on the heart than the majority of sedentary benthic Antarctic nototheniids (Axelsson et al. 1992; Axelsson et al. 1994; Franklin et al. 2001), and may therefore have greater scope to increase inhibitory cholinergic tone and maintain heart rate in the face of increasing temperature. The capacity for changes in blood oxygen-carrying capacity, ventilation frequency, and oxygen consumption rate are also higher in *P. borchgrevinki* than in *T. bernacchii*, which may translate into a greater tolerance of energy-demanding stressors. *P. borchgrevinki* may therefore be among the most thermally tolerant of Antarctic nototheniids, and further studies will be required to determine whether such marked warm-acclimation is possible in the benthic trematomids.

In conclusion, the responses to acute thermal change vary between closely-related Antarctic nototheniids, most likely as the consequence of their differing ecotypes. There is no single haematological test which reflects the magnitude of the stress response in these species, but aerobic scope, cardiac scope and prolonged swimming performance appear to be good indicators of the effect of changing temperatures on the organism as a whole. One factor which became apparent in this study was that acclimation of Antarctic nototheniids to elevated temperatures (even temperatures only ~2°C above environmental temperatures) results in an increase in thermal independence of a variety of physiological parameters. Most notably, the notothenid *P. borchgrevinki* was demonstrated to acclimate prolonged swimming ability, cardiac performance and osmo-regulatory capacity after 4-5 weeks at 4°C, questioning the commonly held belief (Huey and Hertz 1984; Huey and Kingsolver 1989; Angiletta et al. 2003) that specialisation to a narrow range of environmental conditions occurs at the cost of response to short-term

environmental change. The results are thus in agreement with the conclusion drawn by Mongold et al. (1996), following studies on bacteria, that adaptation to a historical environment does not necessarily impede the rate of adaptation to a novel environment.

The current contrasting thermal trends in different regions of the Antarctic (Doran et al. 2002) pose challenges to the designers of climate and ecosystem change models and highlight the unpredictable nature of the climate in this region. Nevertheless, if substantial and rapid warming should eventuate, the phenotypic plasticity of *P. borchgrevinki* bodes well for the species' survival even during periods of warming at a greater rate than the maximum evolutionary rate of change. The Antarctic nototheniids may not be as stenothermal as has previously been assumed.

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APPENDIX ONE

Antarctic Fish Physiological Saline

Compound	**Amount (g L^{-1})**
NaCl	15.078
KCl	0.557
$CaCl_2$	0.597
$MgCl_2$	0.161
NaHEPES	1.98
HEPES	0.58
Glucose	1.00

pH adjusted to 8.43 at 0°C