

**The Effect of Chronic and Acute Temperature Exposure on the
Antarctic Notothenioid *Trematomus bernacchii* during Hypoxia
Exercise and Feeding**

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ABSTRACT

Antarctic fish from the Perciform suborder Notothenioidei inhabit arguably the most thermally stable ocean environment on earth. In order to populate the subzero environment Antarctic fish have evolved numerous adaptations. However, specialisation to -1.9°C has incurred a trade off, thermal flexibility is lost likely due to modifications to the cold and as a result Notothenioidei are extremely stenothermic. Climate change mediated warming is predicted to increase the ocean temperature surrounding the Antarctic continent by 2°C within the next century. This increase is projected to affect individuals, populations and the community structures of those inhabiting the area and therefore the physiological study of the acclimation ability and thermal limitations of Antarctic fish is an area scientific interest.

The present study is a series of discrete experiments relating to one species, *Trematomus bernacchii*, a circumpolar benthic Notothenioidei found in nearly all inshore waters surrounding the Antarctic coastline. These studies included investigation of the response of this species to both chronic and acute temperature exposure prior to and following a feeding event, a reduction in environmental oxygen and an exhaustive exercise event, as well as examination of *T. bernacchii* ability to recovery from these challenges.

T. bernacchii demonstrated variable success when acclimated to $+3^{\circ}\text{C}$. Failure appeared to be determined by the recovery period following capture and aquarium housing, 7 days housing following capture resulted in 100% mortality, conversly 3 months resulted in 100% survival. Following successful acclimation *T. bernacchii* showed physiological adjustment as acclimated resting metabolic rate mirrored that of *T. bernacchii* tested at environmental temperature, 20.63 ± 1.3 compared to 22.38 ± 1.02 $\text{mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$.

The previously undefined specific dynamic action response (SDA), in *T. bernacchii* was characteristic of polar species. At environmental temperatures SDA scope was small 14.52 ± 3.52 $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, and lengthy ,72 hours; SDA duration was reduced to 9 hours in acclimated fish. Resting metabolic rate was elevated following acute exposure to $+3^{\circ}\text{C}$, 34.27 ± 2.35 $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, masking the SDA response and associated parameters.

T. bernacchii were relatively sensitive to hypoxia, P_{crit} over four acute temperature exposures, ranged between 69 and 102mmHg, higher than the average range for teleosts (40 – 60 mmHg). Above -1°C P_{crit} increased, rising with acute temperature exposure. Ventilation rate was temperature dependent and completely absent at +4 and +6°C. A bradycardia (beginning at 60 and 70mmHg) was observed at all temperature exposures, this response was consistent as all heart rates reduced by 25%. Recovery from both hypoxia and acute temperature exposure was rapid.

Following an exhaustive exercise event aerobic Scope of *T. bernacchii* was constrained over an acute temperature increase, reducing from 38.58 ± 5.64 to 24.41 ± 4.92 mg.O₂. kg⁻¹.h⁻¹ over a 7°C temperature increase, respiratory scope too was reduced such that at +4 and +6°C scope was absent. Heart rate of *T. bernacchii* was highly constrained at -1°C, increasing by 2.54 ± 0.9 bpm following exercise. Acute temperature increase resulted in an increase in cardiac scope, maximum 6.29 ± 1.2 bpm at +2°C, due likely to a thermally mediated loss of cholinergic tonus following exhaustive exercise. Recovery of all parameters was temperature dependent and rapid upon return to -1°C.

The present study is the first to quantify and assess the effect of acute and chronic temperature exposure on the SDA response of *T. bernacchii*. Furthermore, it supplements the current literature on acclimation ability, acute temperature exposure, aerobic scope and hypoxia tolerance for this species. This work will be of use in future investigations of the effects of rapid climate change on Antarctic notothenioid fish and the interconnected ecosystem.

CHAPTER ONE

General Introduction

1.1 Antarctica- the continent

The Antarctic region is a vast and desolate area situated at the bottom of the planet. Encompassing everything below 60°S (Robinson 2008), the region consists of the Southern Ocean, a handful of dispersed islands and centred at the southern-most point, the continent of Antarctica (90.0000°S, 0.0000°W) (King and Turner 1997). Antarctica is widely recognised as the driest, windiest and coldest place on the planet with average temperatures generally remaining below 0.0°C at all times of the year. The lowest ever reported air temperature documented on earth, - 89.28 °C was recorded at Vostok Station in 1983 (Convey et al. 2009), while satellite technology using thermal imagery techniques have reported a record low temperature of -93.2°C on the East Antarctic Plateau in August 2010 (Phillips 2013).

The continent is approximately 14 million km², twice the size of Australia (Eastman 1993) and reaches altitudes greater than 4000m at its highest point (Maxwell et al. 1998). It is divided into two distinct areas, West Antarctica and the larger East Antarctica which are connected by a thick ice sheet (Convey et al. 2009) and the regions are separated by the Transantarctic Mountains. The ice sheet is on average 2160 metres thick (Eastman 1993), covers more than 97% of the continent and contains over 60% of the fresh water on the planet (Drewry 1983).

Due to its southerly location the photoperiod in Antarctica and the surrounding water is seasonal rather than diurnal (Convey et al. 2009). At higher latitudes in summer, for as much as 4 months of the year there is 24 hours of sunlight; similarly over 4 months of winter the sun is below the horizon (DeVries and Steffensen 2005). Separating complete dark and light are 2 month periods of increasing and declining sunlight hours (Eastman 1993).

Historically, Antarctica, along with present – day Africa, India, Australia, New Zealand and South America, was part of the supercontinent Gondwana (Knox 2007). Continental drift saw

the landmass that is now Antarctica break from Gondwana approximately 180 million years ago (mya). Antarctica's continued isolation led to a progressive cooling period 65 mya, the continent slowly moved southward taking its current position over 40 mya (Kennett 1977). Approximately 40 mya vast amounts of sea ice appeared (Verde et al. 2008) followed 35 mya by the first continental ephemeral ice sheet (Livermore et al. 2005). The separation of Tierra del Fuego (South America), 35 mya from the Antarctic Peninsula forming the Drake Passage (Aronson et al. 2011) and the opening of the Tasman Seaway separating Antarctica from Australia were crucial to forming current oceanic and atmospheric trends. These two key events facilitated the creation of the Antarctic Circumpolar Current approximately 22 mya (Patarnello et al. 2011) delimiting and defining the Southern Ocean, Antarctica and the flora and fauna inhabiting the latitudes below it (Eastman 2005; Clarke et al. 2005). The icesheet shifted from ephemeral to permanent reaching its current size approximately 15 million years ago contributing to the reduction of water temperatures in the Southern Ocean from 15°C to present day temperatures below 0.0°C (DeVries and Steffensen 2005)

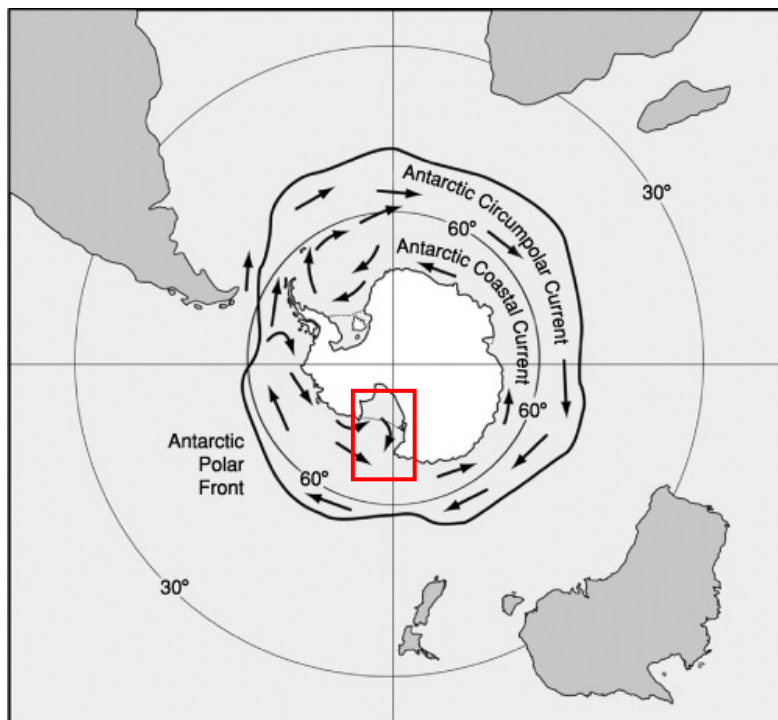


Figure 1.1: The Antarctic Continent, surrounding Southern Ocean, and currents. The box denotes the Ross Sea region. From DeVries and Steffensen 2005.

1.2 The Antarctic Marine Region

The Antarctic Marine Region, consisting of the Antarctic continent and the surrounding Southern Ocean (Figure 1.1), is an old system with water mass and current circulation patterns formed approximately 20 million years ago (Knox 2007). Geographically the Northern boundary lies at approximately 60°S and encompasses areas of the Pacific, Indian and Atlantic oceans; the continent defines the southern boundary. The Southern Ocean is differentiated from those surrounding it by its circumpolar circulation (Verde et al. 2008). The majority of the Southern Ocean depth ranges between 3000 and 5000 metres. Where depths range between 500 and 1000 metres some plateaus are present (DeVries and Steffensen 2005). Water temperatures are cold; at the winter maximum (September) sea ice extends over 20 million km² covering virtually all areas below 60°S, at its minimum (March) sea ice coverage can reduce more than 75%. As with the continent, cold seawater temperatures are regulated by the position of the continent, the presence of sea ice and the permanent ice sheet covering the continent (Eastman 1993). The creation of the Antarctic Circumpolar Current (hereafter ACC) was the major event facilitating the cooling and creation of the conditions associated with the Southern Ocean today (Robinson 2008).

The ACC (Fig 1.1) is one of the largest flowing currents on earth (Coppes Petricorena and Somero 2007); flowing eastward driven by continuous westerly winds it is between 200 and 1200 km wide. The current bottlenecks at the Drake Passage and unlike other ocean currents extends in places to the seafloor (Eastman 1993). The surface velocity of the ACC varies between 25 and 30cms per second and sits longitudinally depending on locality, between 47°S and 60°S (DeVries and Steffensen 2005). Within the ACC lies the Polar Frontal Zone (Fig 1.1 and 1.2), also known as the Antarctic Convergence (Eastman 1993). The Polar Frontal Zone is a region at approximately 50°S and 60°S where dense Antarctic surface water passes below less dense sub-Antarctic water (DeVries and Steffensen 2005); the Polar Frontal Zone creates a sharp temperature and physical differentiation between north and south water bodies. This cold “wall” barrier prevents uni-directional exchange of fish and other fauna (excluding large marine mammals and sea birds), affecting distribution of communities leading to extremely high levels of endemism in the Southern Ocean (Clarke et al. 2005). Water temperature at the Polar Frontal Zone ranges from 4 - 8°C and 1 - 3°C in summer and

winter respectively; below 60°S water temperature rarely exceeds 0°C (Knox 2007) and the water is almost 100% oxygen saturated (Coppes Petricorena and Somero 2007).

The Antarctic Coastal Current (or East - Wind Drift) flows westward around the continent. It flows into the Weddell, Bellingshausen and Ross Seas forming clockwise eddies that feed into the ACC (DeVries and Steffensen 2005). Between the Polar Frontal Zone and the Antarctic Coastal Current are various complicated current systems affecting temperature and salinity ranges, including the nutrient rich, relatively warm upwelling Circumpolar Deep Water and the Antarctic Bottom Water which flow off the continental shelf (Fig 1.2) (DeVries and Steffensen 2005). Close to the continent temperatures remain static barely deviating from -1.9°C with very little thermostratification (Clarke and Peck 2009).

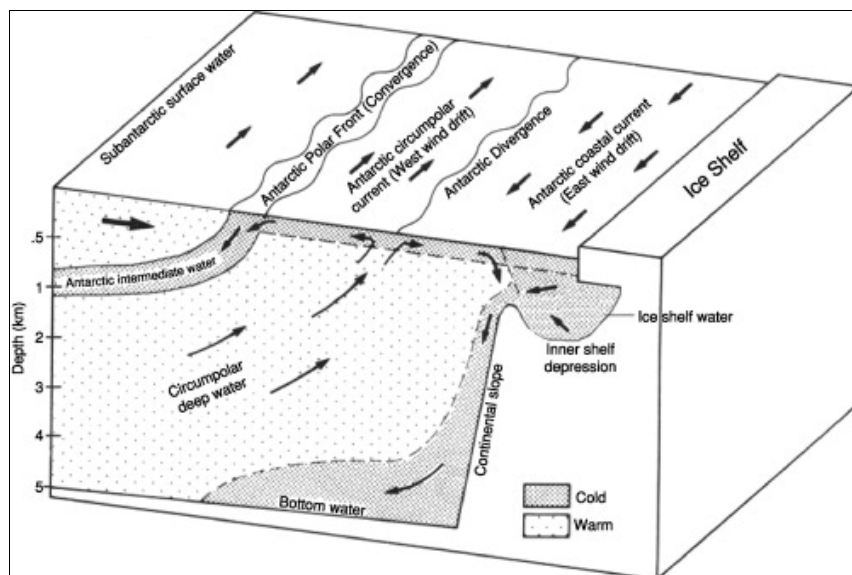


Figure 1.2: Cross Section of the circulating currents in the Southern Ocean. From DeVries and Steffensen 2005.

1.2.1 The Ross Sea and McMurdo Sound

The Ross Sea is a 500 000km² embayment situated in the Pacific sector of the Southern Ocean (Fig 1.1) (Ainley 2002). Its borders are the Ross Ice Shelf to the south, Edward VII Land to the east, Victoria Land on the west and from Cape Colbek to Cape Adare in the north. An unusual feature of the Ross Sea (Fig. 1.1) is the continental shelf which is broad and deep, in contrast to the narrow or non-existent continental shelf areas of Antarctic waters found throughout the rest of the Southern Ocean (Robinson 2008).

Within the Ross Sea is McMurdo Sound. For 10–12 months of the year the majority of the Sound is covered with sea ice, which during a multi - year cycle can reach a thickness of 2 - 4 metres. In summer the sea ice driven by southerly winds and northerly swells, breaks out near the Ross Ice Shelf however, the frequency of ice break - out is variable and the Sound may remain ice covered for several years (DeVries and Steffenson 2005). Water temperature and salinity in the sound is essentially static, hovering at -1.9°C and 34.8 parts per thousand respectively during all seasons (Picco et al. 1999; Sidell and O'Brien 2006). Thick platelet ice on the benthos called anchor ice forms in the sound at approximately mid-August until melting around mid- December. The sea and anchor ice are important habitats for many organisms in the sound (Fuiman et al. 2002), therefore many McMurdo notothenioid fishes, occupy a habitat that is the iciest and coldest in Antarctica (DeVries and Steffensen 2005).

1.3 Climate Change and Antarctica

Carbon dioxide levels are arguably the most recognised source of anthropogenic global climate change. Current atmospheric carbon dioxide levels are approximately 400 parts per million (NOAA 2014). Global carbon dioxide levels prior to the 1880s Industrial Revolution sat relatively static at approximately 280 parts per million (ppm). While atmospheric carbon levels have natural fluctuating seasons (Wang et al. 2014), a steady increase, most likely due to anthropogenic influences such as the burning of fossil fuels has occurred with the current trend in increases predicted to continue (NOAA 2014). Conservative estimates place the current atmospheric CO_2 levels the highest in over 400 000 years while other estimates suggest present levels have not occurred in 20 million years with the current rate of increase unprecedented in the last 20 000 years (Watson et al. 2001). Carbon dioxide and other greenhouse gases such as methane and nitrous oxide increase global temperature by reflecting infrared solar radiation bounced off the Earth, reflecting it back to the surface (IPCC 2007).

Observations over the past 160 years show mean global air temperatures have increased by 0.74°C . Warming has occurred in two discrete phases: firstly, a distinct increase in warming occurred from the 1910s to 1940s when the global average air temperature increased 0.35°C . Secondly, this increase escalated to 0.55°C from the 1970s to the new millennium (Robinson 2008; Enzor et al. 2013). This rate appears to be increasing, between 1995 and 2006 11 of those 12 years global surface temperatures were among the hottest since records began in

1850 (IPCC 2007). Corresponding warming of the troposphere and cooling of the stratosphere have also occurred. Rising sea levels, reduction in Arctic summer sea ice extent and ocean warming are some of the consequences evident today (Watson 2001).

The ocean is a major sink in the carbon cycle absorbing and storing forms of carbon including carbon dioxide. The ocean has also absorbed over 80% of the additional heat added to the atmosphere (Enzor et al. 2013) showing regional variability but equating to a mean sea surface increase of 0.59°C. Below the surface a smaller temperature increase is also observed, averaging 0.39°C at 366 metres (200 fathoms) and 0.12°C at 914 metres (500 fathoms) (Roemmich et al. 2012).

Sub regions of the Antarctic have exhibited some of the fastest temperature increases in both atmospheric and the oceanic systems. The West Antarctic Peninsula has the most rapidly changing climate in the Southern hemisphere; an annual mean atmospheric temperature increase of 3°C has been recorded since 1951 (Meredith and King 2005; Peck et al. 2014), with all meteorological stations on the Peninsula reporting significant and strong warming over this time period. The number of days above 0°C has increased by 74% and the impact of this warming is becoming evident. In a study surveying 244 glaciers, 87% of glacier termini were found to be retreating (Meredith and King 2005) and the rate of decline appears to be increasing. Ten floating ice shelves have lost approximately 14 000km² of ice and seasonal snow cover patterns have decreased (IPCC 2007). The implications of ice shelf collapse is complicated, the melted ice does not alter sea level as the shelves are floating thus seawater is already displaced. But evidence suggests ice shelves act as a buffer between glaciers, the grounded ice sheet and the ocean (Meredith and King 2005); therefore, ice shelf collapse is linked to the acceleration of glaciers retreating. De Angelis and Skvarca (2003) found five out of six major glaciers surveyed on the Peninsula were moving at a rate eight times faster than prior to ice shelf collapse.

The Southern Ocean is poorly sampled in comparison to global ocean sampling, data prior to 1950 is scarce or unreliable and the logistics of satellite sampling is difficult due to the presence of sea ice (Mayewski 2009). However, the trend of increasing water temperature continues in the Southern Ocean. The Antarctic Circumpolar Current mid depth (700 – 1100 metres) temperature has risen by 0.2°C (Robinson 2008), the warming of the ACC may be

impacting waters further south, the Warm Deep Water of the Weddell Gyre has increased by 0.3°C between 1970 and 2000 and it has been hypothesised that the increased temperatures in the ACC contributed to that warming. Antarctic Bottom Water which exports to the South Atlantic has shown decadal warming, the source of this warming is theorised to be due to warming of the cold Weddell Sea Deep Water which is readily distributed north.

Salinity is also important in the Southern Ocean, as at cold temperatures density is regulated by salinity, therefore freshwater input greatly affects stratification, geostrophic currents and mixed layer depth (Mayewski 2009). Boyer et al. (2005) showed a freshening of the Weddell and Ross Seas, noting large decreases in salinity below 70°S.

Corresponding with increased atmospheric temperature, sea surface temperatures in waters adjacent to the Antarctic Peninsula have warmed by more than 1°C in the last 50 years, with models predicting further increase of 2°C in the next 100 years (Somero 2010). Meredith and King (2005) investigated the extreme temperature increase and postulated the water temperature increase, coupled with strong summer salinification, has contributed to a significant reduction in sea ice at the Peninsula since the 1950s. The study concluded the warming patterns observed on the Peninsula are a positive feedback process enhancing atmospheric warming and the reduction in sea ice.

The Southern Ocean plays centre stage in the circulation of the world's oceans, with historical variations in the circulation of the Southern Ocean are believed to have played a fundamental role in driving past global climatic events (Mayewski 2009). The dense Antarctic Bottom Water drives global thermohaline circulation. Some models predict the Southern Ocean initiated breakdown of global thermohaline circulation if Antarctic Bottom Water temperature continues to rise, projecting a 7°C rise in global ocean temperatures (Robinson 2008). The effect of changing circulation and temperature patterns on sea ice is hard to establish, sea ice thickness is hard to measure and is limited to point measurement compilations and therefore no real observations on sea ice have been made. Total sea ice cover has shown no significant decrease, in fact sea ice cover reached a new maximum in 2013 (NASA 2013), however, regional variation has been observed in areas such as the Peninsula (mentioned above) (Mayewski 2009).

The alteration of Southern Ocean temperature, circulation and sea ice extent will have varying impacts on marine species and communities. In some cases if temperature increases species may simply migrate (Peck et al. 2010; Clarke et al. 2012), however, this is not a viable strategy for sessile species or those living at the southern limits (Somero 2010). Reductions in sea ice will reduce productivity in communities that inhabit that sea ice and reduced stratification may diminish the overall productivity of the Southern Ocean (Maxwell et al. 1998). The effects of this are already being observed on the Peninsula; the stock of *Euphausia superba* a krill inhabiting the Scotia Sea, has declined by over 50%, this reduction is believed to be directly associated with the regional decline of sea ice, a major component of this species habitat (Robinson 2008). This species is a key prey item in the Southern Ocean food web; further reductions in sea ice may have massive ecological implications altering distribution of organisms and community structures (Meredith and King 2005).

1.4 Notothenioidei

Fish are the most abundant of all vertebrates. According to FishBase, currently more than 32 000 species from 445 families have been identified worldwide (Froese and Pauly 2011). Although the Southern Ocean covers a large geographical area, the fish fauna within it is less represented than may be expected. The Southern Ocean comprises 10% of the world oceans; however the fish fauna within it are representative of only 1.3% of the globally described species (Coppes Petricorena and Somero 2007). Of that 1.3%, the highly endemic Perciform suborder Notothenioidei is the dominant group in both biomass and species number (Sidell and O'Brien 2006).

Notothenioids are represented over nearly all ecological niches within the entire Southern Ocean representing 35% of all Antarctic fish (Bilyk and DeVries 2010). Benthic species are the major component of the fauna, constituting 55% of the fauna in sub zero continental shelf and slope areas (Coppes Petricorena and Somero 2007; Garofalo et al. 2009). Notothenioids are endemic to the Southern Ocean and several of the surrounding oceans, the shoreline of the continent is the southern boundary of the taxon (Bilyk and DeVries 2010), while some members can be found north in the lower latitude cold temperate waters of New Zealand, Australia and South America (Eastman and Eakin 2000). Notothenioids are the only teleosts found in shallow water environments in the Southern Ocean (Aronson et al. 2011).

The evolution of the Antarctic fish fauna is widely accepted to correspond with the formation of the ACC approximately 22 million years ago (Kock 2005). Notothenioids most likely evolved from a small ancestral benthic fish, lacking a swim bladder, a characteristic of the fish fauna in the temperate continental shelf preceding the cooling of the Southern Ocean (Garofalo et al. 2009). High endemism, biomass and speciation coupled with ecological and morphological diversity are distinctive of an ichthyofauna species flock (Patarnello et al. 2011). Reoccurring retreating of the icesheets coupled with a lack of competing fauna provided new habitat (Verde et al. 2003) allowing a rapid diversification to occur within the notothenioids. Dating of the radiations is debated in the literature. However, morphological evidence and modern DNA techniques corresponding with accepted dates of ocean cooling suggest the radiation occurred between 15 – 10 mya (Near et al. 2005).

The current Notothenioidei comprises of eight families and 43 genera, of which six families can be found below the Polar Front (Figure 1.3) (Garofalo et al. 2009). The three small families, Bovichtidae, Pseudaphritidae and Eliginopidae, consist of temperate water species distributed in the southern waters of New Zealand, Australia and South America (Eastman 2005). Molecular and biochemical evidence show these families lack genes required for antifreeze glycoprotein synthesis, suggesting they established before the cooling of the Southern Ocean prior to the radiation of Antarctic notothenioids, and did not evolve the adaptations required for survival in cold water. Among the families Channichthyidae, Harpagiferidae and Nototheniidae 15 species have non-Antarctic distributions. The presence of non functioning antifreeze glycoproteins genes in these non- Antarctic species suggest they have Antarctic origins diverging approximately 11mya when the Antarctic Convergence moved northward. All members of Artedidraconidae and Bathydraconidae are endemic to Antarctic waters (Coppes Petricorena and Somero 2007).

The success of notothenioids has been facilitated by the ability of the group to survive and thrive in water that is predominantly below 0°C. Physiological, biochemical and morphological modifications have evolved in the group to allow success at cold temperatures (Davison 2005). These adaptations include buoyancy variation, enzyme and lipid adaptations, haematological properties, metabolic cold adaptation, heat shock protein synthesis and perhaps most crucially, the synthesis of anti-freeze glycoproteins (Garofalo et al. 2009).

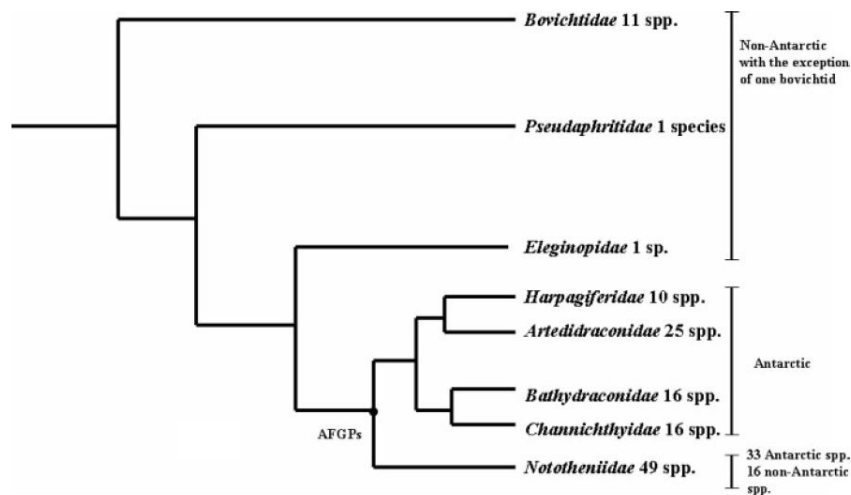


Figure 1.3 Phylogenetic relationship of the Perciform suborder Notothenioidei to the level of family. Adapted from Verde et al. 2008.

1.5 Notothenioidei Adaptations

1.5.1. Buoyancy

Neutral buoyancy conserves energy due to the physical mass of a neutrally buoyant fish being equal to the mass it displaces in the water column; therefore no energy is required to provide hydrodynamic lift (Eastman 1993). Many marine teleost fishes are neutrally buoyant due to the presence of a swim bladder. Approximately 5% of the fish's bodyweight is made up of the swim bladder, therefore a fish lacking a swim bladder or buoyancy modifications has to support that 5% of its body weight (in air), in the water. Although they have radiated and inhabit a variety of niches within the water column swim bladders are absent in all notothenioids, presumably a trait retained from the hypothesised benthic ancestor, (Eastman and DeVries 1982, Near et al. 2012). The morphology and distribution of Notothenioidei species corresponds with the buoyancy and weight of each species skeleton; species found in pelagic habitats have greater buoyancy and fusiform body shape compared to their benthic counterparts (Eastman 1993; Patarnello et al. 2011). Skeletal paedomorphy, the reduced skeletal ossification with the presence of cartilage, increases buoyancy allowing movement into the pelagic water column (Near et al. 2012). Eastman and DeVries (1982) compared the buoyancy and skeletal mass between notothenioids at different depths; they found that as

species habitat deviated from the benthos neutral buoyancy increased and skeletal weight decreased (Table 1.1).

Table 1.1: The buoyancy, ashed skeletal weight and position of 3 Antarctic notothenioid species in the water column. Adapted from Eastman and DeVries 1982.

Species	Buoyancy	Skeletal Weight	Habitat and Depth
Nototheniidae	(Wt. in seawater / Wt. in air) x 100	(Ashed skel. wt. / Total body wt.) x 100	Range (m)
<i>Dissostichus mawsoni</i>	0	0.59	Pelagic: 300 – 500m
<i>Pagothenia borchgrevinki</i>	2.75	0.69	Cryopelagic near platelet ice: 0 – 6m
<i>Trematomus bernacchii</i>	3.37	1.08	Shallow and deep water benthic: 30 – 550m

Other adaptations for buoyancy include ctenoid scales, which are weakly mineralised in neutrally buoyant pelagic species and extensive lipid deposits (Eastman and DeVries 1982; Pörtner 2006).

1.5.2. Enzyme Adaptations

Enzyme functionality is sensitive to temperature because stability and flexibility in distinct areas of the protein must be maintained at equilibrium. Molecular elasticity is required to preserve a suitable catalytic rate, while stability is necessary for the geometry of the active site of the enzyme to ensure substrate recognition (Coppes Petricoreno and Somero 2007). Research suggests that many teleost enzymes have a thermal optimum range of 20-40 °C. In contrast Antarctic fish enzyme thermal optimum is close to 0°C (Kawall et al. 2001). Theoretically at lower temperatures, such as those exhibited in Antarctic waters, enzyme catalysis rates should not be fast enough to sustain and regulate metabolic and locomotive processes (Somero 1991). Evidence suggests Antarctic fish have evolved two adaptations to facilitate high rates of enzyme catalysis at low temperatures (Coppes Petricoreno and Somero 2007). Firstly, notothenioids have higher concentrations of intracellular enzymes, particularly those associated with aerobic respiration (Eastman 1993). Secondly, Antarctic fishes have higher catalytic activity at each active site on the enzyme than homologous temperate species,

this increase equates to elevated metabolic activity via pathways of fewer, faster working enzymes (Somero 1991). Crockett and Sidell (1991) compared the maximal activities of two enzymes associated with aerobic metabolism, citrate synthase and cytochrome oxidase, in the Antarctic fish *Notothenia gibberifrons* and a temperate ecotype similar fish *Myoxocephalus octodecimspinosus*. Maximal activities were found to be 1.5 – 5 fold higher in the cold water adapted *N. gibberifrons*.

1.5.3. Lipid Adaptations

Lipid modifications play multiple roles in notothenioid adaptation to cold temperatures. While important in buoyancy, lipids also play an essential role in membrane fluidity which is crucial for efficient cell function in vertebrates. At lower temperatures such as those experienced by Antarctic notothenioids membrane fluidity may be impaired (Pörtner et al. 2007). Homeoviscous adaptation is maintained in notothenioids by increasing the ratio of unsaturated fatty acids in the membrane phospholipids, which have an expanded conformation and lower melting points comparative to saturated phospholipids (Eastman 1993).

Increased intracellular lipid content may increase oxygen delivery to aerobic tissue, function as an oxygen store and decrease cytoplasmic viscosity (Sidell 1991). Muscular hypertrophy, increasing the diffusion distance for oxygen between mitochondria and capillaries is associated with cold acclimated fish species (Egginton et al. 2002). Oxygen solubility is four fold greater in lipids than in aqueous cytosol, therefore an increase of lipid droplets and mitochondrial densities decreases diffusion distances and increases oxygen flux in notothenioids (Eastman 1993). Lipid may also function as an intracellular oxygen store, this adaptation may be important as myoglobin function is either impaired at low temperatures or absent in notothenioids (Sidell and O'Brien 2006).

1.5.4. Haematological Properties

The primary role of haemoglobin is to carry and deliver oxygen to vertebrate tissues (Verde et al. 2008). The notothenioid fish oxygen transport system is characterised by an adaptive reduction in red blood cell count and haemoglobin content (Di Prisco 2000). This is taken to the extreme by the Channichthyidae, the most phylogenetically derived family. The white blooded

‘ice fish’ have very few erythrocytes and are the only vertebrate lacking haemoglobin in the adult life stage (Tota et al. 1997; Pörtner 2006). Notothenioids also have low concentrations of myoglobin in muscle (Eastman 1991) compared to temperate species or in the case of some species is completely absent (Eastman 1991). Increased blood viscosity has potential negative physiological effects creating higher energy demands for the cardiovascular system and impairing delivery of oxygen (Wells 2005). It is widely accepted that the reduction in erythrocyte numbers observed in notothenioids is an adaptation to counterbalance the increase in blood viscosity (Davison et al. 1994). Concurrently, a temperature related increase in oxygen solubility allows more oxygen to be carried in plasma, while metabolic oxygen demand is reduced at low temperatures. These adaptations allow a reduction in the amount of oxygen required to bind to haemoglobin without impaired oxygen delivery, partly compensating for the loss of erythrocytes (Verde et al. 2008). Under times of metabolic demand, such as increases in environmental temperature and exercise challenges, teleost fish are able to increase circulating erythrocytes by releasing additional red blood cells from the spleen (Wells 2005). When stressed, temperate fish exhibit an increase in haematocrit upwards of 60%, the elevation of haematocrit is much greater in red blooded notothenioids. Davison et al. (1994) found that *Pagothenia borchgrevinki* a semi pelagic notothenioid increased haematocrit by more than 120% when exposed to exercise or temperature stressors. The same study showed the benthic sluggish notothenioid *Trematomus bernacchii* increased haematocrit by 70 % when exposed to high temperatures or hypoxic conditions. This study suggests that red blood sequestration is greater in Antarctic teleosts and strongly correlates with lifestyle.

1.5.5. Metabolic Cold Adaptation

Cold adaptation incorporates all aspects of physiology that allows an organism to inhabit polar environments. Early research by Krogh (1914) showed the resting oxygen consumption and locomotion in the goldfish, *Carassius auratus*, decreased exponentially as temperature decreased. This research suggested that at polar temperatures, metabolic rate would not be sufficient to support survival (Kawall et al. 2002). Krogh postulated if polar fish achieved compensation in locomotive ability comparative to sluggish rates exhibited by temperate fish, a similar elevation could be expected in the respiration in those polar fish. Scholander et al. (1953) and Wohlschlag (1957) built on Krogh’s work, and termed the elevation in respiration

of polar fish metabolic cold adaptation (MCA). However, Holeyton (1974) questioned the validity of MCA, suggesting elevated respiration rates observed in polar fish species was an artefact of poor experimental design and interpretational problems. Holeyton (1974) concluded previous studies of polar fish resting metabolic rate did not allow enough recovery time for animals therefore “resting” metabolic levels were actually elevated due to stressors. The study also questioned the validity of the theoretical background of MCA. Holeyton stated that although it may be advantageous to have an increased metabolic range, a high resting or basal metabolic rate suggests a high degree of maintenance costs diverting energy from areas such as reproduction or growth. Hochachka (1988) suggested that polar fish exhibit a two fold increase in basal metabolic rate, not a seven fold increase as suggested in earlier research. Hochachka (1988) stated that maintenance of electrochemical gradients across the cell membrane, which is a costly process (in various tissues and species can account for between 20-50% of the basal metabolic rate), might be a key factor in cold sensitivity in many organisms and/or tissues. In cold sensitive cells, as temperature drops, the cells are increasingly unable to maintain the correct balance between metabolically-linked and diffusion based ion fluxes (e.g. K^+ and Ca^{2+} efflux and influx). This research suggests that polar fish have to increase the number of functional ion pumps to maintain gradients across cells, thus MCA does exist, albeit as an unavoidable cost. Numerous arguments exist in the literature both for (E.g. DeVries and Eastman 1981; Somero 1991) and against (Davison et al. 1990; Drud Jordan et al. 2001) and metabolic cold adaptation is still highly debated in the literature, although it is generally accepted that metabolic rates of polar fish are lower than that of their tropical and temperate counterparts (Johnston et al. 1991).

1.5.6. Heat Shock Proteins

Antarctic notothenioids are well adapted to life at sub-zero temperatures, however when faced with increased temperatures mechanisms employed by other fishes are lacking or greatly reduced (Coppes Petricorena and Somero 2007). The production of constitutive and inducible heat shock proteins (Hsp) as a response to heat stress was believed to be the only known ubiquitous response to stress in all fish species studied (Clark and Peck 2009). Normally when challenged with heat stress, cells synthesise heat shock proteins, a broad family of chaperone molecules. Hsps aid the folding of polypeptides and chaperone the internal movement of polypeptides in normal cell states (Coppes Petricorena and Somero

2007). In regards to their role in heat stress the Hsps function to assist in restructuring proteins that have become denatured due to heat and assist in the proteolysis of permanently damaged proteins (Hofmann et al. 2005). Numerous studies have shown that Antarctic notothenioids display no increase in Hsp 70 mRNA (a widely studied Hsp) and Hsp 70 protein synthesis when thermally challenged (Hofmann et al. 2005, Place and Hofmann 2004: Place et al. 2004). Hofmann et al. (2000) showing categorically that while *T. bernacchii* lack the classic heat shock response, they and two other Southern Ocean species, *Harpagifer antarcticus*, and *Pagothenia borchgrevinki* perpetually express inducible forms of Hsp 70 (Place et al. 2004). However, although Antarctic notothenioids show no upregulation of inducible Hsp, expression of Hsp genes do occur, suggesting a decoupling between high thermal stress and the production of the proteins (Pörtner et al. 2007). Mapping of the phylogeny of notothenioids shows that non - Antarctic New Zealand species *Bovichtus variegatus*, believed to have migrated from cold waters within the last 5 – 20 million years, and *Notothenia angustata* have maintained the ability to upregulate the classical heat shock response when thermally challenged. This suggests the classical heat shock response in Antarctic notothenioids was lost after a period of evolution in the cold stenothermic waters sometime in the last 12 million years, as fish were no longer exposed to thermal damage that would elicit Hsp synthesis (Hofmann et al. 2005). Thus maladaptive loss of heat shock response chaperoning protein function has truncated the tolerance of highly stenothermic Antarctic notothenioids to elevated temperatures (Somero 2010).

1.5.7. Antifreeze Glycoproteins

Perhaps the most crucial adaptation in Antarctic notothenioids is the synthesis of antifreeze glycoproteins (AFGPs). Antarctic notothenioids have the highest measured levels of antifreeze protein (AFP) in blood and other body fluids of any teleosts (20-30mg/ml) (Cheng et al. 2006).

This adaptation directly addresses the perpetual threat to survival of freezing to death in sub zero waters (Coppes Petricorena and Somero 2007). The freezing point of a solution is controlled by the osmolality of the medium. Salt concentration of seawater is approximately 1030 mOsm, this salinity lowers the freezing point of seawater to -1.9°C (DeVries and Cheng 2005). Marine teleost fish living in polar regions have higher NaCl plasma content than temperate species (DeVries 1988), the highest reaching levels up to 600 mOsm (Hudson et al.

2008), these levels depress the freezing point equilibrium of the fish to approximately -1.1°C (DeVries and Cheng 2005). Many notothenioid fish spend their entire lives at temperatures below the expected freezing equilibrium, thus to survive in waters habitually close to -2°C and as low as -2.2°C a mechanism, the ability to synthesise AFGPs evolved further depressing the internal freezing point of Antarctic notothenioids. (DeVries and Steffensen 2005). Cheng et al. (2006) showed that in notothenioids production of AFGPs occurs in the pancreas and anterior segment of the stomach, rather than hepatic synthesis as previously proposed. Four distinctive classes have been described (Fletcher et al. 2001); however all AFGPs function in a similar manner and are composed of a repeating alanyl-alanyl-threonine basic glycopeptide unit ranging between four and 55 repeats. AFGPs reduce freezing points of notothenioids via adsorption-inhibition (Garner and Harding 2010). Ingested food poses a danger of acute ice formation, AFGPs travel through the gut cavity and bind to the face of the ice, adsorbing onto the ice crystal acting to inhibit the growth of the lattice (Eastman 1993). AFGPs change the formation of ice growth from hexagonal to specular and environmental temperature must be lowered before growth of the ice lattice can resume. This creates a hysteresis between the observed freezing point and the equilibrium melting point (Garner and Harding 2010), thus depressing freezing point of the fish while keeping crystals small, and preventing damage (DeVries 1988; Fletcher et al. 1998). The thermal hysteresis (the difference between the observed melting point and observed freezing point) is commonly $1 - 2^{\circ}\text{C}$, allowing Antarctic notothenioids to survive and thrive in -1.9°C temperatures (Garner and Harding 2010). It is unclear how antifreeze moves from the gut into circulation or how the ice crystals are excreted (Coppes Petricorena and Somero 2007), but it is assumed that they must be excreted or metabolised via an unknown mechanism because if AFGP bound to ice crystals were perpetual, accumulation would impair cell function (DeVries 1988).

AFGPs are permanently expressed in 5 families of notothenioids (Nototheniidae, Harpagiferidae, Artedidraconidae, Bathydraconidae and Channichthyidae) and are absent or greatly reduced in the temperate water notothenioid families Elegendinidae, Bovichtidae and Pseudaphritidae and provide insight into the evolutionary history of the notothenioid Perciform.

1.6 The Nototheniidae Family

The most diverse family of the notothenioid Perciform suborder is the nototheniids in regards to habitat, distribution, size and body form. Consisting of 13 genera and 49 species nototheniids are distributed throughout the Southern Ocean, Subantarctic waters, 15 non Antarctic species inhabit the coastal regions of South America, Australia and New Zealand (Eastman 2005). The majority of species occupy benthic habitats although numerous species have deviated from the benthos, (assisted by several of the adaptations mentioned previously) and can be found in cryopelagic, semipelagic, epibenthic and pelagic habitats. The diversification of the nototheniids is believed to have occurred recently with the divergence of the *Trematomus* genus, consisting of 15 species diverging between seven and two million years ago (Kuhn and Near 2009, Eastman 1993).

1.6.1 *Trematomus bernacchii* (Emerald Notothen)

Trematomus bernacchii (Fig 1.4) is a benthic fish, endemic to Antarctic waters. It inhabits depths up to 20m and has been found at depths below 700m; however, it is most commonly found in the upper 200m. *T. bernacchii* is a circum-polar species, it has been found in approximately all inshore areas surrounding the continent and some offshore islands in the Southern Ocean including the South Shetlands and Elephant Island.

T. bernacchii is a sexually dimorphic species; females reach upwards of 175mm, while males are slightly smaller reaching up to 145mm, the species is generally long-lived surviving up to 10 and 5 years respectively (Dewitt et al. 1990). *T. bernacchii* is a sluggish inactive species with a comparatively heavy skeleton (Table. 1.1) lacking buoyancy adaptations (Eastman and DeVries 1982), and has a dorso- ventral flattened body shape reflecting its lifestyle. The species has two colour morphs, one is mottled brown while the second morph is mottled brown with a white head plate (Dewitt et al. 1990). The diet of *T. bernacchii* covers a wide trophic spectrum showing variability with location and seasonality; however those inhabiting McMurdo Sound largely consume polychaetes, isopods, molluscs, amphipods, fish eggs and fish (Montgomery et al. 1993; La Mesa et al. 2004). *T. bernacchii* lie and wait for prey to ambush or employ the hunt and peck strategy when catching food (La Mesa et al. 2004).



Figure 1.4: *Trematomus bernacchii* Emerald Notothen

1.7 Antarctic Nototheniidae and Stenothermy

Distribution of organisms is limited by abiotic and biotic influences; organisms can only survive short periods outside their critical tolerance limits (Miller and Stillman 2012). Environmental temperature is crucial in determining the distribution of ectothermic organisms as body temperature mirrors environmental temperature (Somero 2010) and changes in temperature alter the rates at which biological and physiological processes occur. Metabolic scope is the difference between resting and maximum metabolic rate, and is used as an indicator of the ability of an animal to function in its environment, especially the ability to catch food or escape from predators (McBryan et al. 2013). Temperature has a major effect on resting metabolic rate, but a much less effect on maximum rate. Thus as temperature increases, metabolic scope decreases, and current hypotheses suggest that upper lethal temperatures are at least in part defined by the point where metabolic scope becomes too small to allow activity, in particular when any activity requires a move into anaerobic metabolism (Pörtner and Lannig 2009). When oxygen supply becomes limiting above or below critical temperatures, resting and maintenance metabolism utilise and exceed all metabolic scope and death of the animal occurs (Robinson 2008).

Differentiating between primary, secondary and tertiary mechanisms that lead to thermal death is challenging. Several mechanisms have been suggested including the denaturation of the secondary and tertiary structure of enzymes, disrupting cell activity resulting in necrosis and ultimately organism death (Seebacher et al. 2005; Somero 2010). The collapse of physiological systems due to thermal stress also results in death of the organism, for example, within the cardiovascular system as temperature increases and approaches lethal thermal limits cardiac function abruptly decreases creating a mismatch in oxygen supply and demand resulting in organism death (Somero 2010). However, determining which processes are ultimately responsible for organism death is difficult as processes leading to death are undoubtedly linked.

Acclimation and acclimatisation is the adjustment of an organism to abiotic changes in the surrounding environment. It can be measured by a multiplicity of mechanisms including; long term survival, stabilisation of oxygen consumption, thus metabolic rate (Robinson and Davison 2008b), the ability to perform standard functions e.g. processing food and the modification of gene expression. Acclimation is the organism's behavioural or physiological response to change in one variable in a laboratory setting, acclimatisation is the physiological or behavioural adjustment to the organism in the natural environment to several abiotic variables. Both of these processes require long term stability in physiological condition in the study organisms before and after the variation in external environments (Peck et al. 2010). Acclimation is generally considered to be successful when at both ambient and acclimation temperatures, mechanisms (as mentioned above) either mirror each other. E.g. Heart Rates are the same at both temperatures. Or, when mechanisms have stabilised at new levels above or below ambient rates. Acclimation has been associated with modification of thermal tolerance in some Antarctic marine species (Pörtner et al. 2007).

Evolutionary theory predicts that specialisation to optimal performance within a narrow temperature range should occur to organisms living in habitats where temperature is stable (stenothermy), while those organisms inhabiting environments with variable temperatures can acclimatise to different ambient temperatures (Seebacher et al. 2005). The selection pressure on organisms inhabiting fluctuating environments favour those with strong acclimatory and phenotypic plasticity in regards to temperature, those species are termed by Huey and Hertz (1984) "Jack of all temperature, master of none".

Many ectotherms inhabiting temperate areas experience daily and seasonal variations in temperature and thus are eurythermal. Phenotypic plasticity in eurythermal organisms permits adjustment to physiological processes in response to increases and decreases in temperature. This allows eurytherms to succeed well below thermal optima (Miller and Stillman 2012).

In comparison, the endemic ectotherms of the Southern Ocean have inhabited extremely thermostable waters for several million years (see above); as a result they have become extremely specialised (Robinson and Davison 2008a). Due to the distinctive physiological challenges that occur with inhabiting Antarctic waters, resident organisms have evolved numerous adaptations to exist in such extreme conditions. In conjunction with those adaptations covered above, slow growth rates, gigantism, slow rates of activity, (Clarke and Peck 2009), low metabolic rates, slow larval growth and reduced fecundity (Aronson et al. 2011) have also occurred as a result of occupying cold stable water. Isolation and adaptation to the physical environment have resulted in Antarctic marine species living at the edges of an extreme, narrow adapted range (Bargagli 2008). The lowest possible metabolic rates in nototheniids are measured at -1.9°C , the point at which seawater freezes (Johnston et al. 1991), while the upper lethal limits have been reported as low as 6°C (Somero and DeVries 1967), this narrow temperature range is further reduced when pejus temperatures, the range in which for nototheniids can perform vital biological function such as feeding and reproduction are considered.

In those Antarctic species studied the ability to acclimate appears to be non – existent or limited to very narrow temperature range (Clarke et al. 2012), and disparity occurs between phyla, Antarctic marine invertebrates appear to have lower acclimation ability and ranges than their vertebrate counterparts (Peck 2002). Peck et al. (2008) showed the brittle star *Ophionotus victoriae* is an extreme stenotherm. Acclimation to 3°C and 2°C resulted in 100% mortality, all animals bar one died within 32 and 68 days respectively. However acclimation ability is variable, the starfish *Odontaster validus* can survive up to 12°C and showed greatest function at 7°C suggesting this species would flourish in warmer waters.

Although some nototheniids may be able to use behavioural strategies to cope with increasing temperatures, for example, those located at the Peninsula may be able to move south towards cooler waters; the presence of the continent sets a boundary to those habitat shifts (Aronson et al. 2011). A handful of studies have shown some species of nototheniid have been successfully acclimated (Lowe and Davison 2005; Seebacher et al. 2005; Robinson and

Davison 2008a); Robinson and Davison (2008b) showed perfect oxygen consumption and ventilation compensation in *Pagothenia borchgrevinki* when acclimated to 4°C for 28 days. However with over 4000 Antarctic marine animals described (Peck et al. 2004) *P. borchgrevinki* may be the exception to the rule (Robinson and Davison 2008b).

The extreme stenothermy and specialisations of Antarctic nototheniids to stable cold water may have led to a high level of disaptation followed by a rapid adaptive recovery and an evolutionary loss of function on physiological processes vital to surviving and thriving in warmer waters (Garofalo et al. 2009). With increasing water temperatures due to climate change effects expected, the resulting genetically fixed upper temperature limits of nototheniids may mean the extinction of many species over the next few centuries is a real possibility (Somero 2010).

1.8 Thesis Aims

The extreme adaptations and stenothermy observed in the majority of Antarctic species thus far investigated suggests that physiological plasticity and acclimation ability to even slight temperature increases is very limited. Studies investigating the ability of a single species to physiologically compensate when exposed to acute and chronic increases in temperature are limited.

This research aims to investigate the metabolic scope of the Nototheniid *Trematomus bernacchii*, in particular, the species ability to acclimate to increased temperature and evaluate the difference between acute and acclimated animals when presented physiological challenges over several temperatures. Acclimatory ability was assessed in fish acclimated for four weeks to 3°C and the ability of *T. bernacchii* to complete challenges in acute and chronic temperature exposures was measured.

Specifically three questions were addressed:

1. Does Specific Dynamic Action, the post prandial increase in metabolic rate differ in ambient, acclimated and acute temperature exposure treated fish

2. Do the cardiovascular responses to environmental hypoxia across several temperature ranges differ between ambient and acclimated *T. bernacchii*
3. Does chronic and acute temperature change affect the metabolic scope and cardiovascular responses of *T. bernacchii*

CHAPTER 2

General Experimental Methods

2.1 Experimental Animal Collection and Transportation

Fish were collected over two Antarctic summer seasons, 2011-12 and 2012-13 between October and December. All fish were caught using hand-lines lowered through drill holes in the sea ice. All hooks used were barb-less to allow for easy removal of hook and reduction of physical damage to the animals during capture. Hooks were removed while animals were at the surface submerged in -1.9°C water then quickly transferred to holding tanks to prevent ice damage caused by low air temperatures. Hand-lines were baited with small pieces of fish and Gulp soft bait lures.

Two types of fishing holes were used to collect specimens. A single hole, one metre in diameter was melted through two metres of sea ice using a specialised heated melting coil. An insulated diesel heated wannigan (temporary structure) was transported to the site and located over the fish hole to prevent the hole from freezing (Fig 2.1a). This fish hut was located in McMurdo Sound ($77^{\circ} 51^{\circ}.21.4''$ S, $166^{\circ} 32^{\circ} 46.1''$ E) for the entirety of the 2011-12 summer fishing season. Secondly, during both summer seasons, multiple 10 centimetre diameter holes were drilled using an ice auger (Jiffy drill) (Fig 2.1b). Jiffy drill sites were located close to Scott Base at Cape Armitage ($77^{\circ}51'4.47''\text{S}$, $166^{\circ}44'55.15''\text{E}$) and further afield at Cape Evans ($77^{\circ}37'53.90''\text{S}$, $166^{\circ}24'16.35''$) and Evans Wall ($77^{\circ}38'36.97''\text{S}$, $166^{\circ}29'31.73''\text{E}$), approximately 25 km north of Scott Base. Three holes were drilled; hand-lines were lowered to 10 – 25 metres until the benthos was reached where fish were located (Fig 2.1c). Holes were deemed depleted if, after approximately 10 minutes no bites were felt at any of the fishing holes, at which time, three new jiffy holes were drilled approximately 20 - 50 metres from the previous site. The number of collection points was determined by the amount of specimens caught, the time animals were kept in holding tanks at the site and travel time from the site to the wet lab facility at Scott Base.

Sites were selected based on sea ice condition, knowledge of areas historically known to give high yields, and proximity to the shoreline (Fig 2.2). All animals were transported from collection site to the Scott Base web lab facility in 45 litre insulated tanks and seawater was continuously aerated during transportation. December 2011-12 season fish were flown from Scott Base, Antarctica to a temperature controlled aquarium at the University of Canterbury, Christchurch, New Zealand. Specimens were flown to Christchurch on a Boeing C17 Globemaster III in continuously aerated seawater insulated 45 litre tanks. Temperature was maintained by addition of sea ice; no more than 10 animals were placed in each tank. Flight time was approximately five and a half hours. No mortality occurred during transportation.



Figure 2.1a: Fishing in McMurdo Sound. Semi-permanent insulated Wannigan located near Scott Base.



Figure 2.1b: Drilling a 10 centimetre transient fishing hole in McMurdo Sound.



Figure 2.1c: Fishing in a 10 centimetre transient fishing hole in McMurdo Sound.

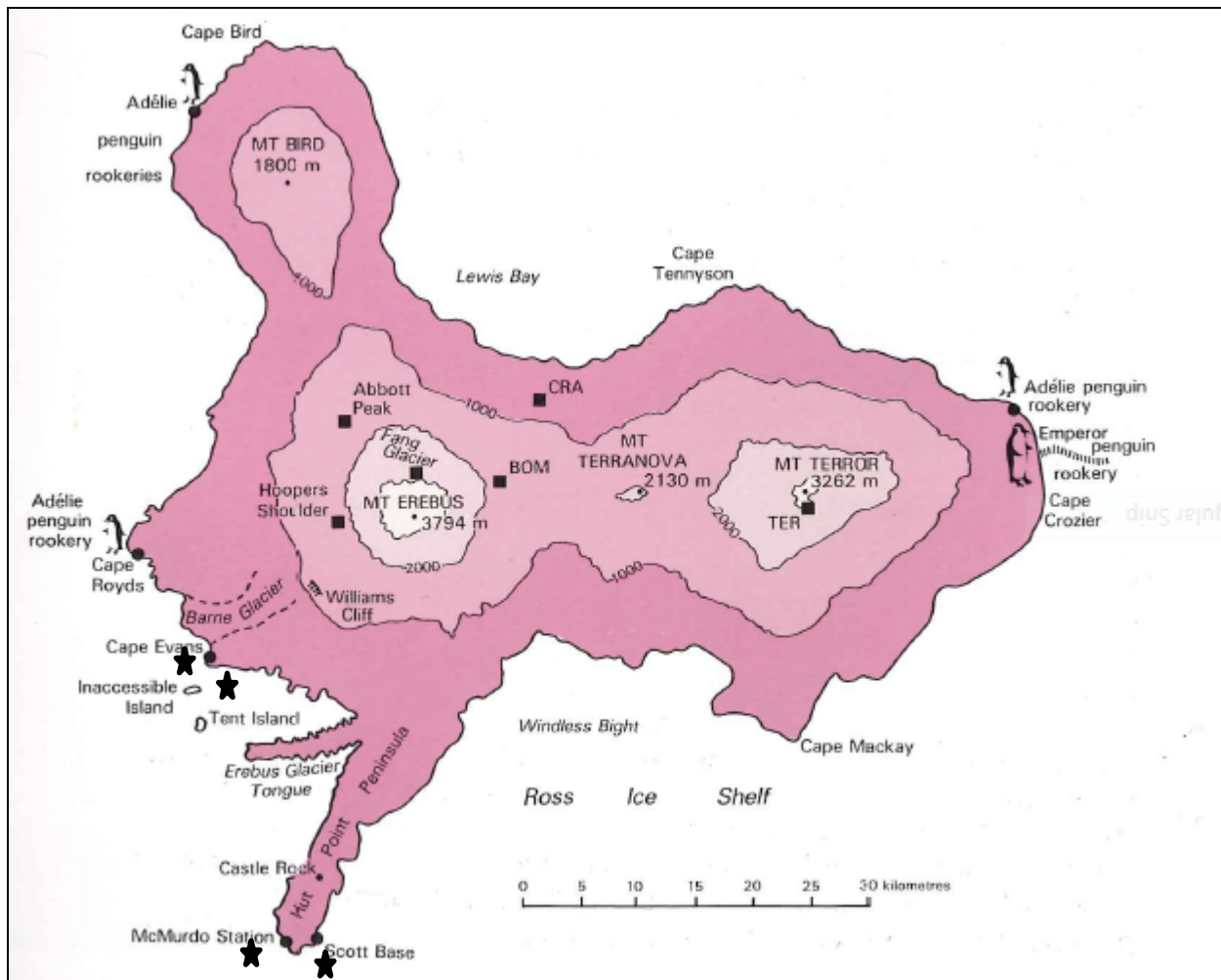


Figure 2.2: Ross Island. Fishing sites are denoted by ★. Figure amended from Hatherton 1990.

2.2 Research and Aquarium Facilities

2.2.1 Research Facilities

Research occurred at two scientific facilities: Scott Base, Pram Point, Ross Island McMurdo Sound Antarctica ($77^{\circ}51'0.01''\text{S}$, $166^{\circ}46'6.17''\text{E}$), the New Zealand Antarctic Programme, and the University of Canterbury, Christchurch, New Zealand ($43^{\circ}31'21.47''\text{S}$, $172^{\circ}35'2.30''\text{E}$).

2.2.2 Scott Base Wet Lab Facilities

Specimens were transported to Scott Base and immediately relocated to the Wet Lab facilities. Fish were housed in a flow through aquarium system consisting of 12, 60 litre tanks. Water temperature was maintained at -0.8 ± 0.3 °C, salinity at 35 ‰ and on a 24 hour photoperiod corresponding to the ambient summer Antarctic light regime. All animals were held for at least four days before experimental use to allow recovery from any stress caused during capture and transportation to aquaria. No fish were fed while housed at Scott Base.

2.2.3 University of Canterbury Aquarium Facilities

Fish were housed in a specialised Antarctic Room facility in a dual isolated recirculating seawater system at the University of Canterbury. Each system was maintained with seawater collected from Lyttelton Harbour, Banks Peninsula (43°36'39.15"S, 172°42'13.49"E). Seawater temperature was held at -0.1 ± 0.3 °C by circulating air temperature maintained between -2 and 0.0°C and salinity was kept at 35‰. Water quality was maintained with biological filters, cleaning and regular water changes. Photoperiod was maintained with 24 hour light cycle simulating Antarctic summer conditions. Fish were fed pieces of fish, common warehou (*Seriotelella brama*), every 7 – 10 days; experimental fish underwent a different feeding protocol explained in the materials and methods in the chapter concerning Specific Dynamic Action.

2.3 Temperature Acclimation Protocol

2.3.1 Scott Base 3° C Temperature Acclimation

Fish ranging in mass between 120 – 180 grams were selected at random and placed in a seawater 300 litre static tank held at -1.8°C; water temperature was increased by 0.1°C per hour until 3°C was reached and held constant for the remainder of the acclimation period (3.3 ± 0.4 °C). Water was heated using two heat exchangers connected to a fresh water tank maintained at 3°C by a water heater. Water was refreshed at least twice per day by removing and replacing at least half the volume of water with 3°C seawater. No significant

modification of water temperature occurred during the water change process. Once 3°C was reached, animals were acclimated for a minimum of 3 weeks.

2.3.2 University of Canterbury 3 °C Temperature Acclimation

Fish were selected at random and moved to one independent circulating system in the Antarctic Room facilities. As the room, thus water temperature was air temperature controlled, 3 × 30°C submersible aquarium water heaters were used to heat aquaria. Water temperature was checked daily and maintained at $3 \pm 0.1^\circ\text{C}$. Water quality was monitored and changed using 3°C seawater; this made no significant modification to water temperature.

2.4 Respirometry Protocol

Respirometry was the technique employed to obtain all measurements in this study. Two forms were used: Continuous sampling and periodic sampling respirometry.

2.4.1 Continuous Sampling Respirometry

Closed boxed respirometry procedures were used. Respirometers consisted of an airtight opaque PVC cylinder-shaped chamber with a clear Perspex lid. Respirometers were positioned in a 20 litre water bath maintained at a constant temperature determined by individual experiments. Oxygen partial pressure, (PO_2), was measured using a Strathkelvin oxygen electrode with polypropylene membrane attached to a Strathkelvin oxygen meter (models IL 1302 and 781 respectively; Glasgow, Scotland). The electrode was fitted in the lid of the respirometer via an airtight bung, allowing continuous sampling. In experiments requiring heart rate and ventilation traces, electrocardiogram (e.c.g) electrodes were inserted subcutaneously close to the heart of the experimental animals (explained below in 2.5 Surgery Protocol). The e.c.g electrodes were fed through the lid of respirometer from the animal to an Animal Bio Amp (model 136) through the same opening as the oxygen electrode. The oxygen meter and Animal Bio Amp were connected to a PowerLab data acquisition system (model 26T), raw data was filtered to display heart (e.c.g) and ventilation (deviation from baseline) signals on a laptop computer using Chart v.7.3 software (Animal Bio Amp, PowerLab and Chart all ADI Instruments, Waverly, N.S.W, Australia) (Figure

2.3). This system allowed continuous recording of oxygen tension, fish heart rate and ventilation rate.

Routine fish activity in the respirometer was limited and sporadic; therefore a small galley pump (model 06302, 378 L per hour; TMC Technology Corp., Taiwan; run at 4v on an adjustable voltage adapter) was placed in the water bath connected via tubing to the respirometer. This flow provided sufficient mixing, but was slow enough to not disturb the fish. The respirometer was opened by detaching tubing from the inflow of the galley pump, allowing flushing of the respirometer with fully oxygen saturated seawater. The point at which the respirometer was flushed with oxygen saturated water was specific to each experiment and is described in the relevant chapter methods section. Two volumes of respirometer were used: 1.4 litres and 1.6 litres.

2.4.2 Periodic Sampling Respirometry

Closed box respirometry was used. Each respirometer was placed in individual 30 litre flow through water baths. As above, respirometers were connected to a small galley pump to allow sufficient mixing to allow a consistent PO_2 reading. Respirometer lids had dual 30mm diameter ports sealed with bungs bearing 20 gauge needles. To prevent damage to fish sharp ends of the needles were blunted and covered with cannula tubing. Use of ports was twofold: to remove air bubbles prior to sealing with as little disturbance to fish as possible. Secondly for sampling: A 1 ml water sample was extracted from the chamber using a syringe. The sample volume was replaced in the respirometer via suction from a reservoir 20 ml syringe located on the opposing side of the lid to avoid sampling of reservoir water. The sample was injected into the sample chamber of a water jacketed oxygen microcell, water from the recirculating experiment system was passed through the water jacket to maintain the constant temperature of samples. The microcell housed an oxygen electrode connected to an oxygen meter (Strathkelvin model MC 100, IL 1302 and 782 respectively). After injection of the sample the oxygen meter was given two minutes to stabilise after which a reading was taken. Oxygen consumption was determined daily (unless stated otherwise in relevant chapters methods), by determining the difference in oxygen tension during a 1 hour period. The first sample was made directly after sealing respirometers, the second occurred after 60 minutes at which point the respirometer would be opened until the subsequent run 24 hours later.

To determine background oxygen consumption a complete system was run without a fish for each experiment, this was determined to be minimal in every treatment.

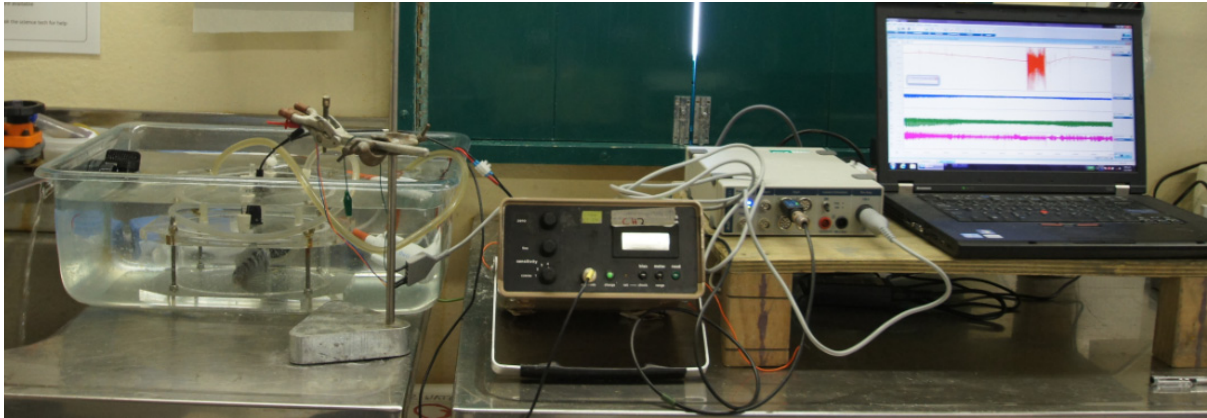


Figure 2.3a: Continuous sampling respirometer experimental set up at Scott Base Wet Laboratory Facilities, Antarctica.

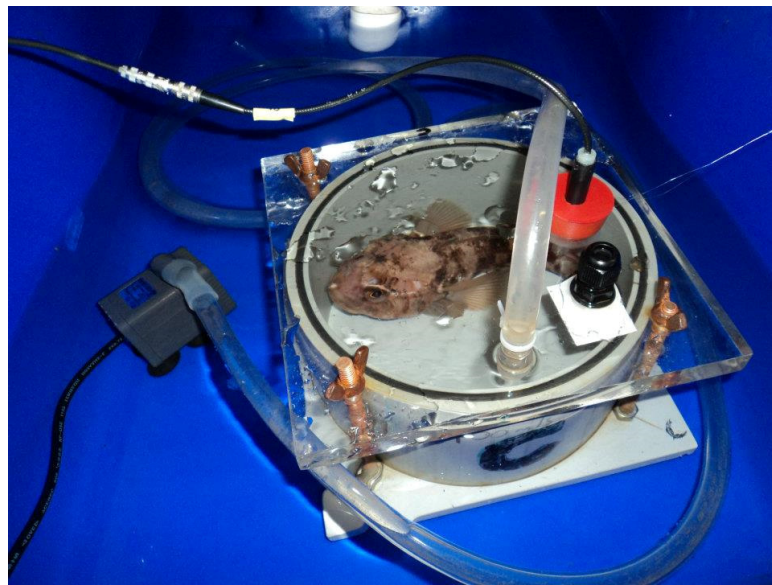


Figure 2.3b: Respirometer and water bath set up with fish.

2.4.3 Calibration of Respirometry Equipment

The oxygen meter and electrode were calibrated prior to each experiment. The zero level was calibrated using an oxygen free solution of 3.80 g. l⁻¹ sodium borate, 1 g. l⁻¹ sodium sulphite. Aerated seawater was used to obtain the upper level, according to the equation:

$$PO_{2(AS)} = 0.2094 (P_B - P_{WV})$$

Where:

$PO_{2(AS)}$ = the air saturated partial pressure of oxygen (mmHg) in air saturated solution,

0.2094 = mole fraction or volumetric fraction of O_2 in the atmosphere,

P_B = barometric pressure (mmHg), and

P_{WV} = water vapour pressure at the specified experimental temperature (mmHg).

2.4.4 Calculation of Metabolic Rate

Preceding the beginning of all experiments air bubbles were removed and respirometer was sealed with as little disruption to the fish as possible.

Metabolic Rate (MO_2 , $mg.O_2.kg^{-1}.h^{-1}$) was calculated using the following calculation:

$$MO_2 = \left(\frac{\Delta PO_2 \times 1 \times C \times \left(V - \left(\frac{M}{1000} \right) \right)}{t \times M} \right) \times 31.99$$

Where:

ΔPO_2 = change in oxygen partial pressure over the incubation period (mmHg),

C = oxygen capacitance of seawater ($\mu mol\ l^{-1}.mmHg^{-1}$),

31.99 = molecular weight of oxygen,

V = volume of water in the respirometer (l),

t = time interval between measurements (h), and

M = mass of the fish (g)

2.4.5 Calculation of the Q_{10} Temperature Coefficient

Q_{10} values were calculated using the equation:

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

Where:

R = the Response Rate,

T = the Temperature (Celsius or Kelvin) ($T_1 < T_2$), and

1 and 2 = the corresponding Rate to Temperature

2.5 Surgery Protocol

Heart rate and ventilation measurements were taken using electrocardiogram (e.c.g.) electrodes inserted subcutaneously. Fish were anaesthetised in 0.1 grams per litre of seawater MS - 222 (3-aminobenzoic acid ether; Sigma) and placed on an operating sling. Two hooked e.c.g. electrodes were inserted using a 23 gauge needle ventrally near the heart. E.c.g. electrodes were secured in place by ventral and dorsal sutures. Post- surgery fish were placed in open respirometers and left to recover for 24 hours (Robinson et al. 2011).

2.6 Ethical Consent

All experiments undertaken in this study were performed with the approval of the University of Canterbury Animal Ethics Committee, permit number 2011/08.

2.7 Statistical Analysis

All statistical analysis was executed on GraphPad Prism 5 for Windows (GraphPad Software Inc., San Diego, U.S.A) and SigmaStat 3.5 (Systat Software, San Jose, CA, U.S.A). Throughout this study, unless otherwise stated, statistical significance is set to $p < 0.05$.

CHAPTER THREE

The Effects of Chronic and Acute Temperature Change on the Specific Dynamic Action of *Trematomus bernacchii*

3.1. Introduction

In order to maintain life all heterotrophic animals are dependent on an external reoccurring supply of food. Regardless of what an organism consumes, food intake provides essential nutrients, the organic raw materials for biosynthesis of complex molecules and fuel to power cellular metabolism based on the oxidation of energy rich fuels, carbohydrates, fats and proteins (Jobling 1993). These materials are converted and utilised for metabolic processes and growth of new tissue (Vanella et al. 2010).

The metabolic cost associated with food intake was first described in 1902 by Max Rubner. He recognised that heat produced, thus metabolic rate, varied between meal portions in fasted dogs. This metabolic cost is recognised as an increase in energy expenditure that occurs during ingestion and assimilation of a meal, and continuing from Rubner's research has been investigated and quantified in many vertebrate and invertebrate taxa (Secor 2009). The mandatory rise in metabolic rate associated with postprandial physiological processes has been labelled Specific Dynamic Action (SDA), the Heat Increment of Feeding (HIF), (Vanella et al. 2010), diet-induced thermogenesis (DIT) and the thermic effect of feeding (Secor 2009). Typically SDA is characterised by a sharp rise in oxygen consumption and heat production following a feeding event, reaching a species specific peak level, followed by a steady decline until metabolic rate returns to resting levels (Peck 1998; Luo and Xie 2009).

Two methods are typically used to measure metabolic rates. Direct calorimetry is a measure of heat production and is commonly employed when measuring the metabolic rates of birds and mammals. However, as the metabolic rates in fish are usually low and heat capacity of water is high this method is not commonly used when measuring metabolic rates in fish (Cech. 1990). Oxygen consumption as an indirect measurement of metabolic rate is used to measure the metabolic cost of SDA (Jobling 1993). Oxygen consumption is a measure of the

rate at which energy reserves are converted, and utilised as fuel via aerobic pathways. The aerobic regeneration of ATP requires oxygen; thus the need for oxygen is driven by biological and mechanical processes and the rate at which those processes occur (Clarke 1998). All animals have a minimum level of energy required to maintain basic metabolic processes essential to sustain life, known as basal metabolic rate. However, it is not pragmatic to measure metabolic rate at basal levels as it requires an exact balancing point at which food intake balances body mass, therefore in the present study, resting metabolic rate is used to describe fish in a quiescent, non active resting state (Cech 1990; Clarke and Fraser 2004). Due to the absence of food no part of the metabolic rate is constituted by processes associated with SDA (Jobling 1993). SDA has been defined numerous times in the literature (Secor 2009), the definition in this study is the accumulated energy expended from the ingestion, digestion, absorption and assimilation of a meal (Jobling 1994).

Numerous hypotheses have been proposed to explain the physiological processes that generate the SDA response. In fish those proposed are biochemical and/or mechanical processing of food, production of waste products and macromolecule synthesis (Secor 2009). It is generally accepted that the rise in metabolic rate can be grouped into three categories: (1) Preabsorptive processes including gut peristalsis, protein catabolism and intestinal remodelling; (2) Absorptive processes, including nutrient transport across digestive membranes and intestinal absorption; (3) Postabsorptive processes which include general costs associated with growth, protein synthesis, ammonia production and renal extraction (McCue 2006). Physiological processes and energetic costs are difficult to attribute to one category as processes are predictably connected (Secor 2009). For example, the intestine is responsible for both peristalsis and absorption; preabsorptive and absorptive processes respectively. The associated metabolic costs of SDA are therefore difficult to attribute *in vivo* (McCue 2006). However, the energetic cost of feeding and peristalsis, the mechanical component of SDAs are now generally accepted as being small (Boyce and Clarke 1997), with the postabsorptive component of the response generally accepted as constituting the highest energetic cost. This has been shown in studies where nutrients and amino acids intravenously dispensed to catfish, starfish and alligators have increased metabolic rate to levels mirroring those observed in SDA response studies of those species (Peck and Veal 2001).

SDA parameters vary between taxa. In reptiles between 17 and 20% of ingested energy is allocated to SDA, in amphibians this increases to over 23% (Secor 2009). In fish the proportion of the total energy budget allocated to SDA is substantial; the energetic cost ranges from five to 20 percent of total ingested energy (Fitzgibbon et al. 2007).

In ectotherms such as fish environmental temperature is one of the most significant variables affecting metabolic rate (Vanella et al. 2010). Metabolic rates and biochemical processes are directly affected by environmental temperature, increasing as temperature rises (Clarke and Johnston 1999; Seebacher et al. 2005), the resting oxygen consumption of polar fish inhabiting 0°C waters is between 8 to 27 times lower than that of fish living in 30°C water. Factorial scope is defined as the maximum metabolic rate divided by resting metabolic rate (Robinson and Davison 2008b; Chapter 1.7); the majority of studies to date have concluded environmental temperature has very little impact on SDA factorial scope in fish; species that share lifestyles, habitats and behaviours have similar scope regardless of latitude (Johnston and Battram 1993; Secor 2009). The SDA response in marine fish typically has a peak factorial SDA response two to five times higher than pre-feeding resting metabolic rates (McCue 2006) and studies so far suggest this to be true of Antarctic fish (Peck and Veal 2001; Secor 2009).

Duration of the SDA response is temperature dependent. Assuming the total amount of energy assigned to SDA does not vary between tropical, temperate and cold water species, the total metabolic energy expended in the SDA response theoretically should not change with body temperature (Secor 2009). Evidence suggests this is true of the SDA response in Antarctic marine ectotherms whose SDA is characterised by lower peaks and longer durations when compared to temperate and tropical species (Peck 1998). Johnston and Battram (1993) investigated the length of the SDA response in fish from the Southern and Indian Oceans. They found the extent of the SDA in the Antarctic species *Notothenia neglecta* was approximately four times longer (208 hours) than an Indian Ocean species of similar lifestyle *Cirrhitichys bleekeri* (57 hours).

What is less clear is the effect of temperature on the energy used during SDA relative to the energy of the ingested food (Vanella et al. 2010), known as the Specific Dynamic Coefficient (McCue 2006). Studies investigating the fishes *Pleuronectes platessa*, *Dicentrarchus labrax*, *Odontobutis obscura* and the Antarctic *Notothenia neglecta* found no significant effect of

temperature on the SDA and SDA coefficient in these species (Jobling and Davies 1980; Johnston and Battram 1993; Peres and Oliva-Teles 2001; Secor 2009). Conversely, numerous studies have shown that altered water temperatures, thus body temperature alter the SDA and the SDA coefficient in fishes *Gadus morhua*, *Sparus aurata* and *Silurus meridionalis* (Guinea and Fernandez 1997; Luo and Xie 2008; Secor 2009).

While the capability of individuals within a species to survive in a specific ecosystem may be directly affected by physiological tolerances, the essential criteria in ensuring a species ability to persist and thrive is the capacity of individuals to complete critical biological functions (Peck et al. 2008). If individuals are unable to perform numerous processes such as locomotion and feeding due to thermal stress, mortality may occur (McGaw and Whiteley 2012). Numerous studies have examined the SDA response and SDA coefficient in different species across different latitudes, and evidence suggests the factorial metabolic scope produced in postprandial marine ectotherms may be stable at water temperatures within a species thermal range (Vanella et al. 2010). However, few studies have investigated the effect of temperature within a species at particular temperatures outside the species natural physiological temperature range over both chronic and acute temperature exposures.

Temperature is considered a major stressor in fish (Hudson et al. 2008). Antarctic nototheniids respond to rises in water temperature by increasing their metabolic rate; however the response is limited by their extreme stenothermy (Aronson et al. 2009) with temperatures above 3°C generally resulting in mortality (Davison personal communication). As SDA is characterised by a sharp increase in metabolic rate and has been quantified as being a prolonged response in polar ectotherms, what is unclear is the effect increases in water temperature may have on nototheniids' already elevated metabolism during SDA and if acclimation to increased water temperature is possible with reference to SDA. Investigation into the SDA response of species within this family over different temperatures is necessary as the extreme stenothermy and limited adaptive abilities of nototheniids (Chapter 1) may make Antarctic nototheniids particularly vulnerable to the metabolic consequences of increasing water temperature.

This research aims to investigate and quantify aspects of the metabolic SDA response within one species of Antarctic fish *Trematomus bernacchii* when exposed to ambient water temperature, an acute elevated temperature and a chronic elevated temperature exposure.

3.2. Methods

3.2.1. Fish

All *Trematomus bernacchii* in this experiment were sourced from McMurdo Sound, Antarctica during the 2011 / 2012 summer season. Fish were collected, transported and housed at the University of Canterbury, Christchurch, New Zealand as described in Chapter 2.2.1. Fish were selected based on weight and length. *T. bernacchii* mass ranged from 46.9 - 91.92 g, mean 68.91 ± 13.95 g (standard error). Length ranged between 161- 201mm, mean 183.1 ± 11.72 mm (standard error), $n = 20$. Breakdown of mass and length of animals used in each treatment is covered in Table 3.1. Fish were housed in the Antarctic aquarium at the University of Canterbury for at least three months prior to the beginning of the experiment.

3.2.2. Specific Dynamic Action Feeding Protocol

Fish were fasted for 28 days prior to the beginning of each experiment; this timeline was chosen as in order to quantify the SDA, starvation periods should be as long as or longer than the SDA response (Boyce and Clarke 1997). Previous studies have shown a SDA response lasting 10 days in *Notothenia coriiceps* (previously *Notothenia neglecta*) and longer than 16 days in *Harpagifer antarcticus* (Boyce and Clarke 1997; Vanella et al. 2010). Personal observations at Scott Base showed some fish regurgitated food after 21 days of fasting; therefore a starvation period of 28 days was deemed appropriate.

Animals were housed in individual respirometers to allow control of water temperature and oxygen saturation and to enable measurement of oxygen consumption and thus metabolic rates. Just prior to the feeding event individual fish were removed from the respirometers and placed into a four litre tank containing 0.1 g of MS-222 anaesthetic (3-aminobenzoic acid ether; Sigma) per litre of seawater. Fish were considered anaesthetised when they were unable to right themselves after being turned ventral side upwards.

Fish were removed from the anaesthetic bath; forceps were used to push a ration of food past the oesophagus into the stomach. Fish were returned to the respirometer and closely monitored to ensure no food was regurgitated. At each treatment temperature a control was run, control animals underwent the exact same protocol as treatment animals, however, no food was implanted.

Each fish was fed *Seriolella brama*, common warehou. This was chosen as fish constitutes a large part of the diet of *T. bernacchii* in McMurdo Sound. Fish were fed 2.5% of their body weight. This portion size was chosen based on Montgomery et al. (1989) who investigated the contents of a fish species related to *T. bernacchii* and found the highest content was 5.5%, while average content in the stomach was 1.6 %. Thus, a percentage sitting between the average and the upper limit was chosen. A similar ration size was chosen by Sandblom et al. (2012) (2% of body mass) in their feeding study of *Pagothenia borchgrevinki*.

3.2.3. Specific Dynamic Action Experimental Protocol

All SDA treatments were run using periodic sampling respirometry techniques described in 2.4.2. Oxygen saturation levels were kept above 70% to ensure fish did not experience hypoxic conditions. The SDA experiment was run over three treatments. 1: Ambient temperature -1°C exposure, 2: Acute exposure to +3°C and 3: fish acclimated for 4 weeks to +3°C.

Once placed in the respirometers animals were left for 24 hours to adjust to their new surroundings (Johnston et al. 1991), examination of the literature suggests this time period is sufficient to allow recovery from any stress experienced during transfer (Jobling and Davies 1980; Chakraborty et al. 1992). Water samples were taken every 24 hours, for five days in the -1°C treatment and the +3°C Acclimated treatment and for three days in the +3°C Acute treatment prior to feeding to establish resting metabolic rates. Animals were fed according to 3.2.2. feeding protocol. Samples were taken one, six, nine, 12 and 24 hours after the feeding event and then every 24 hours thereafter until SDA response was deemed finished. The first eight hours of sampling after the feeding event was not included in the calculation of SDA or related parameters in any of the temperature treatments. This was to ensure any metabolic rise that may have occurred as an artefact of anaesthesia was not misinterpreted as the SDA response. SDA response was considered finished when metabolic rates were not statistically different from resting rates for 48 hours or more (Johnston and Battram 1993). The light regime was maintained at 24 hours light to mirror ambient summer conditions. Fish showed no visible signs of stress when confined.

3.2.4. -1°C Ambient Specific Dynamic Action Temperature Exposure

Animals were placed in individual respirometers each housed in separate flow through tanks maintained at $-0.9 \pm 0.2^{\circ}\text{C}$, $n = 10$. Samples were taken for five days prior to feeding to establish resting metabolic rates.

3.2.5 +3°C Acute Specific Dynamic Action Temperature Exposure

Animals were taken from holding tanks maintained at $-0.92 \pm 0.2^{\circ}\text{C}$ and placed in individual respirometers each housed in separate flow through tanks in water heated to $3 \pm 0.1^{\circ}\text{C}$, $n = 5$. Measurements for resting metabolic rates were taken over three days. This varied from -1°C and $+3^{\circ}\text{C}$ Acclimated treatments as it was considered important to feed the animals and measure the SDA response before fish started to acclimate.

3.2.6. +3°C Acclimated Specific Dynamic Action Temperature Exposure

Animals were acclimated to $+3^{\circ}\text{C}$ for one month (28 days), $n = 5$ (as described in 2.3.2). Animals were placed in individual respirometers each housed in separate flow through tanks at $3 \pm 0.1^{\circ}\text{C}$. Metabolic rate (MO_2) samples were taken every 24 hours for five days prior to the feeding event to establish resting metabolic rates.

One empty system with a respirometer was run during each treatment to establish background oxygen consumption; at all treatments background oxygen consumption was negligible.

3.2.7. Calculations and Statistical Analysis

Data were analysed using ANOVA, Repeated Measures ANOVA and when parameters of normality were violated the non – Parametric Kruskal – Wallis test. When necessary, post – hoc Tukey Range and Dunnett’s Multiple Comparison Tests, were used to identify means that significantly differed.

Absolute and factorial (or relative) aerobic metabolic scope was calculated as defined in Robinson and Davison (2008b). Absolute scope was calculated as resting metabolic rate subtracted from the maximum metabolic rate of an organism. Factorial metabolic scope was calculated as the ratio of peak metabolic rate for each fish divided by the resting metabolic rate of each fish.

The Specific Dynamic Action response was calculated as the additional consumption of oxygen after a single feeding event or the area under the curve above the resting metabolic rate. The ratio used to convert the area under the curve was 1 mg. O₂: 0.01406 kJ (Johnston and Battram 1993; Vanella et al. 2010). The calorific content of food ingested was calculated from a previously determined value of 514 kJ per 100 grams wet weight of *S. brama* (Vlieg 1988).

The Specific Dynamic Coefficient is the energy used during SDA relative to the energy of the ingested food. It can be measured using the following equation (McCue 2006):

$$C_{SDA} = (E_{SDA} / E_{meal}) * 100$$

Where:

C_{SDA} = SDA Coefficient

E_{SDA} = The metabolic energy used for SDA

E_{meal} = The energy within a meal

3.3. Results

The length, mass and the amount of food given to each fish (2.5 % of body weight) are presented in Table 3.1. Figures 3.4, 3.5 and 3.6 show the daily values obtained every 24 hours except the day of feeding where data are presented one, six, nine, 12 and 24 hours after feeding and where measurements were deemed outliers using the extreme studentized deviate test. Unless otherwise stated all rates and ratios given are means \pm SE.

3.3.1. Pre-feeding Resting Metabolic Rates

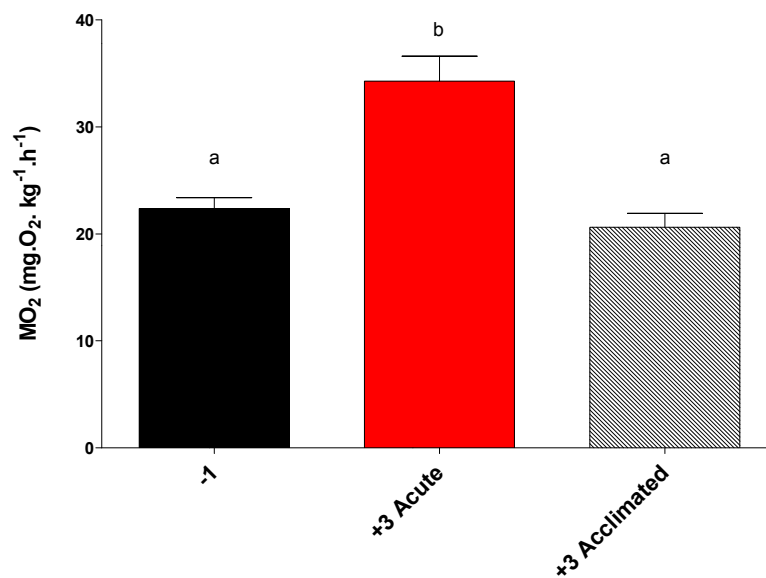


Figure 3.1: The Resting Metabolic Rate of *T. bernacchii* over 72 hours.: Different letters denote statistically significant differences between treatments.

Resting metabolic rates were determined by recording the resting rate for each fish over a determined time period prior to the feeding event and averaging the sum of all resting rates within that treatment. Resting metabolic rate was deemed appropriate as all fish in respirometers remained extremely still for the duration of the experiment and used anal and pelvic fins to maintain a tripod stance as observed in the natural environment (Wells 1987; Cech 1990). There was no statistical difference between resting metabolic rate taken at 72 and 120 hours, therefore resting metabolic rates established after 72 hours were used in this study. Resting metabolic rates of *T. bernacchii* after 72 hours were 22.38 ± 1.02 mg. O₂. kg⁻¹.

h^{-1} , $34.27 \pm 2.35 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and $20.63 \pm 1.3 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in the -1°C , $+3^\circ\text{C}$ Acute and $+3^\circ\text{C}$ Acclimated treatments respectively (Fig 3.1). Resting metabolic rates were significantly different (Kruskal- Wallis statistic 21.83, $p < 0.0001$), post- hoc Dunnett's multiple comparison test showed the $+3^\circ\text{C}$ Acute resting rate was significantly different from both -1°C and $+3^\circ\text{C}$ Acclimated metabolic rates. The -1°C and the $+3^\circ\text{C}$ Acclimated resting metabolic rates were not significantly different (Figure 3.1). Q_{10} values for resting metabolic rates were 2.90 and 0.82 for the $+3^\circ\text{C}$ Acute and $+3^\circ\text{C}$ Acclimated exposures respectively (Table 3.1).

3.3.2. Specific Dynamic Action Metabolic Peak

As data were not normally distributed SDA metabolic peak was analysed using a Kruskal-Wallis test. The SDA peak was not significantly different between treatments. The peak MO_2 was highest in the -1°C treatment, $36.89 \pm 3.12 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, followed by the $+3^\circ\text{C}$ Acute treatment which peaked at $36.01 \pm 1.07 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. The $+3^\circ\text{C}$ Acclimated treatment had the lowest peak MO_2 at $32.43 \pm 1.36 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Table 3.1).

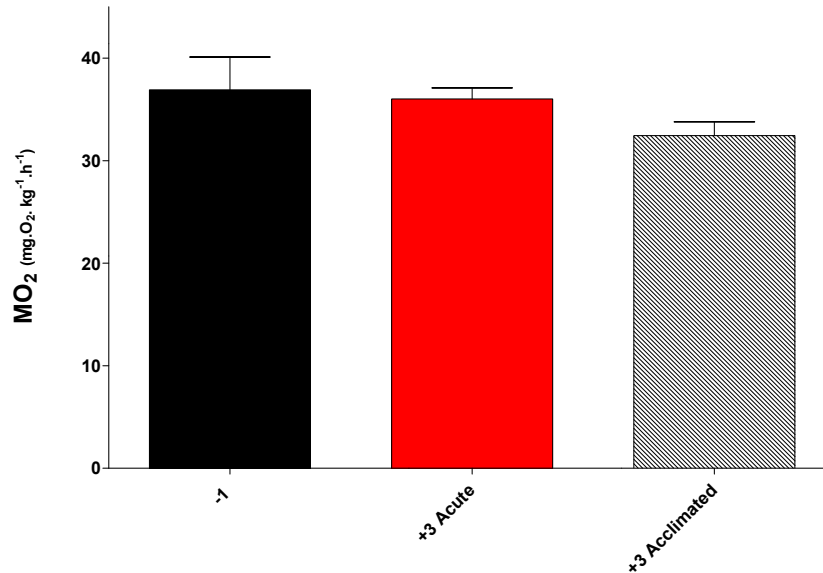


Figure 3.2: The peak MO_2 (mg. $O_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) during the Specific Dynamic Action Response at each treatment.

3.3.3. Specific Dynamic Action Metabolic Scope

The SDA absolute and factorial scopes were investigated over all treatments using a One-way ANOVA (Figure 3.3).

Due to large variation around the mean absolute scopes were not significantly different between treatments. The -1°C absolute scope was $14.52 \pm 3.65 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, the $+3^{\circ}\text{C}$ Acute was much smaller at $1.74 \pm 3.01 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Table 3.1). The $+3^{\circ}\text{C}$ Acclimated treatment absolute metabolic scope was $11.80 \pm 1.97 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Table 3.1), this scope sat between the other treatments (Fig. 3.3a).

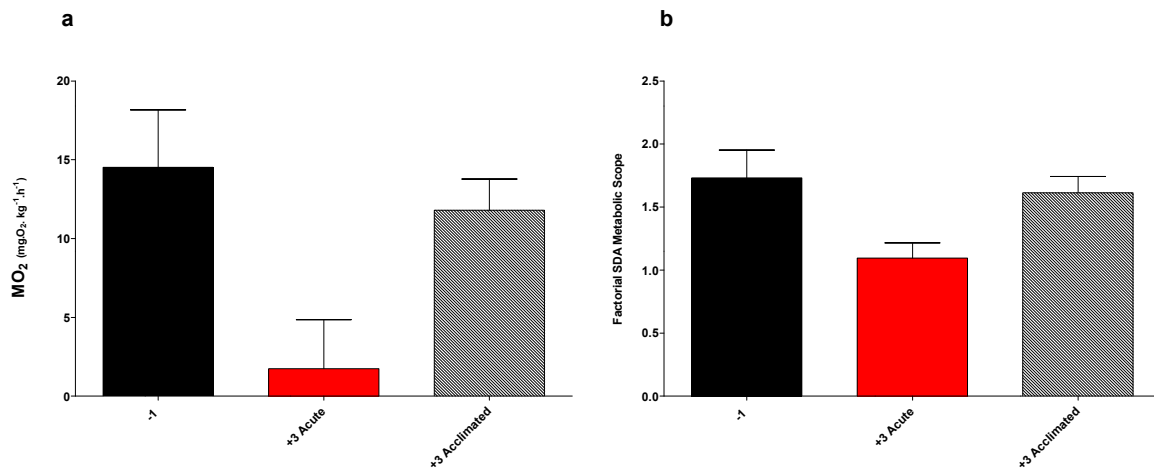


Figure 3.3: The Specific Dynamic Action metabolic scope of *T. bernacchii* over treatments -1°C , $+3^{\circ}\text{C}$ Acute and $+3^{\circ}\text{C}$ Acclimated following a feeding event. **Figure a:** Absolute Scope MO_2 ($\text{mg.O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). **Figure b:** Factorial Scope

Factorial SDA metabolic scope was not significantly different between treatments. The -1°C treatment factorial scope was 1.73 ± 0.22 , the $+3^{\circ}\text{C}$ Acute treatment 1.10 ± 0.12 and was 1.61 ± 0.19 in the $+3^{\circ}\text{C}$ Acclimated treatment (Figure 3.3b; Table 3.1).

3.3.4. -1°C Specific Dynamic Action Treatment

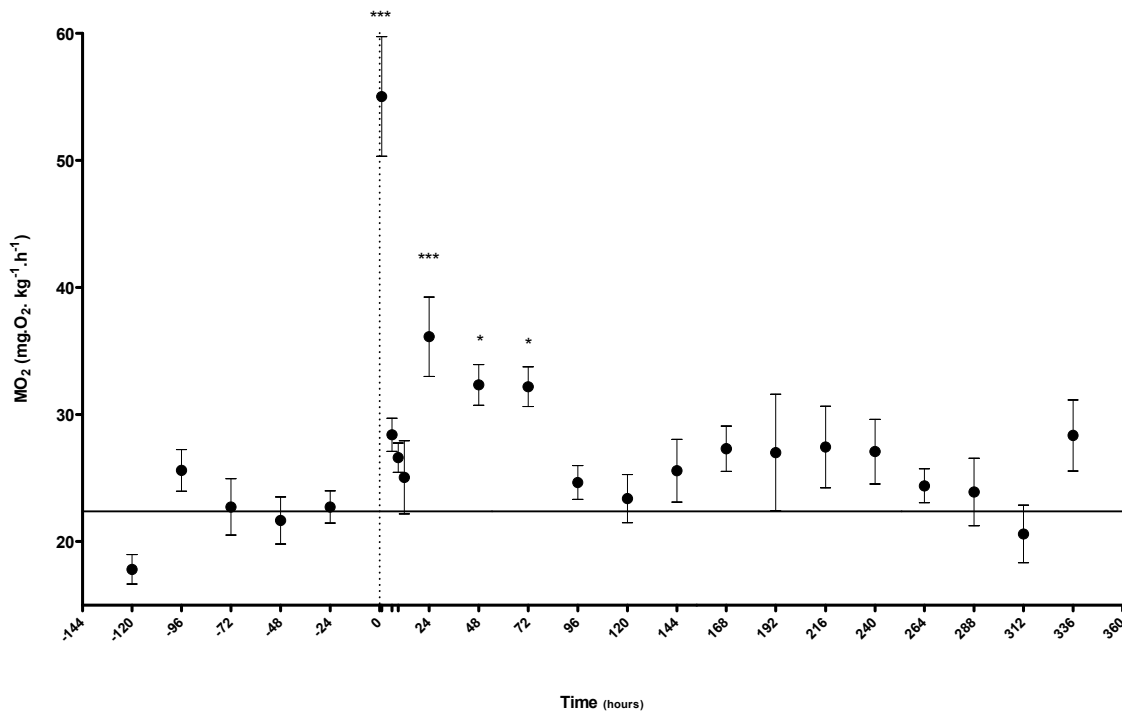


Figure 3.4: -1°C Specific Dynamic Action. Black circles denote MO_2 rates for that time point. The feeding event is represented by the vertical dotted line; the resting metabolic rate, $22.38 \pm 1.02 \text{ mg. } O_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ is represented by the horizontal solid line. Asterisks mark values that are significantly different from the resting metabolic rate (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

The average fish mass and length for the -1°C treatment, $n = 10$, was $70.57 \pm 16.11\text{g}$ and $180.4 \pm 14.96\text{mm}$ respectively. Values were tested for normality using the Kolmogorov–Smirnov test (K–S test), all data passed normality ($\alpha = 0.05$). MO_2 rates over time were analysed using a Repeated Measures ANOVA, means were significantly different ($F_{23, 207} = 12.8$, $r^2 = 0.586$, $p = < 0.0001$). Differences between time points were analysed using a post- hoc Dunnett’s Multiple Comparison Test, which compared each mean MO_2 ($\text{mg. } O_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) value to the resting metabolic rate $22.38 \pm 1.02 \text{ mg. } O_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Figure 3.1). The Dunnett’s Multiple Comparison Test identified four time points that differed statistically from the resting metabolic rate, Hour one, ($p < 0.0001$), hour 24 ($p < 0.0001$), hour 48 ($p < 0.05$ and hour 72 ($p < 0.05$) (Fig. 3.4). The postprandial consumption of oxygen rose rapidly, the MO_2 not including the first eight hours after the feeding event due to anaesthetic, peaked at $36.89 \pm 3.12 \text{ mg. } O_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Table 3.1), this value occurred 24 hours after the feeding event. The duration of the SDA response was 96 hours (Fig. 3.4). The -1°C treatment showed a secondary prolonged rise in MO_2 after the initial postprandial peak. Similar findings were

reported in Boyce and Clarke (1997), although this secondary rise was not statistically significant in the present study due to large standard errors (Figure 3.4).

3.3.5. +3°C Acute Specific Dynamic Action Treatment

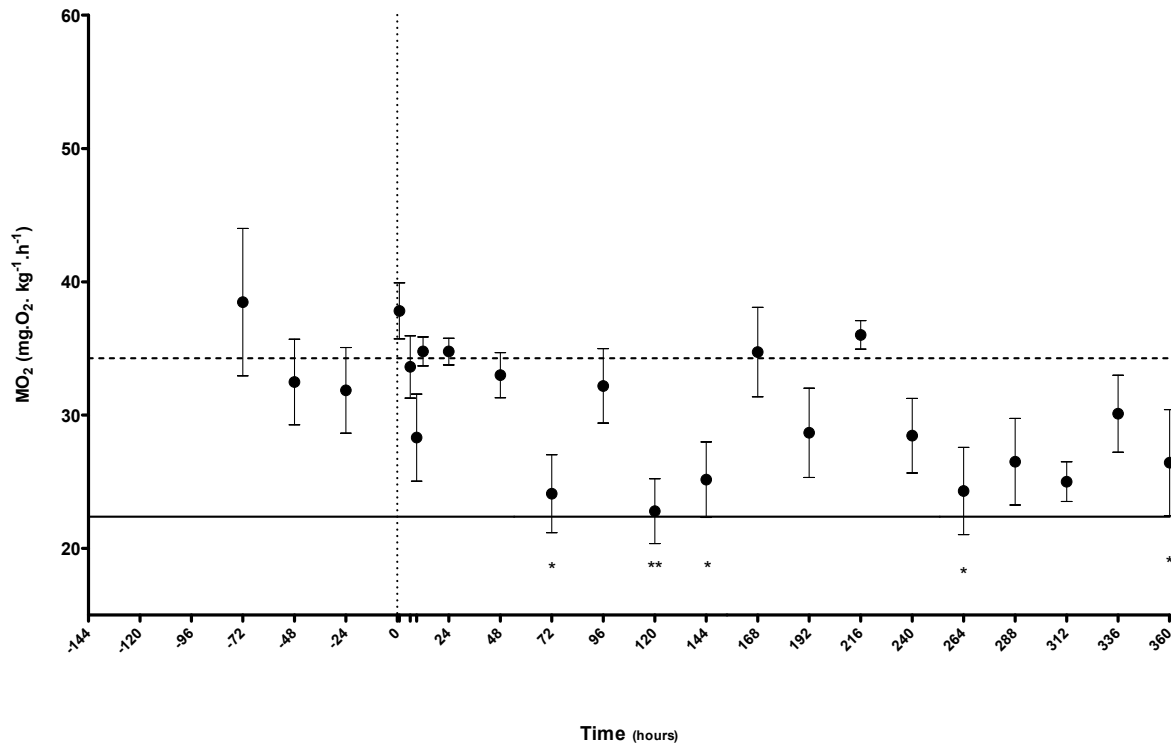


Figure 3.5: +3°C Acute Specific Dynamic Action. Black circles denote MO_2 rates for that time point. The feeding event is represented by the vertical dotted line; the resting metabolic rate, $34.27 \pm 2.35 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ is represented by the horizontal dotted line. The horizontal solid line represents the -1°C treatment resting metabolic rate, $22.38 \pm 1.02 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Asterisks mark values that are significantly different from the resting metabolic rate (* $p < 0.05$, ** $p < 0.01$).

The fish mass and length for the +3°C Acute treatment was $68.1 \pm 13.69\text{g}$ and $186.6 \pm 8.38\text{mm}$ respectively. Values were tested for normality using the K- S test, all data passed normality ($\alpha = 0.05$). MO_2 rates over time were analysed using a Repeated Measures ANOVA, means were significantly different, $F_{19,76} = 4.36$, $p < 0.0001$. Differences in MO_2 between time points were analysed using a post- hoc Dunnett's Multiple Comparison Test, which compared each mean MO_2 ($\text{mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) value to the resting metabolic rate (Figure 3.1). The peak MO_2 in this treatment was $36.01 \pm 1.07 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, this value occurred 216 hours after the feeding event (Table 3.1). However, the post- hoc test indicated

no MO_2 after the feeding event was significantly greater than the resting metabolic rate, therefore SDA duration was deemed 0.

MO_2 was significantly lower than resting metabolic rates at time points 72, 144, 264 and 360 hours ($p < 0.05$) and at time point 120 hours ($p < 0.01$) (Fig. 3.5). There was no MO_2 derived SDA response presented by fish in the +3°C Acute temperature treatment.

3.3.6. +3°C Acclimated Specific Dynamic Action Treatment

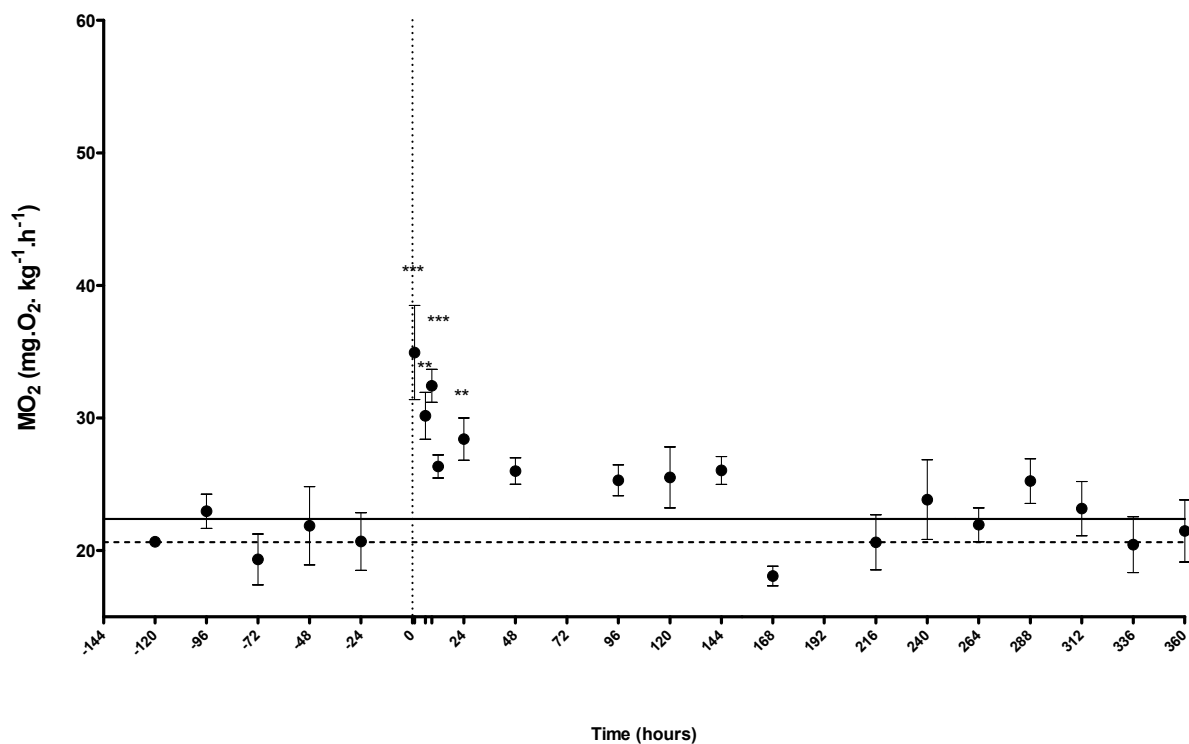


Figure 3.6: +3°C Acclimated Specific Dynamic Action. Black circles denote average MO_2 rates for that time point; squares represent a control fish. Feeding is represented by the vertical dotted line; the resting metabolic rate $20.63 \pm 1.3 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, is represented by the horizontal dotted line. The horizontal solid line represents the -1°C treatment resting metabolic rate, $22.38 \pm 1.02 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Asterisks mark values that are significantly different from the resting metabolic rate (** $p < 0.01$, *** $p < 0.001$).

The average fish mass and length for the +3°C Acclimated treatment was $66.4 \pm 11.78\text{g}$ and $185 \pm 6.4\text{mm}$ respectively (Table 3.1). Values were tested for normality using the K- S test, all data passed normality ($\alpha = 0.05$). MO_2 rates over time were analysed using a Repeated Measures ANOVA, means were significantly different, $F_{17, 68} = 6.08$, $p < 0.0001$. Differences between time points were analysed using a post- hoc Dunnett's Multiple Comparison Test,

comparing each mean MO_2 ($\text{mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) value to the resting metabolic rate $20.63 \pm 1.3 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Figure 3.1). The post – hoc test found four time points that differed significantly from the resting metabolic rate, hour one ($p < 0.0001$), six ($p < 0.001$), nine ($p < 0.0001$) and 24 hours ($p < 0.01$) (Fig. 3.6), therefore the duration of the SDA response was 24 hours (Figure 3.6 and Table 3.1). The MO_2 , when removing the first eight hours to account for anaesthetic after feeding peaked at $32.43 \pm 1.36 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Table 3.1, Figure 3.2), this value occurred nine hours after feeding (Figure 3.6)

3.3.7. Specific Dynamic Action Response and Coefficient:

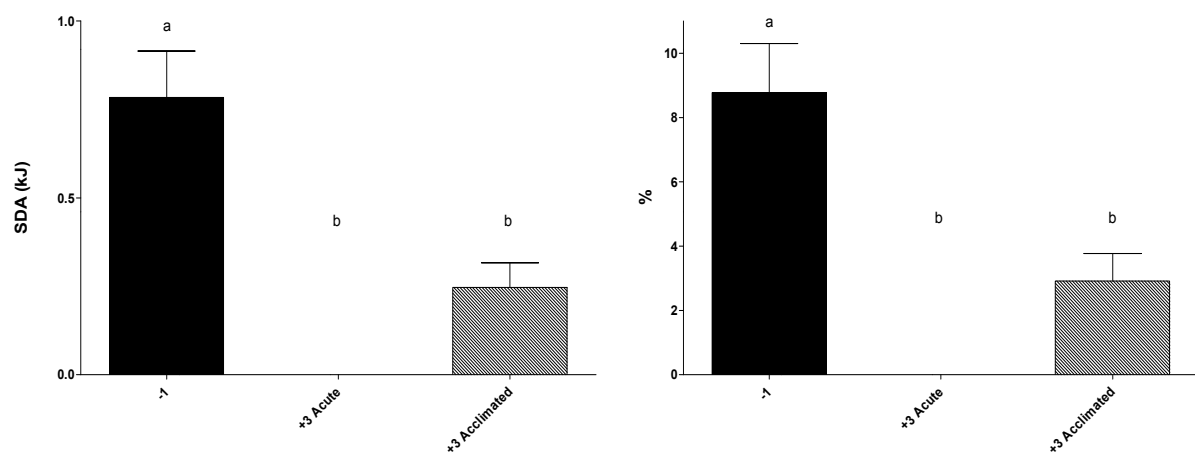


Figure 3.7: a: The Specific Dynamic Response (kJ) at each treatment. **b:** The SDA Coefficient at each treatment. Differing letters denote statistical significance ($p < 0.05$).

The SDA response, the total amount of energy above the resting metabolic rate devoted to SDA (kJ), was significantly different between treatments, One - Way ANOVA $F_{2,17} = 12.11$, $p = 0.0005$. The +3°C Acute treatment had the lowest SDA at 0 kJ. The +3°C Acclimated treatment expended $0.25 \pm 0.06 \text{ kJ}$, while the -1°C treatment showed the greatest amount of energy usage after the feeding event with $0.78 \pm 0.13 \text{ kJ}$ (Table 3.1). A post- hoc Tukey test showed that the -1°C treatment was significantly higher ($p < 0.0001$) than the +3°C Acute temperature treatment and the +3°C Acclimated treatment (Fig.3.7a).

The energy used during SDA relative to the energy of ingested food is the SDA coefficient. As variances between treatments were significantly different, the SDA coefficients between

treatments were analysed using a One- Way ANOVA , means were significantly different, $F_{2,17} = 10.94$, $p = 0.009$. The -1°C treatment SDA coefficient was $8.78 \pm 1.6\%$, the $+3^{\circ}\text{C}$ Acclimated treatment had the second highest SDA coefficient at $2.91 \pm 0.86\%$ The $+3^{\circ}\text{C}$ Acute treatment did not show a SDA response therefore the SDA coefficient was 0% (Table. 3.1), in a post- hoc Tukey Comparison Test the -1°C treatment was significantly different ($p < 0.0001$) from both the $+3^{\circ}\text{C}$ Acute and the $+3^{\circ}\text{C}$ Acclimated treatments (Fig. 3.7b).

Table 3.1: Parameters of the Specific Dynamic Action Experiment: Mass, Length, Condition Factor, Resting Metabolic Rate, Peak Metabolic Rate, Absolute Scope, Factorial Scope, Duration, Meal Size, Meal Energy, SDA and SDA Coefficient of fish over all treatments, +1° C, +3°C acute and +3°C acclimated and control fish. Differing letters denotes statistical significance.

Treatment	N	Fish Mass (g)(<i>sd</i>)	Fish Length (mm) (<i>sd</i>)	Resting Rate (mg. O ₂ kg ⁻¹ h ⁻¹)(<i>se</i>)	Peak (mg. O ₂ h ⁻¹ kg ⁻¹)(<i>se</i>)	Time of Peak (h)	Absolute Scope (MO ₂) (<i>se</i>)	Factorial Scope (<i>se</i>)
<i>T. bernacchii</i> -1°C	10	70.57 ± 16.11	180.4 ± 14.96	22.38 ± 1.02 a	36.89 ± 3.12	24	14.52 ± 3.65	1.73 ± 0.22
<i>T. bernacchii</i> +3°C Acute	5	68.1 ± 13.69	186.6 ± 8.38	34.27 ± 2.35 b	36.01 ± 1.07	216	1.74 ± 3.01	1.10 ± 0.12
<i>T. bernacchii</i> +3°C Acclimated	5	66.4 ± 11.78	185 ± 6.40	20.63 ± 1.3 a	32.43 ± 1.36	9	11.80 ± 1.97	1.61 ± 0.19

Treatment	Meal Size (g)	Meal Energy (kJ)	SDA (kJ)	SDA Duration (h)	SDA Coefficient (%)	Resting Rate Q ₁₀ from -1°C	Condition Factor (<i>se</i>)
<i>T. bernacchii</i> -1°C	1.76 ± 0.13	9.07 ± 0.65	0.78 ± 0.13 a	72	8.78 ± 1.6 a		1.21 ± 0.09
<i>T. bernacchii</i> +3°C Acute	1.70 ± 0.15	8.75 ± 0.79	0 b	0	0 b	2.90	1.04 ± 0.08
<i>T. bernacchii</i> +3°C Acclimated	1.66 ± 0.29	8.53 ± 0.68	0.25 ± 0.06b	24	2.91 ± 0.86 b	0.82	1.04 ± 0.09

3.4. Discussion

As the global trend in atmospheric and oceanic warming is expected to continue, the response and ability of organisms to acclimate may be one of the most important mechanisms in predicting the ability of a species to survive, in particular when organisms such as *T. bernacchii*, are unable to migrate due to geographical restrictions (Peck et al. 2010). True acclimation of a whole animal occurs through acclimation of the individual cells and tissues allowing the organism concerned to perform essential biological processes (Robinson 2008) including the processing and assimilation of food.

Feeding studies typically use one of two feeding methods; offering a specific amount of food, or anaesthetised force feeding, both of which have disadvantages. Due to the previous success of Sandblom et al. (2012), in the present study anaesthetic and force feeding was used. Anaesthesia ensured all animals were fed 2.5% of body weight. Studies have shown variability in ration size alters SDA duration (Chakraborty et al. 1992; Secor 2009), anaesthetic and force feeding ensured control of this parameter not obtainable using offered feeding methods. Secondly, in this study animals in the +3°C Acute treatment were exposed to an acute 4°C water temperature rise, an increase expected from previous studies in *T. bernacchii* to elicit a stress response (Lowe and Davison 2005; Robinson 2008). In fish reduction or cessation of feeding is a classic response to thermal stress; so much so that reinstatement of normal feeding patterns is generally accepted as one of the indicators of acclimation (Peck et al. 2010), therefore fish under thermal stress are unlikely to feed when offered food. Anaesthetising *T. bernacchii* in the +3°C Acute treatment ensured feeding of fish under thermal stress, obviously, for consistency in experimental design, anaesthesia was used in all other treatments. The caveat is interpreting the associated metabolic rise after use of anaesthetic. To avoid this, values obtained within eight hours of anaesthetic, although graphically represented, were not included in calculation of any parameters used to assess the SDA response. This was considered enough time to recover as Wells et al. (1994) found after anaesthesia with MS-222 changes in metabolites associated with stress had returned to normal within that time frame in some species of Notothenioidei. The feeding regime for all treatments of 2.5% of each individual fish's body weight was deemed appropriate for the present study as similar levels have been used in previous SDA studies (Sandblom et al.

2012) and Boyce and Clarke (2007) showed cellular metabolic processes associated with feeding are satiated by low ration levels.

Measuring the SDA response in Antarctic fish is notoriously difficult. Each SDA treatment in this study ran for 49 days consisting of a one month starvation period, 20 days for duration of the SDA experiment and an additional 24 hours to allow each fish to acclimate to each respirometer; the total length of the experiment for three treatments was 147 days. Therefore, due to a limited research season of seven weeks in Antarctica, it was necessary to transport fish and undertake the experiment in Christchurch, New Zealand. Restrictions in transportation and holding facilities in New Zealand meant sample size (n) was small. It is important to note that in Antarctic fish the SDA response is exceedingly small compared with temperate and tropical species. The peak metabolic increase after controlling for anaesthetic was less than two fold in all treatments and although the SDA trend can be observed (Figure 3.4, 3.5 and 3.6), due to small sample numbers and small metabolic increases, the statistical significance of the response may be lost in the natural variation or “ noise ” of the data. However, this and several other studies show similar patterns of SDA in Antarctic notothenioids, suggesting although the response may be too small to be statistically significant it is occurring. For example, Sandblom et al. (2012) found no significant rise in oxygen consumption within the first 24 hours of a feeding event in *Pagothenia borchgrevinki*, however, increases in cardiac output and decreases in gastrointestinal vascular resistance allowing greater blood flow to the gut showed digestion had commenced. Ware (1999) illustrated the SDA response in *Trematomus centronotus* through the catabolism of amino acids via the increase in ammonia, the waste product of this process. In her study metabolic rate increased by approximately $5 \text{ mL O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ following feeding and was significantly higher than the resting metabolic rate of $14.7 \pm 1.7 \text{ mL O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ at six, 216 and 240 hours only. However, ammonia excretion was continually significantly higher for 96 hours following feeding suggesting SDA was occurring and although the metabolic rise was not significant it mirrored the significant pattern of ammonia excretion. In the present study the non significant secondary increase in metabolic rate found in *T. bernacchii* during the -1°C treatment was also observed by Boyce and Clarke (1997) in *H. antarcticus* fed the same ration size, suggesting it is a part of the SDA. These studies mentioned illustrate the difficulty associated with finding statistical significance in the small rises of metabolic rate associated with SDA in Antarctic fishes, but show the response is occurring by testing other parameters such as ammonia excretion and cardiac output and allocation, these techniques were beyond

the scope of this study and inferences may be made where patterns suggest SDA but is not shown with statistical significance.

Conventionally the SDA response is quantified by 3 parameters: the peak metabolic rate following a feeding event, the factorial scope of the feeding event and the total energy expended above a baseline resting metabolic rate. The amount of energy allocated to assimilation of food (the SDA coefficient) and the duration of the response is also of interest, particularly as duration has been reported to be long in Antarctic ectotherms. The SDA response of *T. bernacchii* has not been described previously in the literature, including the SDA response when acutely and chronically exposed to increased water temperatures. The SDA was investigated at water temperatures as close to ambient temperatures as was possible to replicate in a lab environment, in animals acutely exposed to +3°C and in those acclimated to +3°C for 28 days. Somero and DeVries (1967) identified the upper critical temperature of *T. bernacchii* as +6°C, one of the lowest lethal temperature reported for any organism. Previous acclimation studies have shown *T. bernacchii* exposed to +4°C are, at best only capable of partial acclimation (Enzor et al. 2013; Jayasundata et al 2013) often with high levels of mortality (Carpenter and Hoffman 2000; Weinstein and Somero 1998). Due to this, +3°C was chosen as the acclimation temperature for this study. This acclimation temperature was viewed as a pragmatic increase as +3°C is above the highest sea water temperature rise predicted, 2°C, expected around coastal regions of Antarctica within the next century (Somero 2010) and aimed to reduce mortality of specimens.

3.4.1 Resting Metabolic Rates

The validity of the theory of Metabolic Cold Adaption (MCA) has been long debated in polar literature, first postulated by Krogh (1914) later expanded by Scholander et al. (1953) and Wohlschlag (1957; 1960 and 1964). Previous reporting of *T. bernacchii* resting metabolic rates is varied depending on experimental parameters. Wells (1987) showed a resting metabolic rate of $49.6 \pm 4.7 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (mass $178.1 \pm 31.6\text{g}$) in *T. bernacchii* at -1.5°C. As all fish in the present study were smaller (Table 3.1) than animals in Wells (1987) a higher metabolic rate may have been expected, due to a higher rate of oxygen consumption per unit of body mass. However, resting metabolic rates in the present study were much lower even in treatments where animals were exposed to increased water temperature at 22.8 ± 1.02 , 34.27 ± 2.35 and $20.63 \pm 1.3 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ at -1°C +3°C Acute and +3°C Acclimated treatments respectively (Table 3.1). These values are in closer agreement with Ware (1999) and

Steffenson (2005). Steffenson (2005) measured the resting metabolic rate of *T. bernacchii* at -1°C and found rates of $27.4 \pm 6.9 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, Ware (1999) measured the pre-prandial resting metabolic rate of *T. bernacchii* at -1.5°C as $23.4 \pm 2.9 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Enzor et al. (2013) reported even lower values; they measured resting metabolic rate in *T. bernacchii* at -0.61°C at $12.8 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. The resting metabolic rate of *T. bernacchii* is analogous with another notothenioid of similar benthic sluggish lifestyle; Johnston et al (1991) compared the resting metabolic rate of *Notothenia neglecta* with temperate species of similar lifestyle, the resting metabolic rate of $18.2 \pm 4.2 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (as reported in Ware 1999) was lower than that reported in Wells (1987) and found no evidence to support the theory of MCA.

The resting metabolic rate data in this chapter follows previous bodies of work (Davison et al. 1990; Ware 1999; Drud Jordan et al. 2001; Steffenson 2002; Steffenson 2005 and Enzor et al. 2013) and found no evidence of notothenioid elevated resting metabolic rate and thus no evidence to support MCA in *T. bernacchii*. The resting metabolic rates in Wells (1987) were elevated compared with the present study, this may be due to experimental variation or residual SDA driven elevated metabolism as the animals in Wells (1987) were starved for half the time of those in this study. However, assuming fish consumed food prior to starvation; the present study shows the resting metabolic rate of $49.6 \pm 4.7 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in *T. bernacchii* cannot be attributed solely to SDA. The metabolic rate of *T. bernacchii* between 240 and 336 hours after feeding was at its highest point $28.35 \pm 2.8 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, almost half that of the resting metabolic rate reported in Wells (1987) (Figure 3.4). Animals in Wells (1987) also spent considerably less time in laboratory conditions before resting metabolic rates were measured, several days compared to several months in the current study. Oxygen consumption has been shown to decrease after several months acclimation to captive conditions and may be attributed to animals becoming adapted to disturbances associated with a laboratory environment (Hop and Graham 1995).

In Antarctic ectotherms oxygen demand increases due to an increase in metabolic processes as environmental temperature rises; oxygen uptake must match this demand. In this study *T. bernacchii* resting metabolic rate showed a classic response to acute temperature increase, reflected in both the differences in resting metabolic rates and Q_{10} values. Q_{10} , the measure of a rate of change of a biological or physiological process over a 10°C change in temperature, in Antarctic ectotherms is approximately 2 (Clarke and Johnston 1999), and data in this study reflect this. The Q_{10} value of 2.90 (Table 3.1) in the Acute $+3^{\circ}\text{C}$ treatment when compared to

the -1°C ambient treatment supports previous findings that metabolic rate increases with increasing temperature. It has been suggested that the increase is driven by higher kinetic energy of cellular molecules when temperature is increased resulting in a direct acceleration of metabolic rate, this hypothesis is known as the Universal Temperature Dependence (UTD) (Gillooly et al. 2001). Clarke and Fraser (2004) and Clarke (2004) have debated the validity of the UTD, they argue a temperature dependence on the rate of ATP production would be a waste of precious resources and have suggested that temperature induced elevated metabolic rate is a result of the temperature causing variation in passive leaking of protons across the inner mitochondrial membrane coupled with a higher turnover of proteins. However, regardless of the mechanism, the increased cellular activity results in an increase in oxygen demand therefore increased consumption.

Table 3.2: The physiological responses to acute temperature change in ectotherm organisms, as described in Peck (2002)

Compensation	Physiological Response
No Compensation	Physiological rates move to new rates, up or down and remain at the new rate.
Perfect Compensation	Physiological rates return to levels prior to temperature increase.
Partial Compensation	Physiological rates return some of the way to previous rate.
Over Compensation	Physiological rates return to rates beyond the initial rate.
Inverse Compensation	Physiological rates continue to move away from the initial rate with a change in temperature. This is a rare response.

Organisms can adjust physiological rates and respond to temperature increase by partial, perfect, over and inverse compensation (Table 3.2) (Peck 2002). Previous studies have shown *T. bernacchii* are unable to acclimate or compensate to temperatures when increased to +4°C (Robinson 2005; Enzor et al. 2013). However, in the present study *T. bernacchii* showed perfect compensation of resting metabolic rates after 28 days of exposure to +3°C water, demonstrated by the congruence of the -1°C and +3°C Acclimated treatment resting metabolic rates (Figure 3.1) and reflected in the Q_{10} values between these treatments (Table 3.1). This finding suggests that at water temperatures up to +3°C *T. bernacchii* may not experience the adverse effects related to maintenance of increased metabolic rate for example reduced aerobic scope affecting activities such as locomotion and reproduction.

3.4.2 Specific Dynamic Action Metabolic Scope and Peak

In this study peak metabolic rates (and any associated equations), were taken as the highest value above the baseline beyond 8 hours after the feeding event for each fish: 24, 216 and 9 hours at -1°C, +3°C Acute and +3°C Acclimation respectively (Table 3.1).

In fish after feeding the typical rise in oxygen consumption is 2 to 3 times higher than a baseline level (Jobling 1993). The literature suggests this increase is fairly consistent between species and is independent of temperature when fish are in the relevant species thermal range (Hop and Graham 1995; Peck and Veal 2001). Secor (2009) reviewed the postprandial factorial metabolic scope in 62 fish species of varying latitudes and reported the average scope was 2.36 ± 0.07 . This range appears to be true of polar teleosts, for example the factorial scope in the Antarctic plunderfish *Harpagifer antarcticus* has been reported between 2.1 – 2.5 (Boyce and Clarke 1997) while *Notothenia neglecta* reported range sits between 2.03 – 2.34. In the present study the factorial scope of 1.73 ± 0.22 found in the -1°C treatment and 1.61 ± 0.19 in the +3°C Acclimated treatment does fall slightly below this pattern but similar levels have been found in other polar fish, Hop and Graham (2005) reported the factorial aerobic scope in Arctic cod between 1.3 and 1.6.

In the present study the SDA factorial metabolic scope of *T. bernacchii* was not independent of temperature when animals were exposed to an acute temperature rise. The peak SDA metabolic rate response to increases in temperature varies between fish species, however typically a metabolic rise is reported (Guinea and Fernandez 1997; Secor 2009; Pang et al. 2010; Pang et al. 2011). This was not the case in the present study, peak metabolic rate following the feeding event remained relatively constant regardless of time and temperature exposure (Figure 3.2). This, in conjunction with increased resting metabolic rate (discussed above) resulted in the reduction of factorial scope 1.10 ± 0.12 , in the +3°C Acute treatment, comparative to the -1°C and +3°C Acclimated treatments (Figure 3.3b). Although due to large variation, this reduction of scope was not significant it was greatly reduced comparative to the other treatments and illustrates that environmental temperature does affect the scope of *T. bernacchii* after a feeding event. The similarity in scopes, both absolute and factorial between the -1°C and +3°C Acclimated treatment provide further supporting evidence to that shown in the resting metabolic rates of the ability of *T. bernacchii* to acclimate in terms of oxygen consumption to +3°C (Figure 3.3a and b).

3.4.3 Specific Dynamic Action Response:

While factorial scope is similar across most species of fish, absolute scope is greatly reduced in Antarctic fish (Secor 2009). This reduction is due to a reduced metabolic peak linked to trade-offs associated with adaptations to the cold (Chapter 1.7) when compared to temperate and tropical species. This has implications for the duration of the SDA response. Anabolism of amino acids in protein synthesis uses a fixed number of ATP molecules regardless of temperature (Boyce and Clarke 1997). Evidence suggests the metabolic cost of SDA is fixed therefore, the same amount of ATP is metabolised in Antarctic fish over a greater time length compared to temperate and tropical fish (Ware 1999). As a direct consequence the duration of the SDA, for the identical amount and content of food ingested is prolonged compared with temperate and tropical species. Secor (2009) correlated the duration of the SDA response in 62 fish species from different latitudes quantified throughout the literature. Shortest durations were 3.4, 3.5 and 6.5 hours in *Salmo gairdneri*, *S. salar* and *Gadus morhua* respectively (Smith et al. 1978; Peck et al. 2003) all temperate species. In contrast the 3 longest SDAs, lasting 162, 211 and 390 hours in *Myoxocephalus scorpius*, *N. neglecta* and *H. antarcticus* respectively (Johnston and Battram 1993; Boyce and Clarke 1997) are all species found in polar regions.

In the present study all indications show the specific dynamic action response in *T. bernacchii* is typical of fish but prolonged, with a rapid rise in oxygen consumption followed by a gradual decline returning to resting rates. The duration of the response was 72 hours, however, after 120 hours there was a secondary muted rise in metabolic rate continuing until 288 hours, although this rise was not statistically significant it has been documented previously in polar fish. As stated earlier Boyce and Clarke (1997) found a secondary response in *H. antarcticus* occurring 96 hours after the feeding event lasting until 390 hours. The authors attributed the secondary rise to small ration size (2.5% bodyweight) as fish in the same size cohort fed to satiation did not show a secondary response, but did have the same duration. Further investigation into ration size and a secondary metabolic rise may provide insight into the SDA response but is beyond the scope of the present study.

The +3°C Acclimated response was reminiscent of the typical SDA response, a sharp increase in metabolic rate post- feeding followed by a decline back to resting metabolic rate. Resting metabolic rates in the +3°C Acclimated treatment showed full compensation (as covered above). However, the duration of the response was greatly reduced in this treatment

lasting only 24 hours (Figure 3.6 and Table 3.1), the decrease in duration of the SDA may be a classic response of temperature dependence on chemical reaction rates. The implications of this finding may be profound for the life history and energy budgeting of *T. bernacchii*; Antarctic species have a relatively low maximum of metabolic energy available for growth, reproduction and activity and it has been suggested this low aerobic scope may facilitate a seasonal change in energy allocation between reproduction and growth (Johnston and Battram 1993). Studies have shown in some Antarctic fish a reduction in appetite and not resource availability is responsible for a decrease in growth rate during winter months. It has been postulated this reduction may be due to limitations of energetic resources and allow allocation towards reproduction (Johnston and Battram 1993). A decrease in SDA response duration from 72 hours in ambient fish to 24 hours in acclimated fish may allow growth and reproduction concurrently leading to an increase in growth rate. Furthermore, temperature is a major environmental factor affecting growth (Peck et al. 2004). Assuming perfect compensation to +3°C has occurred in not just oxygen consumption but in all physiological parameters in *T. bernacchii*, and the increase in SDA response speed is a direct consequence of this, the rate at which protein turnover, and thus potentially growth occurs will also increase.

In fish, the postprandial generated metabolic peak generally occurs three to 12 hours following the feeding event (Johnston and Battram 1993; Secor 2009). This peak tends to occur after a longer period of time, between 16 and 52 hours, in Antarctic notothenioids (Johnston and Battram 1993; Boyce and Clarke 1997; Ware 1999). In the present study time to metabolic peak in the -1°C treatment follows that reported for Antarctic fish previously in the literature. Initially this treatment showed a large metabolic spike and rapid recovery, in all likelihood a consequence of anaesthetic and subsequent recovery. Following this recovery, another rise in metabolic rate occurred constituting the beginning of the SDA response reaching its peak 24 hours after the feeding event (Figure 3.4). However in the +3°C Acclimated treatment, the initial rise and recovery associated with anaesthetic was much lower and faster (Figure 3.6) with the SDA response peaking much earlier, nine hours after the feeding event. This reduction in time to a peak metabolic rate in the +3°C Acclimated further shows the effects of temperature on the rate of biological processes, illustrating as temperature increases so too does the rate at which processes occur.

In the +3°C Acute treatment the metabolic rate was at no point significantly higher than the resting metabolic rate, therefore, the duration of the response was deemed 0 hours (Figure 3.5; Table 3.1). Typically, metabolic rate increases when fish are exposed to stress; for example, instrumentation of fish requires an invasive surgical procedure and even after allowing for recovery from anaesthetic fish are still likely to be stressed (Davison personal communication). However, Sandblom et al. (2012) showed despite no significant rise in oxygen consumption following instrumentation of *P. borchgrevinki*, digestion was reduced but occurring, despite invasive procedures. As discussed previously, the resting metabolic rate in this treatment was elevated when compared to the -1°C and +3°C Acclimated treatments (Figure 3.1); regardless if the increase in resting metabolic rate was a thermal stress or a Q_{10} response it is likely this elevation masked the small SDA derived rise expected in *T. bernacchii*. Metabolic rate declined over the entire course of the treatment, this suggests acclimation was occurring and commenced within 24 hours of exposure to increased temperature; 432 hours following introduction to +3°C metabolic rate was analogous with the -1°C resting metabolic rate (Figure 3.5). Information on time point measurements of metabolic responses to increased temperature in Antarctic fish is scarce in the literature (Peck 2002) particularly in *T. bernacchii* in which plasma osmolality and erythrocytes are the parameters traditionally used to measure acclimation (Lowe and Davison 2005; Robinson 2008). The 18 day time frame of this experiment provides a snapshot into the acclimation ability of *T. bernacchii*.

For a positive energy balance to occur following digestion of a meal, the proportion of energy utilised in the SDA process should be small, with the remainder being stored as carbohydrate, lipid and protein. In the case of fish, the SDA coefficient typically ranges between 5 and 20% (Chakraborty et al. 1992; Fitzgibbon et al. 2007). The -1°C treatment coefficient of $8.78 \pm 1.6\%$ falls within that range. Due to the feeding regime, meal size and therefore meal energy were similar at each temperature; similarly the protein content of feed did not differ as all animals were fed the same. Nevertheless, the SDA and SDA coefficient of the -1°C treatment were significantly higher than both other treatments (Figure 3.7a and b). The +3°C Acute treatment had a SDA coefficient of 0% however, as discussed above, energy partitioned to digestive processes was most likely masked in this treatment. The SDA coefficient in the +3°C Acclimated treatment was significantly lower than the -1°C, at $2.91 \pm 0.86\%$. This response is different from that of the literature; where the SDA coefficient tends to increase or remain static as water temperature increases (Johnston and Battram 1993; Boyce and

Clarke 1997; Guinea and Fernandez 1997; Vanella et al. 2010). However this result is not unexpected considering the body of evidence presented above; the SDA coefficient is simply a ratio of resting and - feeding rates and as the duration in the +3°C Acclimated treatment was reduced without a corresponding rise in SDA peak the SDA coefficient in this treatment was reduced.

3.5 Summary

Specific Dynamic Action is very difficult to measure in Antarctic fishes, in polar ectotherms the response is characterised by low rises and long durations in metabolic rate. This was true of *T. bernacchii* which responded in a typical manner when housed at ambient temperatures. Acute exposure to increased temperature resulted in the elevation of the resting metabolic rate in *T. bernacchii*. This rise, due to a thermally induced stress response, a simple Q_{10} response or a combination of both masked the SDA response in that treatment; although the mechanism for the metabolic increase of the +3°C resting rate cannot be determined within this study, this treatment does provide a previously unreported time point snapshot of the acclimation process of the metabolic rate in this species. The ability of *T. bernacchii* to acclimate to increased temperatures is a subject of contention in the literature. This study showed *T. bernacchii* are, after 28 days able to fully compensate resting metabolic rates and scope when exposed to +3°C. Also of significance was the reduction in time to reach peak metabolic rates and the duration of the SDA response in higher water temperature in these acclimated fish. The implication for the life style and energetic budgets of the species can only be inferred from these results. However, it does provide the basis for future further investigation of biochemical parameters associated with digestive metabolism and into the feeding dynamics and acclimation ability to fully quantify the Specific Dynamic Action response at different temperatures in *T. bernacchii*. Preliminary results from this study show it is conceivable that, at least in terms of SDA: *T. bernacchii* may be able to adjust to elevated environmental temperature, providing some optimism for a species facing a warming ocean.

CHAPTER FOUR

Tolerance of Hypoxia and the Effects of Temperature on Cardiovascular Function in *Trematomus bernacchii*

4.1. Introduction

Hypoxia, the reduction of environmental oxygen, is a phenomenon that occurs naturally in many aquatic habitats, particularly in estuarine and coastal environments (Richards 2011). Compared to air, water at normal atmospheric pressure has a low capacitance for oxygen; just a few milligrams of oxygen are present per litre of water (Domenici 2012). Because of this oxygen in water can be depleted by respiring organisms (Richards 2009). Ecosystems vary in oxygen availability and fish, if habitat dictates, have evolved mechanisms to cope with hypoxic conditions (McBryan et al. 2013). Anthropogenically derived increases in coastal eutrophication and climate change have increased hypoxic events both in frequency and duration worldwide (Capossela et al. 2012). Currently dead zones affect a total global area greater than 245, 000 square kilometres (Diaz and Rosenberg 2008) and fish previously existing in high oxygen environments are being exposed to hypoxic and even anoxic waters.

The actual quantitative definition of hypoxia varies throughout the literature; Wu (2002) defined hypoxia as dissolved oxygen less than 91.4mmHg, while the United States Environmental Protection Agency, as reported in Capossela et al. (2012) states oxygen levels exceeding 25 mmHg are required for survival, but levels greater than 55mmHg are necessary for growth in fish. In the approximately 32 000 extant fish species described, the effects of hypoxia are variable and species specific (Mandic et al. 2009), therefore the environmental oxygen tension level at which hypoxia occurs also varies such that hypoxia cannot be universally defined using a finite oxygen tension. Farrell and Richards (2009) described environmental hypoxia as the level at which water oxygen tension first compromises physiological function; this definition was employed throughout this study.

A difference between oxygen tension in the water and the blood of the fish is ultimately what drives the uptake of oxygen into the cells (Jobling 1994). Nearly all (95%) oxygen consumed by fish in normoxic conditions is used to generate ATP in the cells via oxidative phosphorylation (Richards 2009). Oxygen travels from the water down a “respiratory chain”, through a series of diffusive cascades e.g plasma convection and red blood cell diffusion, ultimately ending at tissue and cellular sites (Hughes 1973; Axelsson et al. 1992). Environmental hypoxia may lead to hypoxemia (a low level of oxygen in the blood) via a reduction in the diffusion gradient across the gill lamellae and the cardiorespiratory system may no longer deliver sufficient oxygen to tissues affecting ATP production and thus aerobic metabolism (Capossela et al. 2012).

Hypoxia initially affects fish by limiting maximum metabolic rate, this in turn reduces overall metabolic scope (Domenici et al. 2013). However, a large range of fish species, referred to as oxyregulators, are able to regulate and maintain aerobic metabolism over a large range of environmental oxygen tensions (Jobling 1994). This is achieved by shifting respiratory and cardiac functions (Capossela et al. 2012) via methods such as hyperventilation (Perry et al. 2009), catecholamine instigated lamellar recruitment (Jobling 1993), increased quantities of circulating erythrocytes, changes in haemoglobin oxygen binding affinity (Perry et al. 2009) and changes in heart stroke volume and rate characterised by a bradycardia as oxygen levels approach critical limits (Farrell 2007).

Physiological processes altered by fish in response to hypoxic waters must require a well honed sensory system, this appears to consist of neuroepithelial cells located in the gill and pseudobranch with secondary receptors most likely positioned downstream of the arterial blood (Jobling 1994), though the exact location of receptors is a subject of current debate in the literature (Perry et al. 2009).

As hypoxia increases, a critical point of oxygen tension is reached (P_{crit}). At P_{crit} , as environmental oxygen is reduced, so too is the gradient for diffusion of oxygen into the blood across the gill lamellae, therefore available oxygen is insufficient to maintain standard metabolic rate (the minimal amount of oxygen required for maintenance functions) (McBryan et al. 2013) and oxygen consumption rate switches becoming dependent on environmental oxygen (Pörtner and Lannig 2009). When the P_{crit} threshold is reached a sharp linear decline in oxygen consumption occurs as fish become oxyconformers matching decreasing

environmental oxygen tensions (Schurmann and Steffensen 1997; Pörtner and Lannig 2009), aerobic metabolism decreases or ceases and the demand for ATP must be met by anaerobic metabolism (Capossela et al. 2012).

Anaerobic pathways are metabolically costly; glucose is fermented faster for a highly reduced ATP output. Substrate level phosphorylation of ADP to ATP during anaerobiosis using glucose generates two ATP molecules compared to 36 ATP molecules generated during aerobic respiration (Jobling 1994; Richards 2009). Associated with anaerobic metabolism is the accumulation of harmful end products such as lactate and protons (H^+) (Jobling 1994). If aerobic processes are not reinstated, glycogen stores are quickly exhausted creating a disparity between ATP production and demand leading to failure of critical ATP consuming processes. This, in conjunction with accumulations of end products leads to breakdown in cellular ion regulation and changes in cell pH causing metabolic acidosis and ultimately necrosis (Richards 2009). The hypoxia tolerance of any given species of fish is governed by its ability to simultaneously reduce metabolic rate and reduce metabolic demand and thus extend the availability of anaerobic substrates for fermentation (Bickler and Buck 2007). In the majority of teleosts P_{crit} appears to lie between 40 and 60mmHg, however, this varies between species, e.g. Carp, a sluggish bottom dwelling species is able to maintain aerobic metabolism down to 25mmHg and can survive anoxic conditions for up to 24 hours depending on temperature (Gamperl and Driedzic 2009), conversely P_{crit} for salmonids, highly active animals, sits around 90mmHg (Jobling 1994).

Conventionally, temperature and hypoxia have been treated as two separate environmental stressors, however, studies across all latitudes have shown oxygen supply and thermal limitation are linked (Pörtner and Lannig 2009). Initially postulated by Fry (1971) (as reported in McBryan et al. 2013), this model has been coined the “oxygen and capacity limitation of thermal tolerance” or “OCLTT”. This concept suggests that between lower and upper pejus temperatures, surplus oxygen not required for maintenance metabolism is allocated to aerobic scope, and oxygen supply to tissues works at an optimum. As discussed throughout this thesis, temperature dictates the rate of metabolic reactions, therefore towards the upper boundary of the thermal envelope resting or routine metabolic rate increases, reducing metabolic scope (Pörtner and Lannig 2009; Capossela et al. 2012). Therefore, as temperature rises the thermally induced metabolic increase counteracts the depression of metabolic rate and reduction in ATP utilisation (the physiological defences to hypoxia)

(Enzor et al. 2013). As a result P_{crit} may occur at higher levels of oxygen tension in both acute and chronic exposures to elevated temperature and can be expected to reduce survival times in reduced oxygen environments (McBryan et al. 2013).

The Southern Ocean, due to its low temperature contains more oxygen than temperate waters, e.g. the Ross Sea holds 1.6 times more oxygen than 20°C seawater (Eastman 1993). Temperatures have remained stable in the Ross Sea for several million years; as a result notothenioid fish inhabiting the region are unlikely to have experienced hypoxic conditions. In fact, adaptations to the cold, such as reduced circulating erythrocytes, have most likely evolved as a direct result of the high levels of ambient environmental oxygen (Garofalo et al. 2009). This adaptation is taken to the extreme by the Channichthyidae family, known as the icefishes comprising approximately 25 species all of which lack red blood cells (Kock 2005). Therefore, it is probable that Antarctic notothenioids are sensitive to reductions in oxygen tensions even at relatively high environmental oxygen levels. Current literature suggests responses to hypoxia in notothenioids vary between species (Robinson 2008). These responses include increased respiration, increases in oxygen carrying capacity due to increases in circulating red blood cells facilitated by splenic contraction, decreases in spleen size, decrease in erythrocyte [ATP] and associated increase in oxygen affinity at the gill and increase in heart rate (Axelsson et al. 1992; Davison et al. 1997; Schumann and Steffensen 1997; Wells et al. 1998; Campbell et al. 2009). Previous research has characterised the main responses of *T. bernacchii* to reduced water oxygen by a slight immediate reduction in respiration (Fanta et al. 1989) and a lack of bradycardia, a response typical during hypoxia in most teleosts and elasmobranchs (Axelsson et al. 1992; Robinson 2008; Campbell et al. 2009; Robinson et al. 2011). However, the literature suggests that the bradycardia response in *T. bernacchii* has not been clarified and requires further investigation (Gamperl and Driedzic 2009).

Changes in global temperature due to anthropogenic climate change (Chapter 1.3) means hypoxia may be a very real physiological challenge facing notothenioid fish in the not too distant future. If P_{crit} does increase as water temperatures rises, due to the associated increase in metabolic rate *T. bernacchii* are likely to experience temperature induced hypoxia in the tissues (Robinson 2008) as cardiovascular and respiratory systems fail to match the increased metabolic rate associated with temperature increase (Farrell and Richards 2009). Understanding the physiological responses and tolerances to hypoxia, temperature and the

interaction of these stressors is vital in predicting how these animals will cope in a changing environment.

This research aimed to investigate the cardiovascular responses of *T. bernacchii* to decreased oxygen tension over a range of increasing water temperatures; and, if acclimation to a higher temperature of +3°C altered this response.

4.2. Methods

4.2.1. Fish

All fish were held for a minimum of five days prior to beginning of acute hypoxia or acclimation to allow recovery from stress associated with capture.

Trematomus bernacchii in this experiment were sourced from McMurdo Sound, Antarctica over two, 2011 / 2012 and 2012/2013, summer seasons. Fish were collected, transported and housed at the Scott Base Wet Lab Facilities as described in Chapter 2.1. All hypoxia experiments took place at Scott Base, Antarctica. The mass of Cold Acclimated *T. bernacchii* was $149.81 \pm 7.17\text{g}$ (range 94.9 – 203g) and length was $216 \pm 2.96\text{mm}$ (range 195 – 234mm), $n = 17$. The mass of Warm Acclimated *T. bernacchii* was $132.67 \pm 6.5\text{g}$ (range 88.36 – 183) and length $215.9 \pm 2.74\text{mm}$ (range 195 – 236mm), $n = 20$.

4.2.2 Warm Acclimation and Survival

Fish were acclimated over a 28 day period using the protocol described in 2.3.1. Mean survival time was 17.59 ± 1.52 days. At the end of the acclimation period three fish remained and two days later, 30 days after introduction to +3°C, only one fish remained. Therefore n values were not large enough to complete hypoxia experiments on acclimated fish.

4.2.3. Hypoxia Protocol

Animals were anaesthetised and e.c.g. electrodes were applied as described in Chapter 2.5. Animals were placed in individual respirometers, allowing control of ambient oxygen concentration, in individual water baths, allowing management of water temperature. Fish were allocated 24 hours recovery from the effects of surgery and anaesthetic. Fish are known to be sensitive to handling stress (Boyce and Clarke 1997), however previous studies have deemed a 24 hour acclimation period sufficient to allow Antarctic fish adequate time to adjust

to the respirometer and recover from surgery and handling stress (Wells et al. 1984; Morris and North 1984; Wells et al. 1994; Johnston et al. 1991; Robinson 2008; Robinson et al. 2011).

Fish heart rate (beats per minute), ventilation (breaths per minute) and changes in water oxygen partial pressure (mmHg) were measured using continuous sampling respirometry, covered in Chapter 2.4.1. Manual measurements of ventilation rates to confirm traces were made by counting the number of operculum movements over one minute. Respirometers were open for the first 30 minutes to establish resting heart and ventilation rates in fully saturated water at the treatment temperature. After thirty minutes respirometers were closed. Oxygen was depleted in the individual chambers by the fish themselves utilising oxygen for metabolic function, oxygen levels were reduced to 50 mmHg or until fish showed visible signs of stress. Indications of stress included laboured and enlarged opercular movement, forceful thrashing movements and listing of fish. At this point, the respirometers were flushed with fully aerated water maintained at the treatment temperature. During recovery respirometers were routinely opened and closed to ensure oxygen saturation level was not taken below 70% saturation ensuring fish did not experience hypoxic conditions. Thirty minutes into recovery water in the respirometers was flushed from the treatment temperature to -1°C, the control temperature. Total recovery time was two hours. Fifty mmHg was chosen as the lowest oxygen tension as it matches levels which have elicited a hypoxic response in previous studies of notothenioids (Holeton 1970; Hemmingsen and Douglas 1972; Axelsson et al. 1992).

The Cold Acclimated experiment had four temperature treatments: -1°C, +2°C, +4°C and +6°C, heart rate $n = 8$, ventilation $n = 8$, and metabolic rate $n = 9$. The -1°C treatment was used as a control as it reflected water temperatures in the fish source location, McMurdo Sound.

4.2.4 Statistical Analysis and Calculations

Heart rate, ventilation and metabolic rates ($\text{mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) (Figure 4.1) were the parameters analysed to investigate the response and recovery of *T. bernacchii* to environmental hypoxia at several acute temperature exposures.

Heart and ventilation rates were calculated by counting the amount of each over one minute at the given time or oxygen partial pressure (PO_2) (Figure 4.1), changes in oxygen tension were converted to metabolic rate using the equation in Chapter 2.4.4. P_{crit} was determined using methods similar to Schurmann and Steffensen (1997) and Cook and Herbert (2012). A repeated – measures ANOVA with post-hoc Dunnetts comparison test identified the partial oxygen tensions where metabolic and resting metabolic rate significantly differed. Resting metabolic rate was projected across the entire PO_2 range; a linear regression, constrained through the origin, was fitted through all points below the resting rate. P_{crit} was defined as the intercept of the linear regression and the resting metabolic rate.

Metabolic, heart and ventilation resting rates were calculated by calculating the mean rate of the variable in question at the given treatment temperature above 120mmHg, consistent with resting normoxic conditions. Resting rates were analysed using a one – way ANOVA or a Kruskal - Wallis ANOVA if data were non-parametric, post-hoc analysis was performed using Tukey Range, Dunnett’s Multiple Comparison Test and Bonferroni tests.

During Hypoxia and Hypoxia recovery one-way repeated measures ANOVA with post-hoc comparison Tukey Range, Dunnett’s Multiple Comparison Test and Bonferroni tests were used to determine statistical differences within the temperature treatment for heart rate and ventilation. In the -1°C treatments post hoc analysis compared all values to the -1°C routine rates, recovery was considered at the point where values were not significantly different from the resting value. At temperatures above -1°C post-hoc analysis was used to determine at what point rates were not significantly different from the -1°C treatment resting rates and the resting rates of the temperature treatment concerned. Two - way repeated measures ANOVA determined the difference between temperatures and across time, points of difference were identified using a Bonferroni post-hoc comparison Q_{10} values were calculated using the equation stated in Chapter 2.4.5.

T. bernacchii in McMurdo Sound inhabit waters at -1.9°C (Eastman 1993), therefore the -1°C metabolic, heart and ventilation rates were used as a control. All mean rates were compared to -1°C resting rates and the relevant treatment temperature mean rates.



Figure 4.1: The instantaneous electrocardiogram (e.c.g) recorded of *T. bernacchii* over one minute during the hypoxia experiment using an oxygen electrode and subcutaneous electrodes. a) The oxygen tension (mmHg) of water in the respirometer. b) Short frequency spikes due to cardiac contraction (heart rate). c) Buccal and opercular muscle contraction generating baselines shifts (ventilation rate).

4.3 Results

4.3.1 Cold Acclimated Normoxic Metabolic, Heart and Ventilation Rate

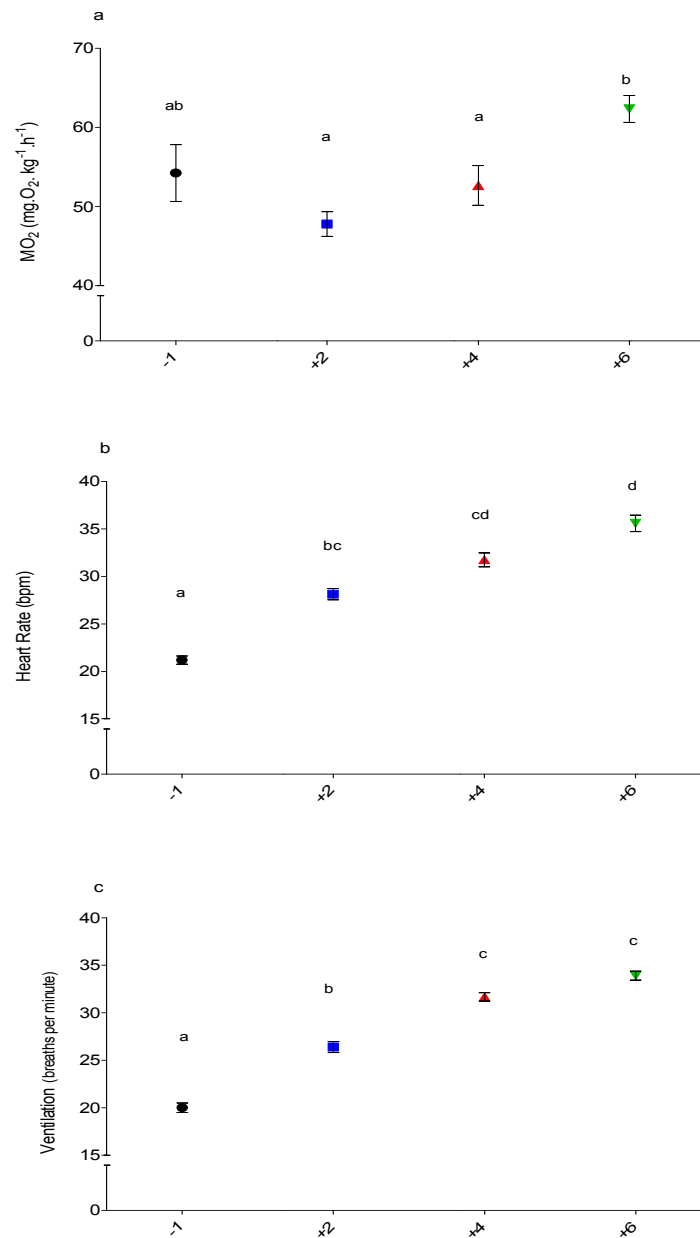


Figure 4.2: Resting metabolic rate of *T. bernacchii* during normoxia at four temperature exposures: -1, +2, +4 and +6°C. a: Metabolic Rate $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ b: Heart Rate bpm c: Ventilation bpm. Differing letters denotes statistical significance in rates between temperatures ($p < 0.05$).

Resting metabolic (MO_2 resting), heart (f_H resting) and ventilation (f_V resting) rates all increased in a stepwise manner as temperature increased, the only exception was the control MO_2 resting -1°C treatment (Figure 4.2a). MO_2 resting was 47.79 ± 1.55 , 52.66 ± 2.5 and 62.35 ± 1.69 mg. O_2 . kg^{-1} . h^{-1} at +2, +4 and $+6^\circ\text{C}$ respectively, the -1°C MO_2 resting was 54.24 ± 3.59 mg. O_2 . kg^{-1} . h^{-1} . MO_2 resting was significantly different between temperature treatments, $F_{3, 140} = 6.01$, $p = 0.0007$. The $+6^\circ\text{C}$ treatment had the highest MO_2 resting, significantly higher than both the +2 and $+4^\circ\text{C}$ treatments (Figure 4.2a).

T. bernacchii f_H resting was significantly different between temperature treatments, Kruskal – Wallis statistic = 84.21, $p < 0.0001$. f_H resting increased in a stepwise manner, 21.19 ± 0.48 , 28.13 ± 0.59 , 31.75 ± 0.74 and 35.6 ± 0.87 bpm at -1, +2, +4 and $+6^\circ\text{C}$ respectively. f_H resting was not significantly different between 2 and $+4^\circ\text{C}$, or between 4 and $+6^\circ\text{C}$, between all other treatments f_H resting increased significantly with increasing temperature (Fig 4.2b).

f_V resting was 20 ± 0.49 , 26.39 ± 0.58 , 31.65 ± 0.43 and 33.91 ± 0.48 bpm at -1, +2, +4, and $+6^\circ\text{C}$ respectively, Kruskal- Wallis statistic = 94.54, $p < 0.0001$. There was no statistical difference between the $+4^\circ\text{C}$ and $+6^\circ\text{C}$; all other treatments were significantly different (Fig 4.2c).

Table 4.1: Q_{10} values of resting metabolic rates (mg. O_2 . kg^{-1} . h^{-1}), heart rate (bpm) and ventilation (bpm) of *T. bernacchii*.

MO_2	$+2^\circ\text{C}$	$+4^\circ\text{C}$	$+6^\circ\text{C}$
-1°C	0.64	0.94	1.22
$+2^\circ\text{C}$		1.62	1.94
$+4^\circ\text{C}$			2.33
Heart Rate	$+2^\circ\text{C}$	$+4^\circ\text{C}$	$+6^\circ\text{C}$
-1°C	2.57	2.24	2.09
$+2^\circ\text{C}$		1.81	1.8
$+4^\circ\text{C}$			1.77
Ventilation	$+2^\circ\text{C}$	$+4^\circ\text{C}$	$+6^\circ\text{C}$
-1°C	2.51	2.5	2.12
$+2^\circ\text{C}$		2.48	1.87
$+4^\circ\text{C}$			1.41

4.3.2 Cold Acclimated Hypoxia response

4.3.2.1 Cold Acclimated Critical PO_2

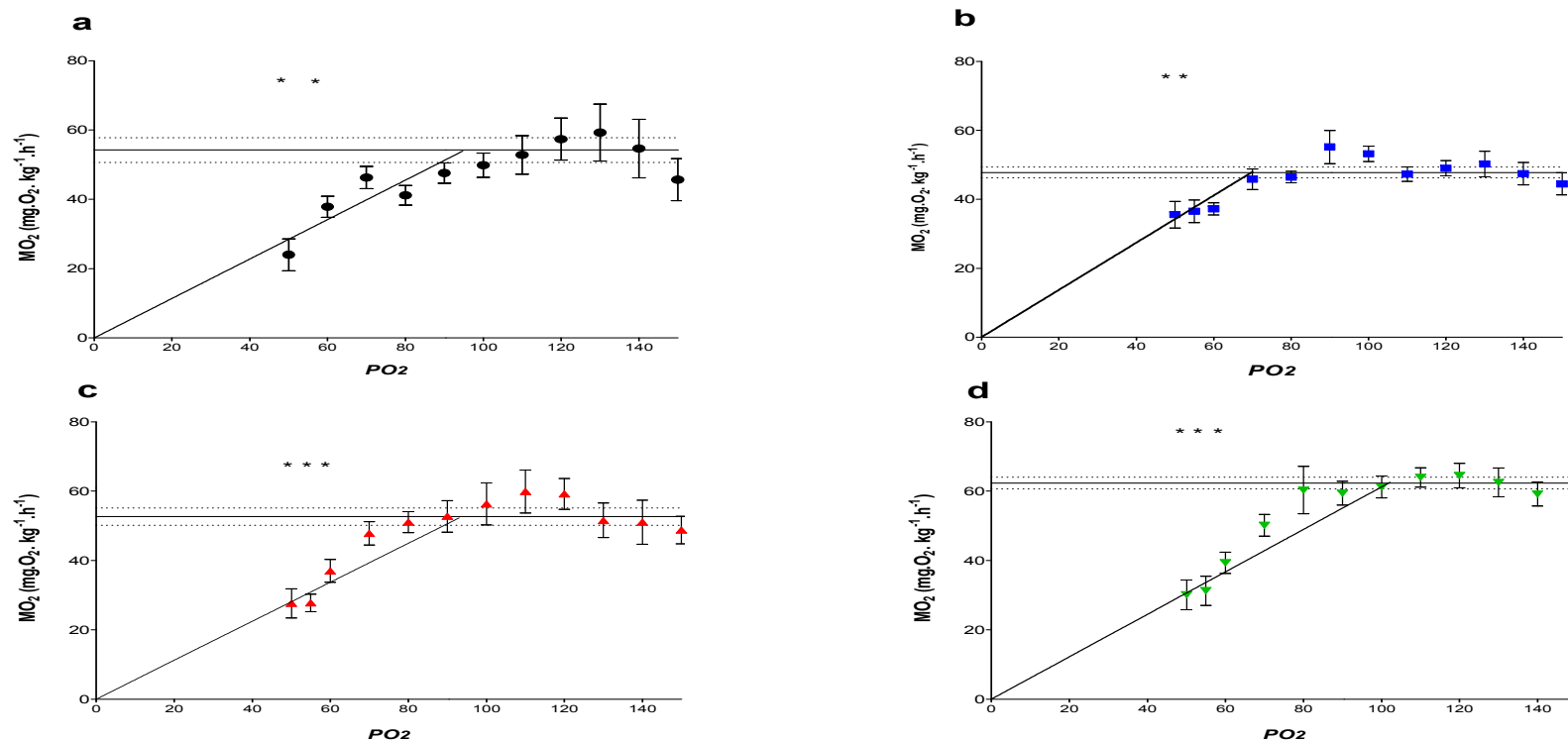


Figure 4.3: Metabolic rate of *T. bernacchii* during a progressive reduction in water oxygen tension (PO_2) at different water temperatures. a: -1°C b: +2°C c: +4°C d: 6°C. Solid horizontal line represents metabolic resting rate during normoxic conditions at that temperature treatment; dotted horizontal lines represent standard error associated with the resting rate. * denotes metabolic rate statistically different from resting metabolic rate, a linear regression was fitted (solid line) through those values statistically different from resting metabolic rate ($n=9$, $p < 0.05$). a) $MO_2 = 0.57 * PO_2$, $Sy.x = 12$ b) $MO_2 = 0.69 * PO_2$, $Sy.x = 10.5$ c) $MO_2 = 0.56 * PO_2$, $Sy.x = 10.21$ d) $MO_2 * PO_2$, $Sy.x = 11$.

Between 150 and 120 mmHg all temperatures exhibited a non significant metabolic rate (MO_2) increase. As oxygen tension decreased below P_{crit} , MO_2 decreased in a linear manner. Over +2, +4 and +6°C, as temperature increased P_{crit} occurred at a higher ambient PO_2 , P_{crit} at these temperatures were resolved at 69.73mmHg (95 % CI 60.9 – 81.56mmHg), 93.67mmHg (95% CI 82.88 – 107.71) and 102.21 mmHg (95% CI 90.36 – 117.64) respectively. The P_{crit} for the ambient -1°C was higher than both the +2 and +4°C treatment, resolved at 95.14 mmHg (95% CI 79.75 – 117.89) (Figure 4.3).

4.3.2.2 Cold Acclimated Heart Rate

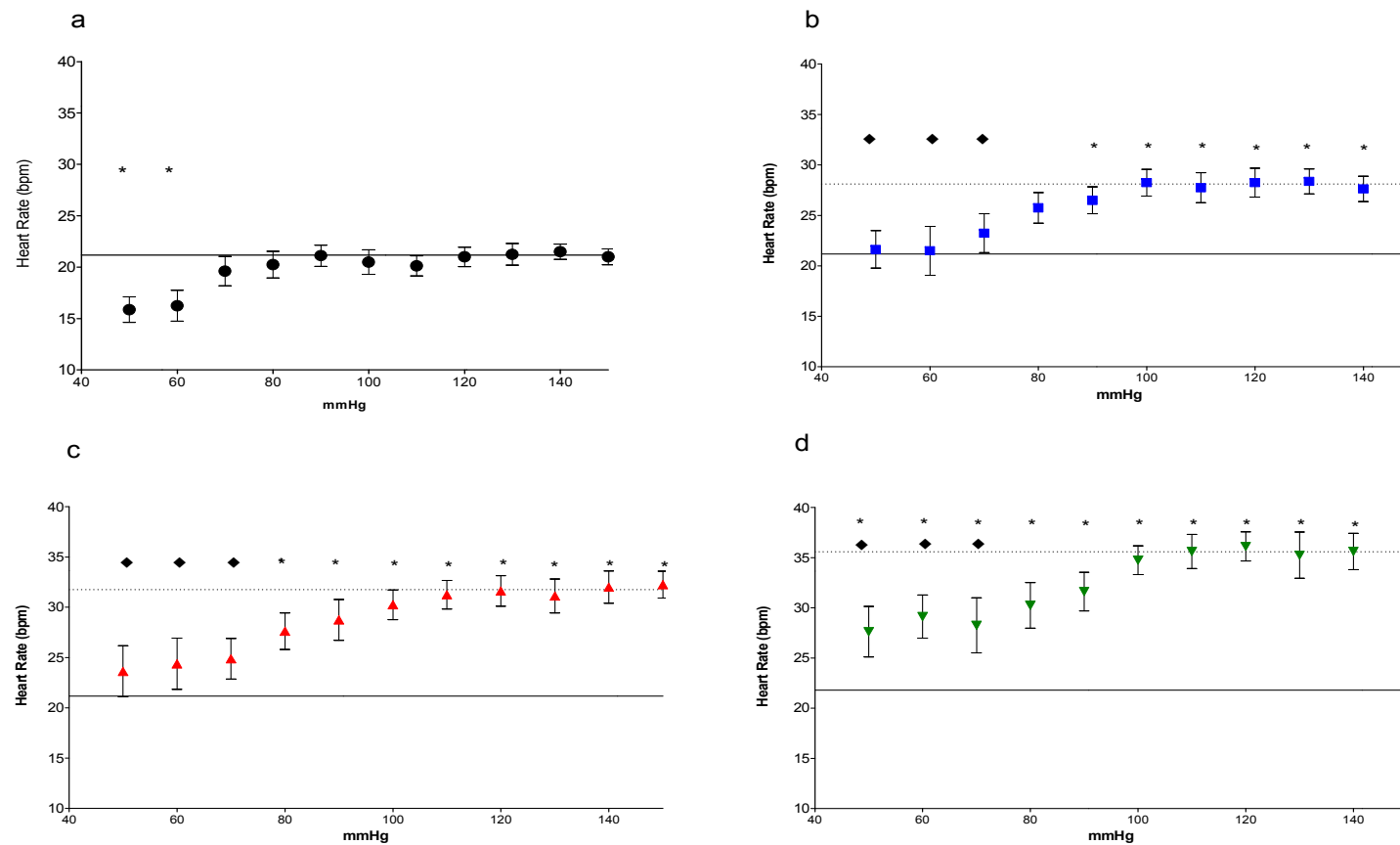


Figure 4.4: Heart rate of *T. bernacchii* during progressive reduction in oxygen tension. a) Water temperature a: -1°C b: +2°C c: +4°C d: +6°C. Solid horizontal line represents routine heart rate at ambient -1°C, dashed horizontal line represents resting heart rate for the given treatment temperature. * denotes statistical deviation from -1°C resting heart rate, ◆ denotes significant deviation from the treatment temperature resting heart rate.

During normoxic conditions (150 -120 mmHg), f_H was maintained independent of ambient PO_2 . In all temperature treatments as oxygen tension progressively decreased, *T. bernacchii* showed a bradycardia response with f_H declining in a linear fashion followed by a plateau between 60 and 50mmHg. In all treatments as oxygen decreased f_H reduced significantly below $f_{H \text{ resting}}$ of that given temperature (Figure 4.4) such that at 50mmHg f_H had decreased by 25% from each temperature treatment's $f_{H \text{ resting}}$ (Figure 4.4). The final f_H measurement at 50mmHg showed a linear increase between temperature treatments, $F_{3, 28} = 5.4$, $p = 0.0048$, Tukey's Multiple Comparison test showed the -1 and +6°C treatments differed significantly (Figure 4.5).

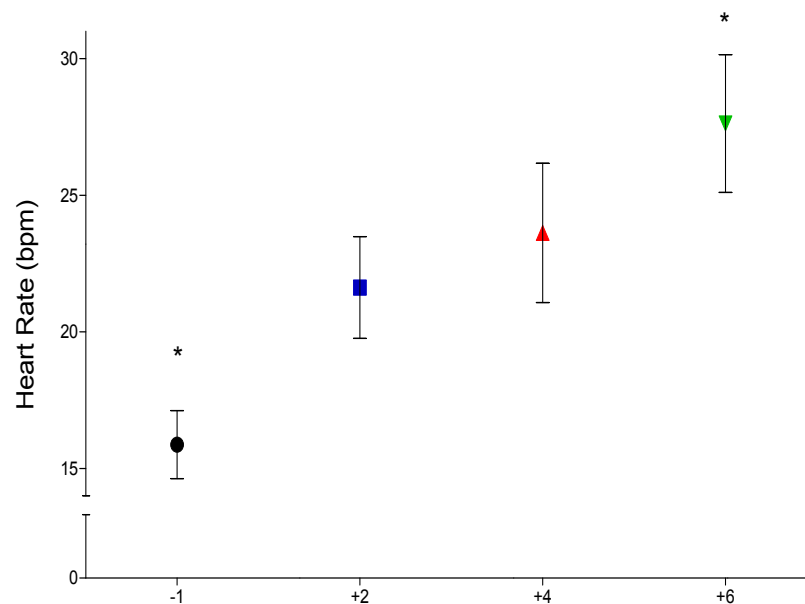


Figure 4.5: Final heart of *T. bernacchii* during hypoxia at 50mmHg partial pressure of oxygen. Black circle represents -1°C, blue square represents +2°C, red triangle represents +4°C and green del represents +6°C. * denotes statistical difference.

4.3.2.3 Cold Acclimated Ventilation

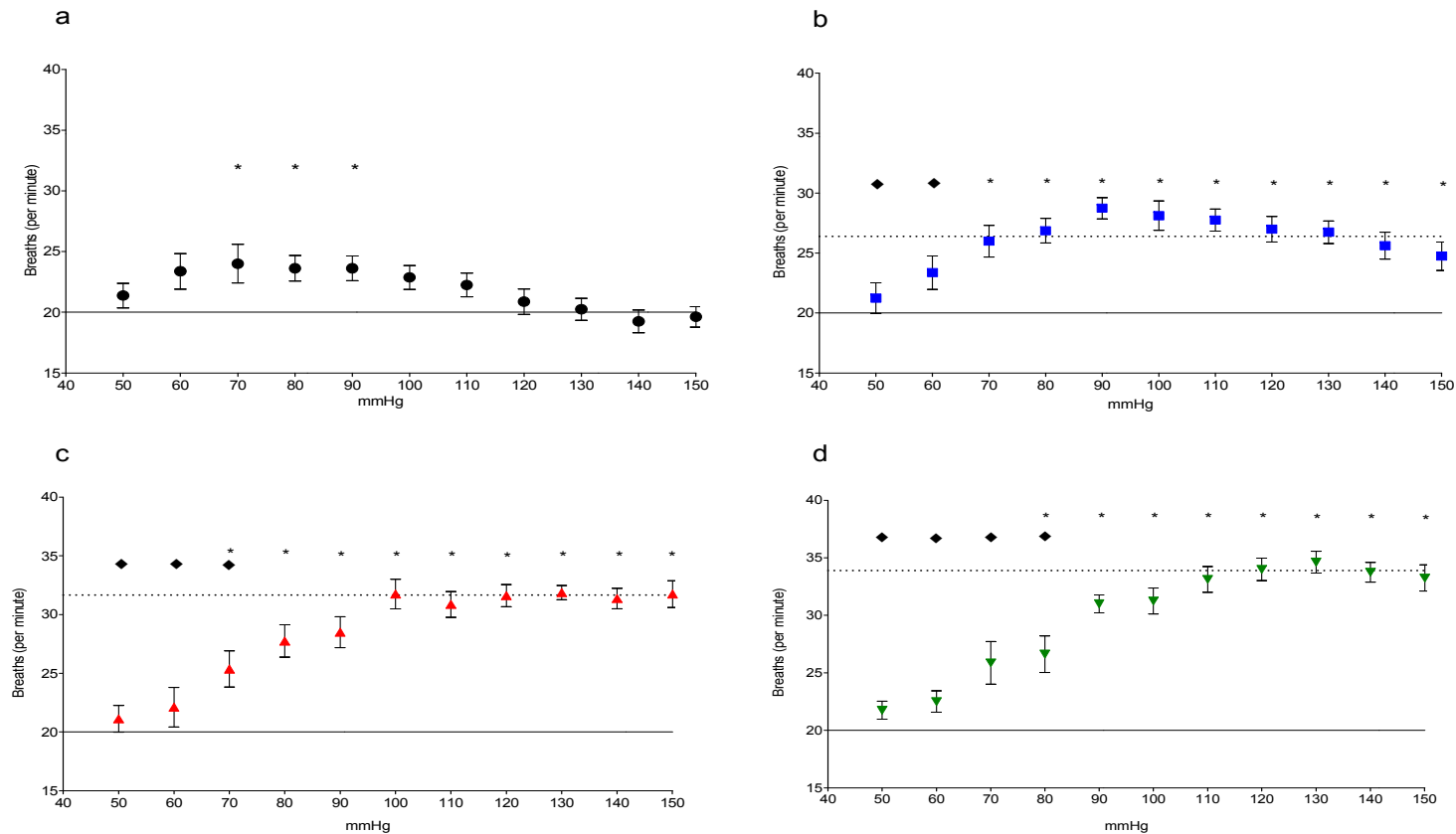


Figure 4.6: Ventilation rate in *T. bernacchii* during continued reduction in partial oxygen tension. a) Water temperature a: -1°C b: +2°C c: +4°C d: +6°C. Solid horizontal line represents resting ventilation rate at -1°C, dashed horizontal line represents resting ventilation rate for the given treatment temperature. * denotes statistical deviation from -1°C resting ventilation rate, ♦ denotes significant deviation from the treatment temperature resting ventilation rate.

In all treatments except +4°C, f_V initially increased to a maximum then steadily declined; in the +4°C treatment the initial rise did not occur. f_V peaked at 24 ± 1.5 , 28.7 ± 0.83 , 31.75 ± 1.08 and 34.63 ± 0.73 bpm, while the lowest observed f_V was 19.25 ± 0.89 , 21.25 ± 1.2 , 21.25 ± 1.08 and 21.75 ± 0.72 bpm at -1, +2, +4 and +6°C respectively. Minimum f_V occurred at 50mmHg in all treatments except the -1°C treatment in which the minimum f_V occurred at 140mmHg (Figure 4.6).

In all treatments except -1°C f_V at 50mmHg, was significantly reduced compared to f_V peak ($p < 0.001$).

At any given temperature f_V was dependent on oxygen tension, two-way repeated measures ANOVA showed f_V was significantly different for temperature, oxygen tension and the interaction effect ($F_3 = 12.96$, $F_{10} = 41.13$ and $F_3 = 15.27$) (Figure 4.7).

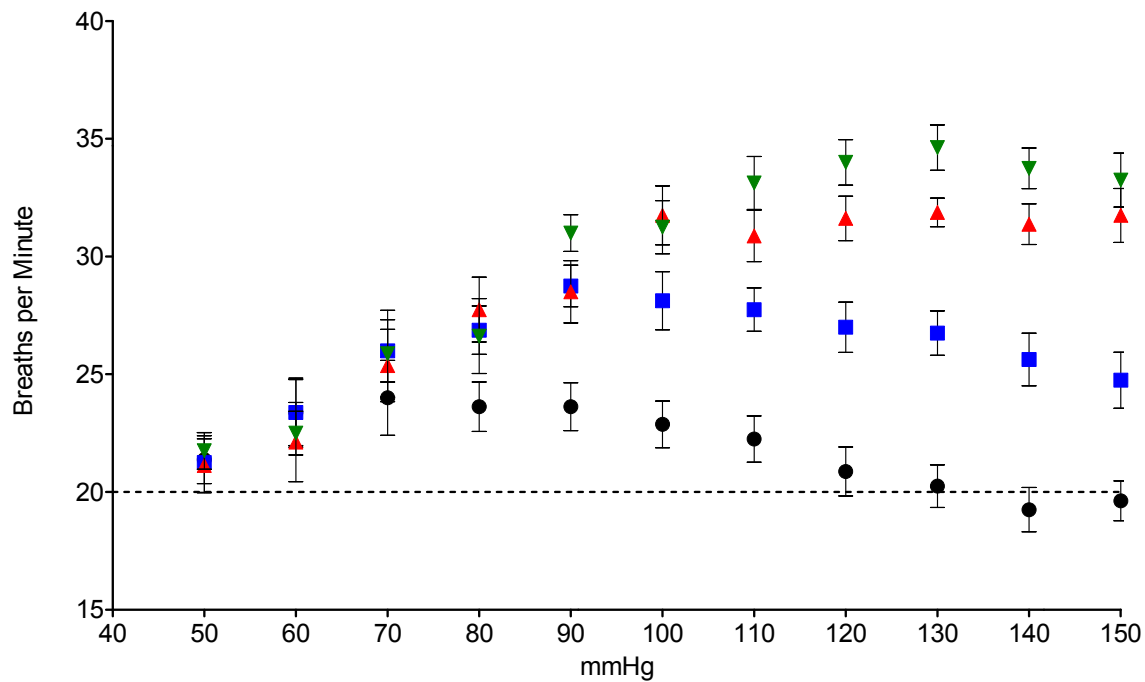


Figure 4.7: Ventilation rate in Cold Acclimated *T. bernacchii* during continuous reduction of ambient oxygen tension, all treatments. Black circles represent fish maintained at -1°C, blue squares represent +2°C, red triangles represent +4°C, green delts represent +6°C. The dotted horizontal line represents the -1°C resting ventilation rate. Statistically different points are reported in Table 4.2.

As oxygen tension decreased to 90 mmHg, there was no significant difference in f_V between any temperature treatments and f_V continually converged until the end of the experiment (Figure 4.7 and Table 4.2).

Table 4.2: Cold Acclimated *T. bernacchii* ventilation during a progressive reduction in oxygen tension. Bonferroni post-hoc comparison of statistically significant differences between temperature exposures, -1, +2, +4 and +6 °C (Figure 4.7).

Temperatures being compared	Point of Statistical Difference (mmHg) ($p < 0.05$)
-1°C - +2°C	150, 140, 130, 120, 110, 100, 90
-1°C - +4°C	150, 140, 130, 120, 110, 100, 90
-1°C - +6°C	150, 140, 130, 120, 110, 100, 90
+2°C - +4°C	150, 140, 130
+2°C - +6°C	150, 140, 130, 120, 110
+4°C - +6°C	No significance

4.3.3 Cold Acclimated Hypoxia Recovery

4.3.3.1 Cold Acclimated Metabolic Rate Recovery

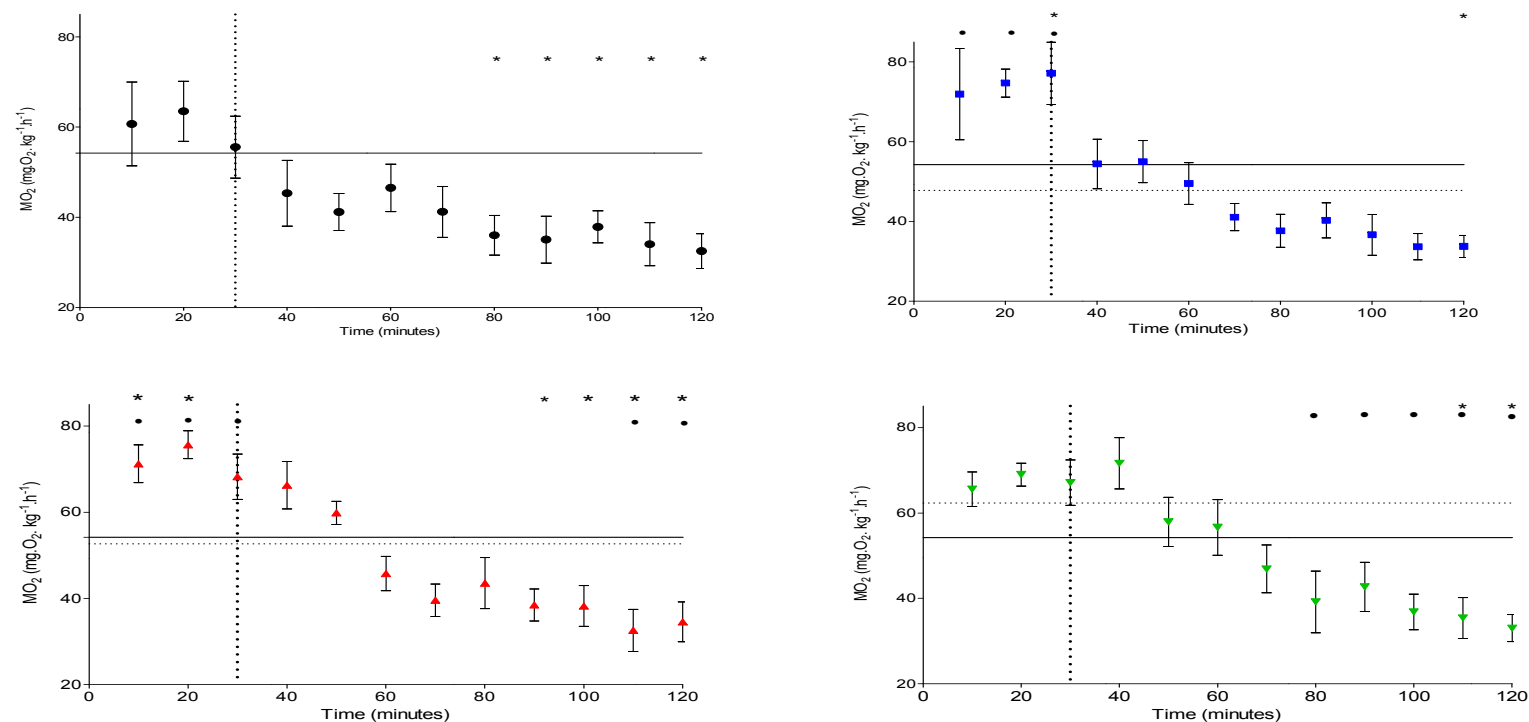


Figure 4.8: Post hypoxia metabolic rate in Cold Acclimated *T. bernacchii* at acute temperature treatments over a two hour recovery period. a: -1°C b: $+2^\circ\text{C}$ c: $+4^\circ\text{C}$ d: $+6^\circ\text{C}$. Dashed vertical line at 30 minutes denotes point water temperature was flushed to -1°C . Solid horizontal line represents -1°C resting metabolic rate (150 – 120 mmHg), dashed horizontal line represents resting metabolic rate at the given temperature treatment. * denotes statistical significance deviation from -1°C resting metabolic rate, ● denotes statistically significant deviation from the given temperature resting metabolic rate.

In all treatments, after the introduction of air saturated water at the given treatment temperature post hypoxia MO_2 increased slightly, this increase from MO_2 resting was significant in the +2 and +4 °C treatments. All treatments were flushed with air saturated control -1°C temperature water 30 minutes into recovery. After return to -1°C all MO_2 rapidly declined to rates significantly lower than the control -1°C MO_2 resting and, lower than the MO_2 resting at that temperature treatment (Figure 4.8).

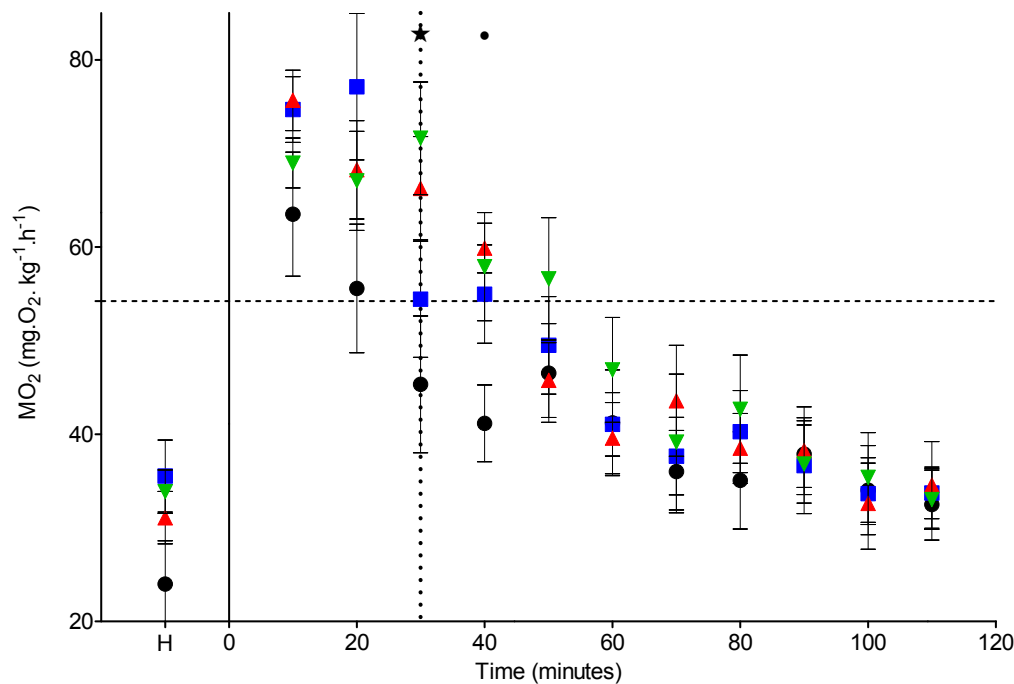


Figure 4.9: The metabolic rate ($\text{mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) of Cold Acclimated *T. bernacchii* over a 2 hour recovery period post hypoxia, all treatments. Black circles represent -1°C, blue triangles represent +2°C, red triangles represent +4°C, green dels represent +6°C. Solid horizontal line represents -1°C resting metabolic rate. The solid vertical line represents flushing of the respirometer back to air saturated water. The vertical dotted line represents the time point water was flushed to -1°C. H represents the resting metabolic rate of *T. bernacchii* at 50mmHg during hypoxia. ★denotes statistical difference between -1°C and +2°C, ● denotes statistical difference between -1°C and +6°C ($n = 9$).

Two-way repeated measure ANOVA with post-hoc Bonferroni showed time had a significantly effect on MO_2 ($F_{11} = 42$) (Figure 4.9).

4.3.3.2 Cold Acclimated Heart Rate Recovery

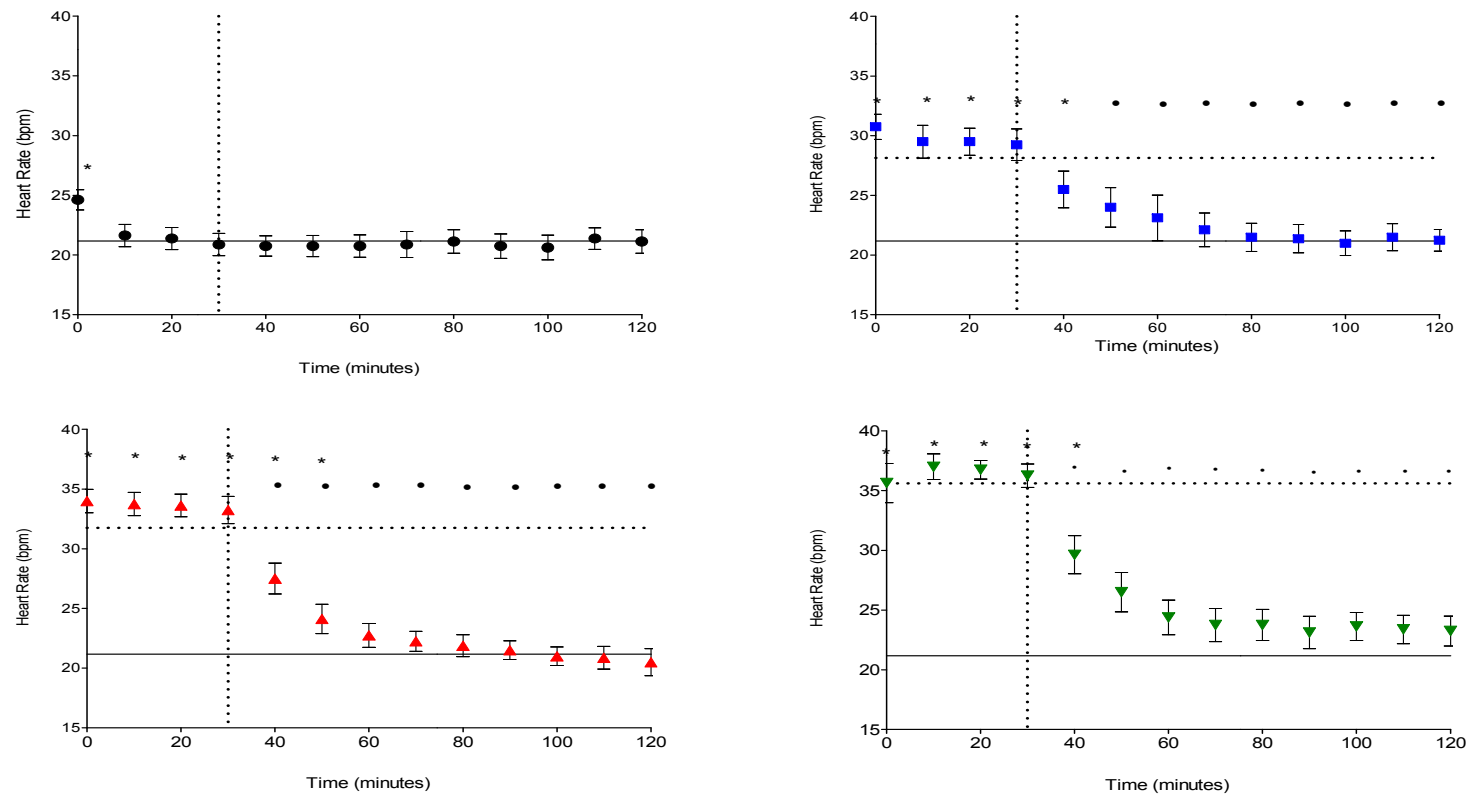


Figure 4.10: Post hypoxia heart rate in Cold Acclimated *T. bernacchii* at acute temperature treatments over a two hour recovery period. a: -1 °C b: +2°C c: +4°C d: +6 °C. Dashed vertical line at 30 minutes denotes point water temperature was flushed to -1°C. Solid horizontal line represents -1°C normoxic resting heart rate (150 – 120 mmHg), dashed horizontal line represents normoxic resting heart rate at the given temperature treatment. * denotes statistical significance deviation from -1°C resting heart rate, ● denotes statistically significant deviation from the given temperature resting .

f_H in all treatments immediately increased from hypoxic f_H to rates mirroring the relevant temperatures $f_{H \text{ resting}}$. The -1°C control treatment was maintained at a constant rate mirroring $f_{H \text{ resting}}$ for the entire recovery period (Figure 4.10a). At all other treatments prior to 30 minutes, f_H was maintained at a constant rate, mirroring that given temperatures resting f_H and was significantly higher than control $f_{H \text{ resting}}$. At 30 minutes immediately following the return of water temperature to -1°C f_H decreased and within 60 minutes all treatments f_H plateaued at the control -1°C $f_{H \text{ resting}}$ (Figure 4.10a, b and c).

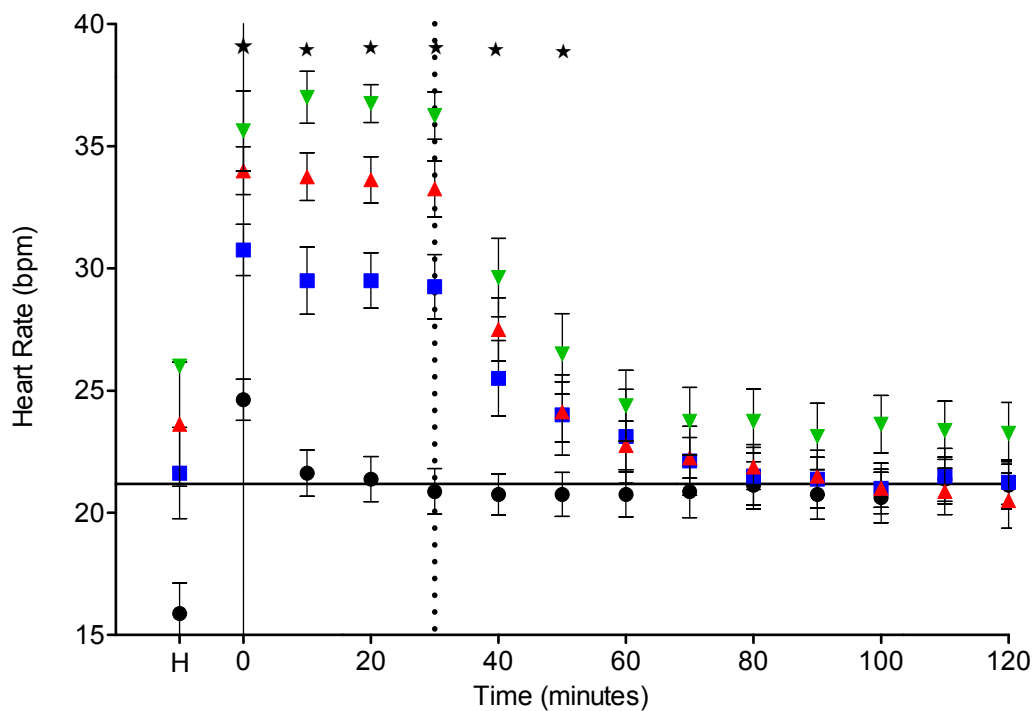


Figure 4.11: The heart rate of Cold Acclimated *T. bernacchii* over a 2 hour recovery period post hypoxia, all treatments. Black circles represent -1°C , blue triangles represent $+2^\circ\text{C}$, red triangles represent $+4^\circ\text{C}$, green dells represent $+6^\circ\text{C}$. Solid horizontal line represents -1°C resting heart rate. The vertical solid line is the time point the respirometer was flushed with air saturated water. The vertical dotted line represents the time point water was flushed to -1°C . H represents the heart rate of *T. bernacchii* at 50mmHg during hypoxia. ★ denotes statistical difference between temperatures, bonferonni post-hoc results recorded in Table 4.3 ($n = 8$).

Two - Way repeated measures ANOVA analysis showed that time, temperature and the interaction had significant effects on Heart rate recovery following hypoxia ($F_{11} = 113.3$, $F_3 = 8.23$ and $F_{33} = 13.99$), meaning recovery varied over time and between temperature treatments and at any given temperature f_H was dependent on recovery time (Fig 4.11). After

50 minutes of recovery there was no statistical difference in f_H between temperature treatments and all were mirroring -1°C rates, suggesting full f_H recovery (Table 4.3).

Table 4.3: Cold Acclimated *T. bernacchii* heart rate during a 120 minute recovery following a challenge to progressive reduced oxygen tension. Bonferroni post-hoc comparison of statistically significant differences between temperature exposures, -1 , $+2$, $+4$ and $+6^\circ\text{C}$ (Figure 4.11)

Temperatures being compared	Point of Statistical Difference (mmHg) ($p < 0.05$)
$-1^\circ\text{C} - +2^\circ\text{C}$	0, 10, 20, 30, 40
$-1^\circ\text{C} - +4^\circ\text{C}$	0, 10, 20, 60, 40
$-1^\circ\text{C} - +6^\circ\text{C}$	0, 10, 20, 30, 40 50
$+2^\circ\text{C} - +4^\circ\text{C}$	No Significance
$+2^\circ\text{C} - +6^\circ\text{C}$	0, 10, 20, 30
$+4^\circ\text{C} - +6^\circ\text{C}$	10, 20, 30

4.3.3.3 Cold Acclimated Ventilation Recovery

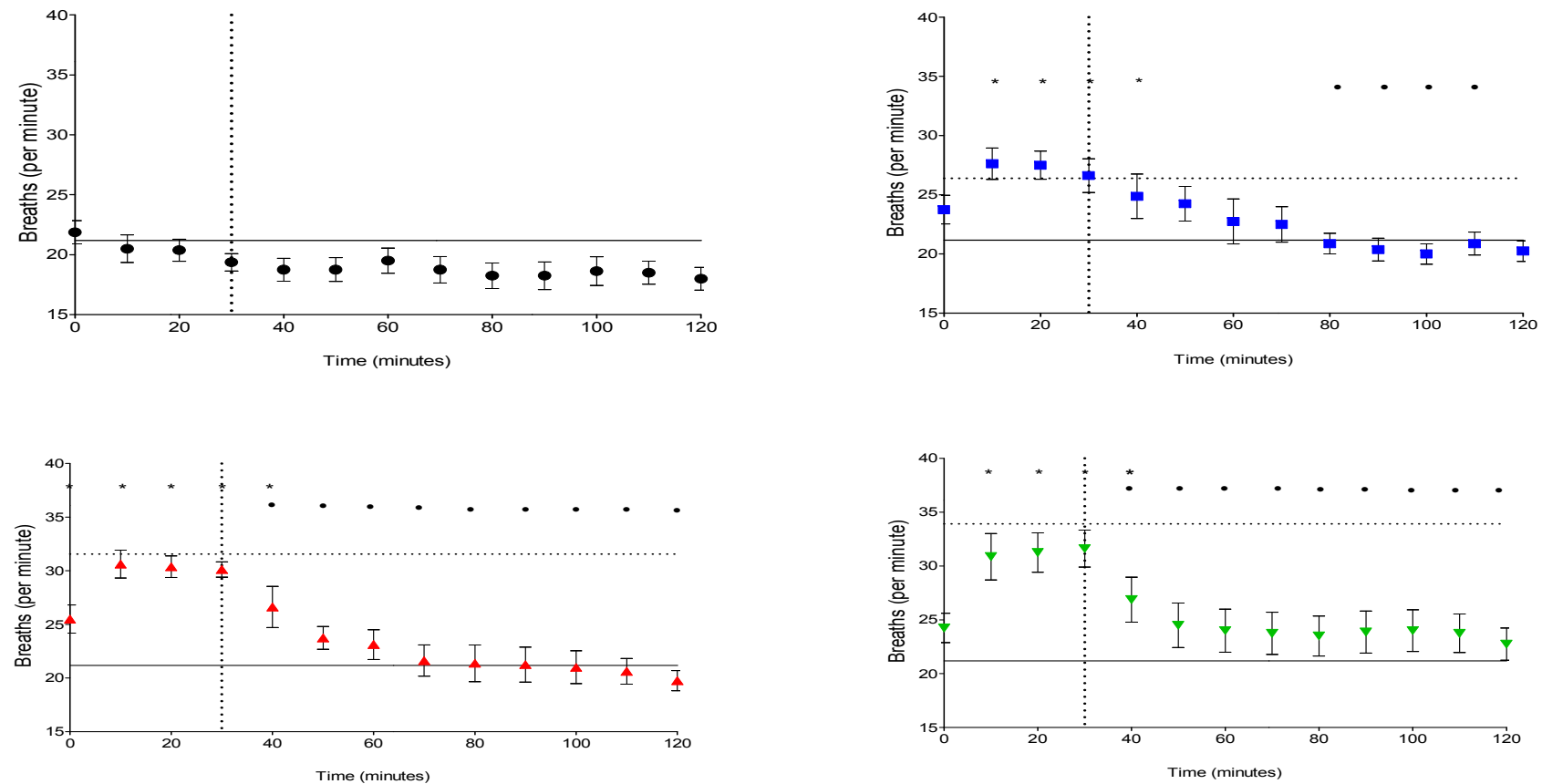


Figure 4.12: Post hypoxia ventilation rate in Cold Acclimated *T. bernacchii* at acute temperature treatments over a two hour recovery period. a: -1 °C b: +2°C c: +4°C d: +6 °C. Dashed vertical line at 30 minutes denotes the time point water temperature was flushed to ambient -1°C. Solid horizontal line represents -1°C ambient temperature normoxic resting ventilation rate (150 – 120 mmHg), dashed horizontal line represents normoxic resting ventilation rates at the given temperature treatment. * denotes statistical significance deviation from -1°C resting ventilation rate, ● denotes statistically significant deviation from the given temperature resting ventilation rate

Directly following return to 100% air saturated water, f_V in the -1°C control treatment began to slightly decline but mirrored resting f_V during recovery at all points, in all other treatments f_V initially increased to levels mirroring the relevant temperature $f_{V \text{ resting}}$. Following a return to -1°C at 30 minutes all f_V steadily declined then plateaued around the control -1°C $f_{V \text{ resting}}$. 50 minutes into the two hour recovery period f_V in all temperature treatments was not significantly different from the -1°C $f_{V \text{ resting}}$ suggesting full f_V recovery (Figure 4.12).

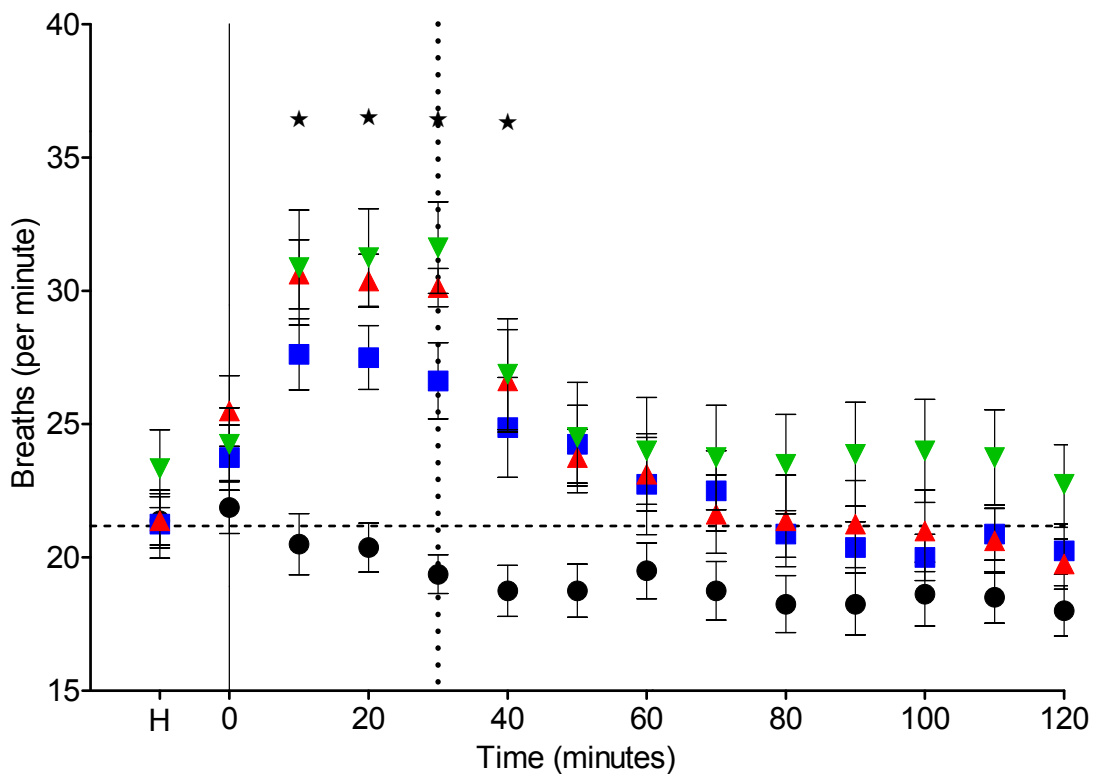


Figure 4.13: The ventilation rate of *T. bernacchii* over a 2 hour recovery period post hypoxia, all treatments. Black circles represent -1°C , blue triangles represent $+2^\circ\text{C}$, red triangles represent $+4^\circ\text{C}$, green dels represent $+6^\circ\text{C}$. Solid horizontal line represents -1°C resting heart rate. The solid vertical line represents flushing of the respirometer with air saturated water at the given temperature treatment. The vertical dotted line represents the time point water was flushed to -1°C . H represents ventilation at 50mmHg during hypoxia. ★ denotes statistical difference between temperatures, ($n = 8$).

Two - Way repeated measures ANOVA analysis showed that time, temperature and the interaction were significant, meaning recovery varied over time and between temperature

treatments and at any given temperature f_V was dependent on recovery time ($F_{11} = 38.18$, $F_3 = 6.399$ and $F_{33} = 3.14$) (Fig 4.13). f_V in the -1°C treatment was significantly lower than all other treatments except at 0 until 50 minutes into recovery at which point and all following f_V not significantly different from the $-1^\circ\text{C } f_{V \text{ resting}}$ (Figure 4.13).

4.4 Discussion

The ability of fish species to tolerate and survive variation in oxygen availability derives from the environment they inhabit and a myriad of species specific physiological changes (Bickler and Buck 2007; Domenici et al. 2013). Antarctic notothenioids inhabit extremely stable well oxygenated waters and may never experience hypoxic conditions; however, some species have been shown to retain physiological responses similar to those found in teleosts which are likely to experience hypoxia (Wells et al. 1998). Environmental temperature affects the hypoxia tolerance in some species of teleosts. As *T. bernacchii* inhabit extremely stable waters and are stenothermic with little tolerance to increases in water temperature, acute temperature change may affect the capabilities of the species to deal with a hypoxic event.

4.4.1 Warm Acclimated Fish Survival

The success of *T. bernacchii* to acclimate to increases in water temperature varies throughout the literature. Several studies have housed *T. bernacchii* at higher temperatures, however, no survival rates were reported (Hoffman et al. 2000; Ream et al. 2003; Podrabsky and Somero 2006; Bilyk and DeVries 2011). Robinson (2008) successfully acclimated the notothenioid *P. borchgrevinki* to $+4^\circ\text{C}$ over both a 28 day and six month period. However, under the exact same laboratory conditions mass mortality of *T. bernacchii* occurred; only one animal survived the entire acclimation period with a mean survival time of 5 ± 0.5 days. The author concluded $+4^\circ\text{C}$ does not fall within the thermal tolerance zone, the temperature range in which 50% of the population can hypothetically survive (Jobling 1981). Enzor et al. (2013) acclimated *T. bernacchii* to $+4^\circ\text{C}$, no mortality was reported, however, resting metabolic rate remained elevated in fish exposed to increased temperature compared to cold acclimated fish, suggesting in this study compensation and acclimation did not occur. Jayasundara et al. (2013) exposed *T. bernacchii* to 4.5°C for 14 days, the authors concluded ventilation rate

indicated partial acclimation of the cardiorespiratory system; however, cardiac acclimation capacity may limit the thermal plasticity in this species.

Due to the variability in the literature of successful acclimation to +4°C in conjunction with the low upper lethal limit of +6°C in *T. bernacchii* (Somero and DeVries 1967) an acclimation temperature of +3°C was used throughout this thesis. Previously in this thesis *T. bernacchii* acclimated successfully to this temperature, showing perfect compensation in oxygen consumption following a 28 day acclimation period (Chapter 3); however in the present study, during an identical acclimation period 90% mortality occurred, rising to 100% over 31 days. This variation in survival to acclimation may be due to the timeframe animals were held in captivity prior to the commencement of acclimation. Metabolic rate has been shown to decrease in animals held over long periods of time in captivity due to acclimation to laboratory conditions (Hop and Graeme 1995); in the present study, resting metabolic rate of control fish approximately seven days following sourcing was $54.24 \pm 3.59 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, elevated compared to *T. bernacchii* resting rates reported in the majority of the literature (Steffenson et al 2005; Robinson 2008; Enzor et al 2013), discussed below. Due to the time constraints associated with the Antarctic research season, animals were placed in +3°C water approximately seven days following capture. The metabolic scope of fish, due to the elevation of resting metabolic rate associated with capture, was likely reduced in fish selected for acclimation, and as metabolic rate accelerates with increased temperature metabolic scope was likely further reduced after relocation to the +3°C acclimation tank. In the present study the metabolic rate of acclimating fish was not measured therefore the reasons for mortality can only be postulated; however, previous research has accredited Antarctic fish heat death to the failure of ventilation and circulation systems to match temperature derived increases in metabolic rate leading to oxygen deficiency at the tissues and an associated build up of anaerobic by products and ultimately death of the animal (Mark et al. 2002; Robinson 2008). It is likely this occurred in the present study, future study of anaerobic by - products may provide an interesting avenue of future research to support this theory.

4.4.2 Resting Rate and Responses to Acute Temperature Change

$MO_2 \text{ resting}$ in *T. bernacchii* varies throughout the literature. Enzor et al. (2013) reported a rate of $12.8 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ one of the lowest described, while Steffensen et al. (2005) reported $27.4 \pm 6.9 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Previous findings in this thesis found $MO_2 \text{ resting}$ of 30.46 ± 2.47 in animals held at Scott Base, Antarctica for at least two weeks (Chapter 5) and 22.38 ± 1.02

mg. O₂. kg⁻¹. h⁻¹ (Chapter 3) in animals acclimated to laboratory surroundings for five months. Wells (1987) reported 49.6 ± 4.7 mg. O₂. kg⁻¹. h⁻¹, while Robinson (2008) had a similar finding of 49.9 ± 8.8 mg. O₂. kg⁻¹. h⁻¹. In the present study MO_2 resting in the control -1°C treatment was high at 54.24 ± 3.59 mg. O₂. kg⁻¹. h⁻¹ (Figure 4.2a), this rate is at the upper end found in the literature. This elevated MO_2 may be due to fish being stressed. Animals used in this experiment were sourced within two weeks of the experiment commencing and were placed in the respirometers 24 hours prior commencement of the hypoxia experiment, a time period shown to be sufficient for recovery from surgery and handling stress (Robinson 2008). However, studies have shown metabolic rate decreases over time in captivity as fish acclimate to the laboratory environment (Hop and Graham 1995). Therefore metabolic rate may have been elevated due to stress associated with capture and housing. Conversely, elevated metabolic rate may be due to accidental measurement of routine, and not resting metabolic rates, due to sporadic movements of fish. This is unlikely in the present study as all fish were stationary within the respirometers adopting the tripod stance, using anal and pelvic fins observed in the natural environment (Wells 1987). In the present study resting metabolic rate under normoxic conditions was calculated using rates taken between 150 and 120mmHg, following methods used previously in the literature (Davison 2002); all treatments showed a slight almost immediate increase in metabolic rate following reduction of oxygen tension (Figure 4.3) and was most pronounced, over 11.5 mg. O₂. kg⁻¹. h⁻¹, in the control -1°C treatment. This increase during normoxic conditions suggests *T. bernacchii* are sensitive to even slight decreases in oxygen tension. This sensitivity may explain the elevated control MO_2 resting compared to resting rates for *T. bernacchii* in the other temperature treatments in the present study and the literature (Figure 4.2a and 4.3).

Exposure to acute temperature change in notothenioid fish leads to an increase in demand for oxygen at the tissues as cellular rates increase (Jayasundara et al. 2013). Between the non control treatments, as expected, resting metabolic rate increased in a stepwise manner as temperature increased (Figure 4.2a), Q_{10} values suggest the increase was a simple temperature effect with values ranging between 0.64 and 2.33 (Table 4.1). However to deduce if the metabolic rise reported was a simple Q_{10} effect, the result of thermal stress or a combination of both, analysis of biochemical parameters associated with thermal stress was required. This was beyond the scope of this study; however this may provide the basis for future research.

The resting heart rate of the control -1°C treatment was 21.19 ± 0.48 beats per minute (Figure 4.2b), this rate corresponds with Davison et al. (1995), who reported resting heart rates of 21.9 ± 0.4 , 21.6 ± 0.6 and 19.5 ± 0.7 bpm and Jayasundara et al. (2013) who reported 21.6 ± 1.4 bpm initial heart rate of *T. bernacchii*. It is higher than the rate observed in Campbell et al. (2009) 14.3 ± 2.4 bpm, and, almost double that found in Axelsson et al. (1992), 10.5 ± 0.9 bpm. This variation may be due to Axelsson et al. (1992) holding animals in a quiescent state via anaesthetic. MS- 222 the anaesthetic administered has been shown to decrease heart rate in several species of fish including *Oncorhynchus mykiss* (rainbow trout), *Salvelinus fontinalis* (brook trout), *Cyprinus carpio* (carp) and *O. tshawytscha* (Chinook salmon) (Randall et al. 1965; Randall and Smith 1967; Houston et al. 1971; Hill 1999). Conversely the authors also found inhibition of the cholinergic control of the heart in *T. bernacchii* via the administration of atropine resulted in a heart rate of 21.7 ± 0.9 bpm, comparable to heart rates found in both Jayasundara et al. (2013) and the present study. Resting heart rate is a balance between adrenergic stimulation and vagal inhibition of the heart (Olsen and Farrell 2006). *T. bernacchii* has one of the highest reported cholinergic tonus of any teleost approximately 80% at resting conditions and recruits only 28%, adrenergic excitatory fibres (Davison et al. 1997); the control of heart rate in *T. bernacchii* varies as the “brake is removed” with very little capacity to “apply the accelerator” (Egginton et al 2006; Robinson 2008). As animals in Axelsson et al (1992) were held in captivity for six weeks prior to determination of resting heart rate metabolism may have been reduced and cholinergic tonus on the heart increased compared to animals in the present study, resulting in reduced heart rate.

Temperature is one of the most important environmental variables determining heart rate in fish, with environmental elevation in temperature resulting in increased heart rate (Axelsson et al. 1992). Exposure to increasing temperature leads to an increase in metabolic rate (as discussed above), resulting in elevated oxygen requirements to drive metabolic processes (Capossela et al. 2012), and this oxygen demand must be met by the cardio - respiratory system (Robinson 2008). In Antarctic fishes increase in cardiac output occurs via increase in heart rate not stroke volume, in fact *T. bernacchii* have shown decreases in stroke volume when exposed to acute temperature increase (Axelsson 1992; Franklin et al. 2007). Axelsson et al. (1992) investigated change in heart rate between 0 and 5°C. The authors found *T. bernacchii* heart rate increased at temperatures 2.5°C and greater, at which point heart rate increased rapidly to a maximum at 5°C of 31.5 bpm with Q_{10} values exceeding 4.

Jayasundara et al. (2013) (supplementary information) showed heart rate in *T. bernacchii* increased from 21.6 ± 1.4 bpm at -1°C to approximately 37 bpm at $+5^{\circ}\text{C}$, however, the change in heart rate across temperatures was not statistically tested. In the present study resting heart rate increased linearly with temperature (Figure 4.2b), with the highest value of 35.76 ± 0.87 bpm at 6°C . This rate sits between heart rate values found in Axelsson et al. 1992 and Jayasundara et al. (2013). Evidence suggests the increase in heart rate is a Q_{10} temperature effect ($Q_{10} = 2.09$) (Table 4.1), however due to the limited thermal range of *T. bernacchii* stress from increased temperature is also likely contribute to elevated heart rate.

The gill and in some fish the skin, is the site at which gas exchange occurs; in *T. bernacchii* the gill is the primary site responsible for oxygen uptake (Davison 2002). In the present study the resting ventilation rate in the -1°C control treatment was 20 ± 0.49 bpm (Figure 4.2c). This rate was similar to the resting ventilation rate of 18 ± 2.37 bpm reported for this species by Forster et al. (1998), 19 ± 3 bpm Jayasundara et al. (2013) and 21.3 ± 3 bpm in *P. borchgrevinki* (Wilson et al. 2002).

In fish as temperature increases so too does ventilatory minute volume (V_w) acting to increase the total volume of oxygen at the respiratory surface, mediation of V_w occurs via alteration of ventilation frequency and/ or ventilation stroke volume. As the pumping of water across the gill is energetically costly (Perry and Wood 1989), changes to minute volume commonly occurs via mediation of stroke volume, however, changes are species specific and increases in ventilation frequency do occur (Graham 2006). Measurement of ventilatory minute volume was beyond the scope of this study, although visual observations of ventilation amplitude, as a proxy of stroke minute volume, were made and are commented on where relevant. In the present study, ventilation frequency increased with increasing temperature (Figure 4.2c), $Q_{10} = 2.12$ (Table 4.1). The increase in ventilation was a function of increased oxygen demand associated with the increase in metabolic rate discussed above, and counters decreases in the diffusive gradient of water and blood PO_2 and oxygen solubility associated with temperature change (Jayasundara et al. 2013). As temperature reaches levels close to the animal's thermal maximum a decline in ventilation occurs. In the present study the increase in ventilation began to decline at 6°C , $Q_{10} = 1.41$ (Table 4.1), this occurred at a lower temperature than Jayasundara et al. (2013) in which ventilation declined in fish acclimated to -1°C after an increase to 7°C . In the present study as stroke volume was not quantified it is not possible to deduce if oxygen uptake across the gill was reduced due to thermal limitations or alteration to

stroke volume occurring to reduce the energetic cost of ventilation and therefore reducing ventilation frequency.

4.4.3 Acute Temperature Change and Critical PO_2 in *Trematomus bernacchii*

P_{crit} , the point at which metabolic processes switch from aerobic to anaerobic due to reduced oxygen tension is used to describe the hypoxia tolerance of any given fish species (Domenici et al. 2013). In nearly all fish species at oxygen levels below P_{crit} survival is limited and if animals are unable to relocate to water containing higher oxygen tension tissue necrosis and ultimately organism death will occur (Boutilier 2001). P_{crit} is dictated by a multitude of factors, including behaviour (Cook et al. 2011) habitat, lifestyle and temperature (Domenici et al. 2013). Temperature affects the oxygen tension at which P_{crit} occurs as higher temperatures elevate the metabolic requirement for oxygen. As oxygen tension decreases and metabolic rate increases, oxygen is utilised faster and P_{crit} within the same species should occur at higher oxygen tensions. Acute increases in temperature elicit greater metabolic responses in fish that have limited acclimation capacity which may further limit the response to hypoxia (Capossela et al. 2012).

In the present study the P_{crit} of *T. bernacchii* rose with temperature in all but the control - 1°C treatment, with P_{crit} resolved at 69.73, 93.67 and 102.21 mmHg at +2, +4 and +6°C respectively. The -1°C P_{crit} occurred at an oxygen tension of 95.14 mmHg (Figure 4.3), however, this P_{crit} may be overestimated due to elevation of the resting metabolic rate in that treatment, as discussed above. The P_{crit} of *T. bernacchii*, in all but the control treatment over acute temperature change follows previous patterns in the literature.

P_{crit} of most teleost species lies between 40 and 60 mmHg (Jobling 1994), the values obtained in this study suggest *T. bernacchii* are sensitive to small reductions in environmental oxygen, switching to an oxyconforming state at moderate to high oxygen tensions. To the knowledge of this author P_{crit} in *T. bernacchii* has not been quantified, however, parameters such as haematocrit (Hct) number (the number of circulating red blood cells in the plasma), as a response to hypoxia has been investigated. Davison et al. (1994) reported a 64% increase in haematocrit, corresponding to a decrease in relative spleen mass, the organ responsible for storage of erythrocytes. This research demonstrates one of the cardiovascular adjustments employed by this species and supports the present study demonstrating the sensitivity of *T. bernacchii* to reduced oxygen. Future investigation of P_{crit} in *T. bernacchii* would benefit

from the investigation of end products associated with anaerobiosis; in particular accumulation of lactate and if levels mirror the point at which P_{crit} is identified to confirm the oxygen tension at which *T. bernacchii* switch to anaerobic metabolic pathways.

4.4.4 Ventilation Responses to Hypoxia and Acute Temperature Changes in *Trematomus bernacchii*.

If fish are unable to employ behavioural strategies such as relocation to avoid hypoxia teleosts respond to early and mild hypoxia by several adjustments to cardiac function (Diaz and Rosenberg 2008; Cook et al. 2011).

Ventilation and perfusion are the two crucial factors determining metabolic rate as the pace at which these occur dictates the rate of gas exchange (Perry et al. 2009). Therefore, alteration in ventilation is perhaps the most important physiological adjustment made by fish when faced with moderate hypoxia (Randall 1982). Hyperventilation is a classic response in fish when decreases in oxygen tension are detected; this occurs via an increase in ventilatory minute volume, acting to elevate the total volume of water, thus, oxygen present at the gill. Hyperventilation acts to maintain the PO_2 gradient between water and the blood, promoting branchial oxygen transfer which in turn sustains high arterial blood content, therefore maintaining the ability of fish to utilise aerobic metabolic processes (Randall 1982; Perry et al. 2009; Capossela et al. 2012). Ventilation represents a major proportion of metabolic cost; in resting fish the energetic cost of powering opercular ventilatory muscle is estimated to be 20% of metabolic rate (Marvin and Heath 1968). As partial pressure decreases, the metabolic cost of elevated ventilation and thus metabolic rate surpasses the advantages associated with those increases and ventilation rate declines. Ultimately ventilation fails to provide sufficient gas exchange, metabolism switches to anaerobic pathways, which can only be sustained for a short period of time and death occurs (McBryan et al. 2013). As extensively covered in this thesis, temperature increases the rate at which biochemical and metabolic process occur. In Antarctic notothenioids slight increases in temperature may cause stress related increases in cardiovascular function as the thermal window in which animals can function is small. Because resting ventilation is elevated via a temperature and/or stress effect, during hypoxia at higher acute temperature exposures, *T. bernacchii* may not have the scope available to increase ventilation to rates required to maintain an adequate transbranchial gradient and thus processes associated with hypoxia may occur at higher oxygen tension.

This appeared to be the case in the present study. At the two lower treatment temperatures the ventilation of *T. bernacchii* responded in a typical manner, the opposite of the decrease reported for this species in Fanta et al. (1989). In the -1°C treatment ventilation increased to a rate 20% greater than the resting value; however, in the +2°C treatment the peak ventilation increased by 8.7% higher, an increase which was at no time significantly higher than that treatment's $f_{V \text{ resting}}$ (Figure 4.6a and b). This suggests at elevated temperature the scope of the ventilatory response is reduced. This is further supported by the highly reduced or absent increase in ventilation in the +4 and +6°C temperature exposures. Additionally as temperature increases so too does the oxygen tension at which peak ventilation occurs, 70, 90, 100 and 130mmHg at -1, +2, +4 and +6°C respectively (Figure 4.6). This peak ventilation suggests that at the two highest acute temperature exposures, due to Q_{10} or stress related responses ventilation maximum rates are reached during normoxic conditions and *T. bernacchii* are unable to respond to reductions in oxygen with the typical elevation of ventilation. Interestingly as oxygen decreased below 90mmHg ventilation of all treatments began to converge (Figure 4.7) and at 50 mmHg all rates were between 19 and 21 beats per minute. This may represent a rate at which ventilation reaches a point of diminishing returns, when the metabolic cost does not outweigh the benefit of increased rate and ventilation plateaus at a rate which is not as metabolically costly, around 20 beats per minute. However this can only be postulated as ventilation rate below 50mmHg was not measured and rates may have diverged at lower oxygen tensions.

4.4.5 Cardiovascular Responses to Hypoxia and Acute Temperature Changes in *Trematomus bernacchii*.

Temperate teleosts respond to hypoxia typically via an increase in vagal tone and increase in dorsal aortic pressure mediated by systemic resistance resulting in a reflex bradycardia (Axelsson 2005), the benefit of which is thought to be twofold. Firstly, increased cardiac control via the negative force frequency effect (Gamperl and Driedzic 2009) and secondly, improved oxygenation of the myocardium due to increased residency time of blood in the heart (Farrell 2007). The bradycardia, via increased pulse pressure, may serve to open vascular spaces in the gill lamellae, allowing better perfusion in addition to recruiting previously unperfused lamellae and providing additional rigidity to the lamellar structure, all of which serve to increase the gill diffusion capability (Randall 1982; Jobling 1994). Previous investigation of hypoxia in *T. bernacchii* has shown a tachycardia response, a direct opposite

to the “typical” bradycardia response (Gamperl and Driedzic 2009). Axelsson et al (1992) investigated cardiovascular function in *T. bernacchii* after reducing oxygen to 50 mmHg, this study concluded *T. bernacchii* did not show the expected bradycardia; in fact a phasic bradycardia combined with an arrhythmia eventuated in a overall tachycardia consisting of a 2bpm increase in heart rate. However, the literature suggests the lack of bradycardia in *T. bernacchii* is by no means confirmed and requires further investigation (Gamperl and Driedzic 2009). In the present study, *T. bernacchii* showed a bradycardia response previously unreported in the literature. At all temperature treatments a 25% reduction in heart rate from resting values occurred, with bradycardia becoming significant at 60mmHg in the control temperature and at 70mmHg in the remaining treatments (Figure 4.4). Obviously, the bradycardia observed in *T. bernacchii* in the present study differs from the hypoxia response reported in the literature. However the lack of bradycardia reported in Axelsson et al. (1992) may be a matter of *T. bernacchii* in that study not being reduced to a low enough mmHg relative to the reported resting heart rate. The resting rate reported in Axelsson et al. (1992) is almost half the resting rate reported in the present study, therefore, oxygen tension at which bradycardia occurred may be lower than the present study due to the difference in resting heart rates and presumably associated costs such as ventilation and metabolic rate. Bradycardia in the control -1°C treatment becomes significant in the present study at 60mmHg, it is possible that to observe a bradycardia associated with reduced oxygen tension in *T. bernacchii* in Axelsson et al. (1992) required a reduction in oxygen tension lower than 50mmHg.

4.4.6 Recovery from Hypoxia and Acute Temperature Change

Immediately after reintroduction of oxygenated water, *T. bernacchii* began to show responses associated with recovery (Figure 4.8, 4.9, 4.10, 4.11, 4.12, and 4.13). Observation of MO_2 directly following reintroduction of fully saturated water was not possible as it takes several minutes to mix re-oxygenated water within the respirometers. However ten minutes following, metabolic rate increased more than twofold at all temperature treatments. This increase reflects, at least in part, the costs associated with gearing up physiological systems, increases in heart and ventilation rate to enhance oxygen delivery to tissues. Previous studies have reported a tachycardia associated with recovery from hypoxia (Axelsson et al. 1992), increase in stroke volume, an increase in cardiac output and a decrease in total vascular resistance all of which are associated with increased oxygen delivery to previously anaerobic

tissues (Campbell et al. 2009) returning to post hypoxic rates. In the present study an immediate tachycardia was observed (Figure 4.11) and prior to return to -1°C all rates returned immediately to $f_{\text{H resting}}$ for that temperature (Figure 4.10). Ventilation in the control treatment showed little variation, mirroring the control resting ventilation for the recovery period with a slight continuous decrease (Figure 4.12a). In all other treatments ventilation increased rapidly back to the relevant treatment's resting rate within ten minutes of recovery (Figure 4.12 b, c and d; Figure 4.13).

In the control treatment, within ten minutes of introduction of aerated water all parameters had returned to resting levels and, in the case of heart rate, recovery was instantaneous with a slight overshoot (Figure 4.10). This lends support to the presence of oxygen detecting neuroepithelial cells situated near or within the gill; as all other variables within the respirometers were held constant *T. bernacchii* must possess a sensitive detection system for instantaneous change to occur. The MO_2 of the control treatment declined immediately after measurements began, dropping below resting rates within thirty minutes of recovery. This suggests at ambient temperatures *T. bernacchii* have the capacity to rapidly clear end products associated with anaerobic load. Conversely, build up of end products may have been minimal due to respirometers being returned to complete air saturation immediately after oxygen tension reached 50mmHg with animals not being held for any significant period of time at the lowest oxygen tension. However, reduction from P_{crit} 95.14mmHg to 50mmHg ranged between 50 and 85 minutes for each fish, this in excess of Axelsson (1992) in which animals were held at hypoxic conditions (50mmHg) for approximately 40 minutes. Further supporting fast clearance of anaerobic processes was the recovery time of the other temperature treatments. As with the control, at all other temperatures directly following introduction of oxygenated water heart rate, ventilation and MO_2 immediately increased. Rates remained close to the resting rate of the given temperature treatment until water was returned to ambient -1°C control temperature. This again suggests *T. bernacchii* are able to recover from the effects of hypoxia rapidly as physiological processes are able to immediately increase following air saturated water. Within one hour of recovery and 30 minutes of return to -1°C all parameters had returned to or dropped below the control resting rates. In all temperature treatments MO_2 lowered to approximately $30 \text{ mg. O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Figure 4.8), this rate is significantly lower than the -1°C $\text{MO}_{2 \text{ resting}}$, and further supports the theory that in that treatment $\text{MO}_{2 \text{ resting}}$ was elevated (Figure 4.2a).

The reduction of MO_2 , heart and ventilation rate in all treatments following return to -1°C suggests *T. bernacchii* appear to be able to recover relatively quickly from both hypoxia and acute exposure to increases in temperature.

4.5 Summary

Trematomus bernacchii have inhabited highly oxygenated, thermally stable water for millions of years (Eastman 1993), therefore regularly encountering hypoxic environments may not currently be likely. However, with anthropogenic climate change altering the earth's weather patterns, sea temperature is expected to rise and the Southern Ocean is not exempt from that predicted change. *T. bernacchii* may well encounter elevated temperatures within the next century. Although hypoxic waters are not currently an issue for Antarctic notothenioids, most likely due to retention of genetic and physiological characteristics from a previous ancestor, research has suggested some notothenioids are able to respond to hypoxic waters. Metabolic rate increased immediately after reduction in water tension suggesting *T. bernacchii* show sensitivity to even slight reduction in oxygen tension. This increase in metabolic rate may have been caused, in part, by the increase in ventilation, a common response of teleosts to mild hypoxia; however at $+4$ and $+6^\circ\text{C}$ fish this ventilation increase was not observed. The average range of P_{crit} in teleosts sits between 40 and 60mmHg, by comparison the P_{crit} of *T. bernacchii* was relatively high, between 69 and 102mmHg, and in all treatments above -1°C increased as temperature increased.

Perhaps most importantly, this study found a reflex bradycardia associated with hypoxia, previously unreported in the literature. Heart rate decreased 25% from resting rates in all temperature treatments. Acute temperature exposure increased the rate at which physiological processes occurred and at higher temperatures the metabolic and ventilatory scopes were greatly reduced with normal increases associated with hypoxia response not observed. This combined with stepwise increases in P_{crit} with increasing temperature suggest *T. bernacchii* will be less adapted to combat hypoxia if sea temperatures increase to levels 4°C and above. However *T. bernacchii* are clearly capable of physiological response to hypoxic waters despite occupying and evolving in a highly oxygenated environment.

Further investigation of the parameters associated with anaerobic metabolism such as lactate accumulation and clearance rate following hypoxia in *T. bernacchii* was beyond the scope of

this study. This highlights an area of future research necessary to continue building the current understanding of *T. bernacchii* and its response to hypoxia.

CHAPTER FIVE

Aerobic Scope and the Effect of Acute Temperature Exposure in *Trematomus bernacchii*

5.1 Introduction

Increasing ocean water temperature due to climate change is predicted to affect individual fish, fish species, the structure of communities and the function of entire ecosystems (Pörtner and Knust 2007); the effects of increased environmental temperature are species, environment and latitude dependent (Nilsson et al. 2009). Ectotherms inhabiting mid temperate latitudes have evolved in waters in which the temperature fluctuates; as a result, these ectotherms have evolved mechanisms to allow exploitation of a thermally fluctuating niche and are therefore eurythermic (McBryan et al. 2013). Those ectotherms inhabiting extreme latitudes, the poles or equator, have evolved in thermally stable environments (Rummer et al. 2014), in particular, ectotherms in the Ross Sea whose evolution has occurred in static water temperatures of -1.9°C for approximately 10 million years (Eastman 1993). As a direct result thermal ranges in notothenioids inhabiting this region are greatly reduced compared to their temperate counterparts and many Antarctic fish are extremely stenothermic (Wilson et al. 2001; Somero 2010).

It is highly probable specialised adaptations required for survival at sub- zero temperatures, such as preservation of high numbers of muscle mitochondria and synthesis of antifreeze glycoproteins, induce a metabolic cost not incurred in temperate fish (DeVries and Eastman 1981; Johnston 1987). Nevertheless, evidence suggests when compared to temperate and tropical fish of similar lifestyle, resting metabolic rate in Antarctic notothenioids is reduced. For example, Johnston et al. (1991) found the resting metabolic rate of the notothenioid *Notothenia neglecta* was lower than several temperate and tropical fish at each given species

normal environmental temperature. This result appears to be reflected in the majority of Antarctic notothenioids studied (Wells 1987).

Aerobic scope (maximum metabolic rate minus resting metabolic rate), is the physiological capacity of aerobic performance (Clarke et al. 2013). Aerobic metabolic scope is important as its breadth dictates the ability of the organism to perform vital processes such as, feeding, reproduction and locomotion which in turn, may dictate survival (Pörtner and Lannig 2009). It has been postulated that reduced resting metabolic rate may allow for an increase in metabolic scope in Antarctic notothenioids (Holeton 1974). However, several studies have shown this is not the case, with metabolic scope reflecting those found in temperate and tropical fish of similar lifestyle (Forster et al. 1987; Lowe and Davison 2006).

Variation in ambient temperature may lead to changes in aerobic scope. Evidence suggests, due to the progressive divergence of circulatory and ventilatory systems, as temperature approaches upper and lower thermal limits, whole animal aerobic scope is the first process to become limiting (Pörtner 2001). As thoroughly discussed in this thesis, temperature has a greater effect on resting, rather than maximum metabolic rates, with the former increasing, as temperature does (Pörtner and Lannig 2009). Mechanistically the effect of temperature on fish performance may be associated with the way in which temperature influences oxygen uptake, transportation and delivery to tissues, also known as the “OCLTT” (the oxygen and capacity limited thermal tolerance) (Pörtner 2001; Pörtner and Knust 2007; Pörtner and Farrell 2008; Rummer et al. 2014). Aerobic scope diminishes at temperatures in excess of thermal optima and disappears at critical temperatures at which point metabolic processes transition from aerobic to anaerobic (Pörtner 2001; Peck et al. 2004) and thereby performance decreases or ceases. In *T. bernacchii* the lowest possible resting metabolic rates are obtained at -1.9°C, the temperature at which seawater freezes (Johnston et al. 1991; Somero 2010); while the upper lethal temperature limit of *T. bernacchii* has been described by Somero and DeVries (1967) as +6°C, at that time, the lowest reported for any organism. However, information on the reduction of metabolic scope in *T. bernacchii* during exposure to acute temperature increase has not been reported.

Geographical distribution of a species may reflect the ecological consequences of thermal effects on aerobic scope. This has been shown recently in the North Sea eelpout (*Zoacres viviparous*), as the water temperature at which population density declines correlates with thermal oxygen limitation delivery in this species (Pörtner and Knust 2007; Robinson 2008).

Therefore, the aptitude for marine fishes to aerobically perform is predicted to control thermal tolerance (Nilsson et al. 2009).

Evolutionary theory predicts stenotherm fish, such as *T. bernacchii*, specialised to life at a constant temperature have limited or no capacity to acclimate to changes in temperature (Pörtner and Knust 2007). This appears to be the case for many Antarctic notothenioids. For example, following 28 days exposure to 4°C both *Trematomus hansonii* and *Trematomus pennellii* were unable to acclimate with mortality occurring in over 50% of animals (Robinson 2008). However, reports of the acclimatory ability of *T. bernacchii* vary throughout the literature (Hofmann et al. 2000; Ream et al. 2003; Robinson 2005; Enzor et al. 2013). The response of marine animals to increased ocean temperature is predicted to be determined by the degree of reduction in aerobic scope (Pörtner and Knust 2007; Nilsson et al. 2009) particularly in those organisms, such as *T. bernacchii* in which relocation is not possible (Peck et al. 2010). A thermally reduced aerobic scope may make species in the thermally stable Antarctic waters particularly susceptible to changes in temperature due to anthropogenic climate change (Somero 2010).

The primary goal of this research was to investigate and quantify the effects of acute temperature increase on the scope for activity of *T. bernacchii* in fish held at temperatures experienced in the natural environment and those acclimated to +3°C. The effect of temperature on the recovery from exercise was also investigated.

5.2 Methods

5.2.1. Fish

Trematomus bernacchii used in this experiment were sourced from McMurdo Sound, Antarctica during the 2011 / 2012 and the 2012 / 2013 summer seasons. Fish were collected, transported and housed at the Scott Base Wet Laboratory facilities as described in Chapter 2.1. All experiments in this chapter took place at Scott Base Antarctica.

The mass of *T. bernacchii* in cold acclimated experiments was 144.53 ± 25 g, range 116.5 - 186g, and length of 215.75 ± 9.57 mm, range 201 – 230mm, n = 8. Animals in the cold acclimated treatment were housed as described in chapter 2.2.2.

Continuous sampling respirometry as described in chapter 2.4.1 was used in this experiment.

5.2.2 Acclimation Survival

As discussed in Chapter 4.2.2, only 5% of animals survived the acclimation period, therefore, there was not enough animals to perform the aerobic scope experiment on acclimated fish. Reasons for fish mortality are discussed in Chapter 4.4.1.

5.2.3 Experiment and Exercise Protocol

Fish were held in the Scott Base Wet Lab aquarium system for at least 96 hours prior to surgery to allow for recovery from stressors associated with capture. Fish were anaesthetised and electrodes were inserted into animals as described in Chapter 2.5. Fish were placed in individual respirometers, to allow measurement of parameters described below, in stand-alone water baths, immediately after surgery. Experiments commenced 24 hours after surgery to allow adequate time to settle into the respirometer and recover from surgery and handling stress; 24 hour recovery time is in accordance with previous research on Antarctic notothenioids (Morris and North 1984; Wells et al. 1984; Wells 1987; Johnstone et al. 1991).

Maximum metabolic rate was tested at four temperatures: -1, +2, +4 and +6°C. The -1°C treatment was used as a control as this parallels the water temperature in McMurdo Sound, Ross Sea, Antarctica, where fish were sourced.

Changes in water oxygen tension, fish heart and ventilation rates were measured using methods described in chapter 2.4.1, manual measurements were taken of ventilation by counting the beat frequency of the operculum over one minute.

The experiment began by gently removing all air bubbles from the respirometer and sealing the chamber for thirty minutes to allow pre-exercise measurements of oxygen consumption, heart rate and ventilation. To ensure fish did not become hypoxic, throughout the entirety of the experiment, oxygen tension was kept above 70% saturation (115mmHg), via flushing of the respirometer with air saturated water. Animals were placed in the relevant treatment temperature for one hour prior to beginning of experiments to allow adjustment to that temperature, after which respirometers were closed for one hour to obtain resting metabolic rates. As *T. bernacchii* refuse to swim when placed in a swim tunnel (Axelsson et al. 1992; Davison et al. 1994; Robinson 2008) animals were removed from the respirometer, placed in

a 40 litre tank filled with seawater at the treatment temperature and continuously exercised for five minutes. Exercise consisted of following the animal with a metal rod to ensure continuous swimming (Davison et al. 1994). Directly following exercise animals were placed in the respirometer, lids were secured, electrodes reconnected, air bubbles were removed from the chamber and respirometers were sealed. This procedure in most cases was completed in less than two minutes. During recovery measurements of heart rate, ventilation and oxygen consumption were taken. Recovery time was four hours: the first two hours of recovery temperature was held at levels in which the resting and exercise had occurred; the respirometers were flushed back to the control temperature of -1.9°C after two hours and held for the remainder of recovery.

5.2.4. Statistical Analysis

Data were analysed using one - way ANOVA, one - way Repeated Measures ANOVA and two - way Repeated Measures ANOVA when data had violated Normality a non - parametric Kruskal - Wallis test was used. Post - Hoc Tukey Range test and Dunnett's Multiple Comparison and Bonferroni test were used to identify values that were significantly different within and between experiment treatments. Paired t-tests were used to determine statistical difference between resting and maximum metabolic, heart and ventilation rates.

Resting metabolic rate for each temperature were calculated by averaging metabolic rates taken at three time points prior to exercise, -60, -30 and -5 minutes. Absolute and Factorial Scope were calculated as described in Chapter 3.2.7. Maximum metabolic rate was calculated as the highest metabolic rate value obtained for each fish.

Recovery was determined by comparing metabolic, heart and ventilation rates at different time points to the corresponding control resting treatment and the relevant treatment temperature resting rate.

5.3 Results

5.3.1 Resting and Maximum Rate

Resting metabolic rates (MO_2 resting), increased in a statistically significant stepwise manner. Values were 30.46 ± 2.47 , 35.61 ± 1.73 , 40.9 ± 2 and 52.17 ± 2.6 mg.O₂. kg⁻¹.h⁻¹ at -1, +2, +4 and +6°C respectively, Kruskal – Wallis statistic 38.43, $p < 0.0001$ (Figure 5.1a). The maximum metabolic rate (MO_2 max) was 69.04 ± 5.17 , 74.4 ± 5.66 , 72.64 ± 5.21 and 76.52 ± 4.92 mg.O₂.kg⁻¹.h⁻¹ at -1, +2, +4 and +6°C respectively. MO_2 max was not significantly different between any temperature treatments. MO_2 max was significantly greater than MO_2 resting at all temperature exposures, $p = 0.002$, 0.0013 , < 0.0001 and 0.0020 at -1, +2, +4 and +6°C respectively (Figure 5.1a).

Resting heart rate (f_H resting) was 23.2 ± 0.7 , 28.58 ± 0.76 , 31.25 ± 0.84 and 35.38 ± 0.56 bpm at -1, +2, +4 and +6°C respectively, $F_{3,92} = 49.64$, $p < 0.0001$ (Figure 5.1b), this increase was statistically significant between all treatments except the +2 and 4°C. The maximum heart rate (f_H max) was 25.75 ± 0.88 , 34.88 ± 0.52 , 37.25 ± 0.90 and 40.5 ± 0.57 bpm at -1, +2, +4 and +6°C respectively and as with f_H resting, f_H max increased significantly with temperature between all treatments except the +2 and +4°C, $F_{3,28} = 73.66$, $p < 0.0001$. f_H max was significantly higher than f_H resting at all temperatures except the control -1°C, $p = 0.0025$, 0.0020 and 0.0006 at +2, +4 and +6°C respectively (Figure 5.1b).

Resting ventilation rate (f_V resting) was 20.46 ± 1.15 , 27.83 ± 1.44 , 33.16 ± 0.98 and 35.08 ± 1.81 breaths per minute (bpm) at -1, +2, +4 and +6°C respectively, Kruskal – Wallis statistic 61, $p < 0.0001$ (Figure 5.1c). Maximum ventilation (f_V max) was 27.38 ± 1.61 , 33.13 ± 1.64 , 36.5 ± 1.87 and 38.38 ± 1.58 bpm at -1, +2, +4 and +6°C respectively. The increase in f_V max across acute temperature increase was significant, $F_{3,28} = 11.36$, $p < 0.0001$. f_V max was significantly greater than f_V resting at -1 and +2°C, $p = 0.0024$ and 0.0048 (Figure 5.1c).

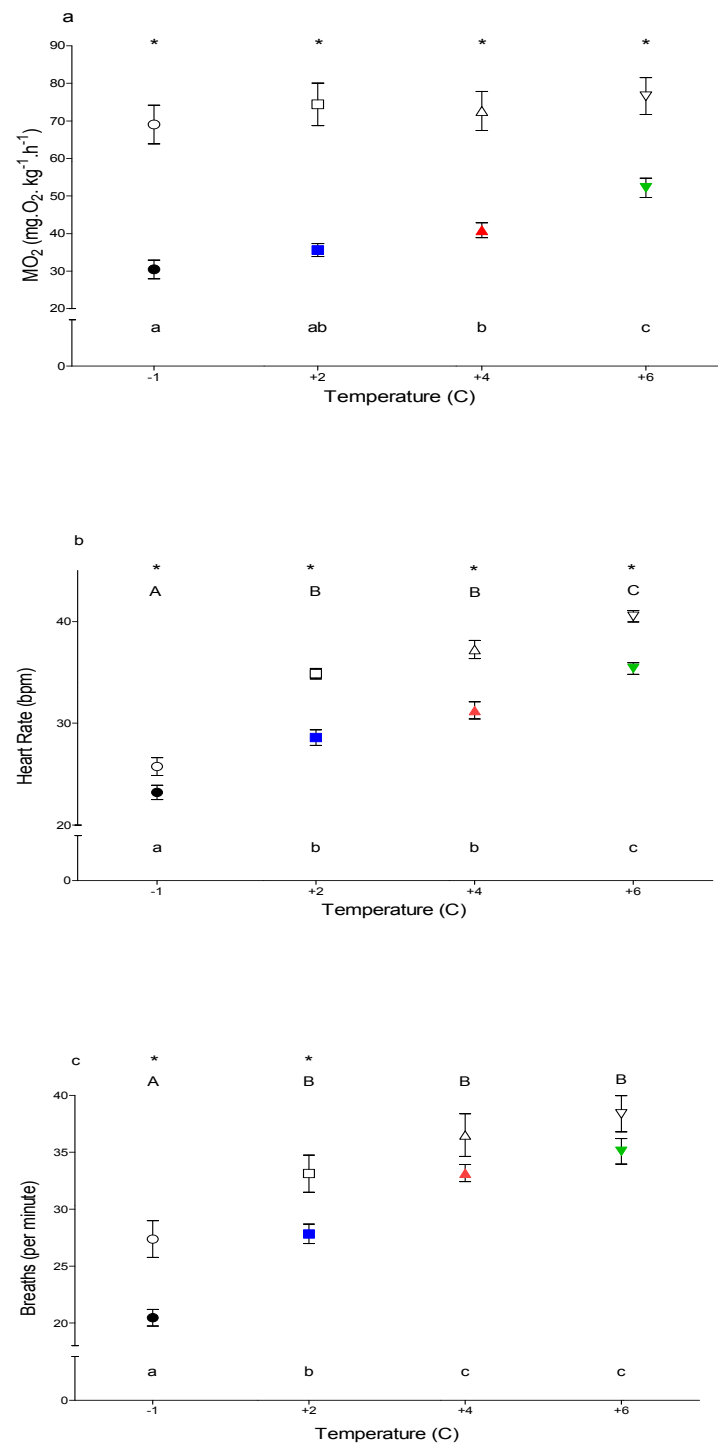


Figure 5.1: The resting and maximum rate of a: Metabolic Rate (mg. O_2 . kg^{-1} . h^{-1}), $n = 8$ b: Heart Rate (bpm), $n = 8$ c: Ventilation Rate (bpm), $n = 8$, of *T. bernacchii* at four acute temperature exposures, -1, +2, +4 and +6°C, prior to and following an exercise event. Open symbols represent maximum rates, closed symbols represent resting rates. Capital letters denote statistically significant differences within maximum rates; lower case letters denote statistical differences within resting rates. * denotes statistical differences between resting and maximum rates at that temperature.

5.3.2 Absolute and Factorial Metabolic Scope

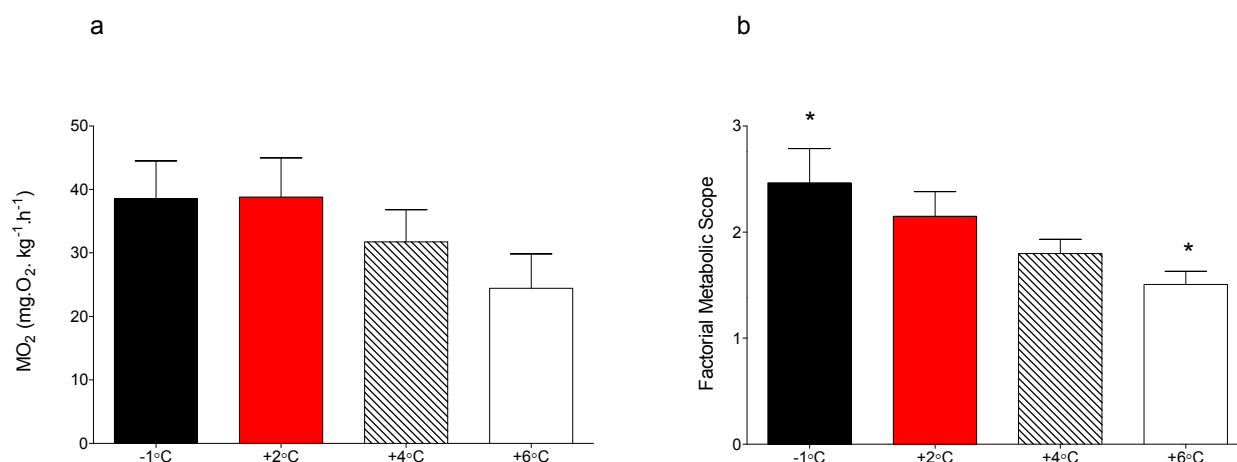


Figure 5.2: Metabolic Scope of *T. bernacchii* at different acute temperature exposures after exercise. a) Absolute metabolic scope (mg. O₂. kg⁻¹.h⁻¹) b) Factorial Scope, * denotes statistically significant differences (n=8).

The absolute aerobic scope of *T. bernacchii* after exercise was similar at -1°C and +2°C; at higher temperatures, +4 and +6°C absolute scope decreased. Absolute aerobic scope after exercise was 38.58 ± 5.64 , 38.82 ± 6.14 , 31.74 ± 5.05 and 24.41 ± 4.92 mg.O₂. kg⁻¹.h⁻¹ at -1, +2, +4 and +6°C respectively (Figure 5.2a).

Factorial aerobic scope decreased in a stepwise manner as temperature increased. Factorial aerobic scope after exercise was 2.46 ± 0.32 , 2.15 ± 0.23 , 1.8 ± 0.14 and 1.51 ± 0.12 at -1, +2, +4 and +6°C respectively. At the highest temperature treatment, +6°C, factorial aerobic scope was significantly lower than the control treatment factorial scope ($F_{3,28}=3.64$, $p = 0.04$), no other treatments differed (Figure 5.2b).

5.3.3 Cardiac and Respiratory Absolute and Factorial Scope

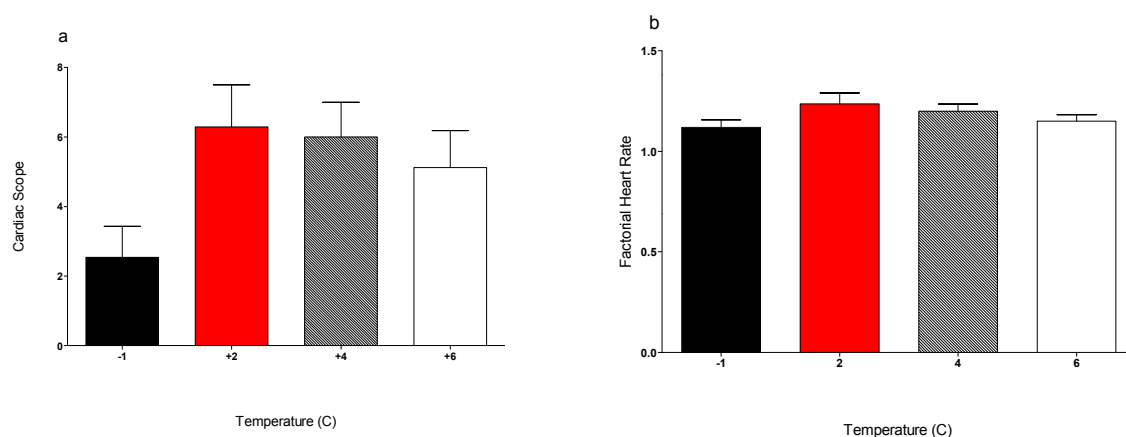


Figure 5.3: Cardiac Scope of *T. bernacchii* at different acute temperature exposures after exercise. a) Absolute cardiac scope (beats per minute) b) Factorial Scope (n=8).

Both the absolute and factorial cardiac slope increased from -1 and +2°C, after which scope decreased as temperature increased. Absolute cardiac scope was 2.54 ± 0.9 , 6.29 ± 1.2 , 6 ± 1 and 5.13 ± 1.06 at -1, +2, +4 and +6°C respectively (Figure 5.3a). Factorial cardiac scope was 1.12 ± 0.04 , 1.24 ± 0.05 , 1.2 ± 0.04 and 1.15 ± 0.03 . Change in both absolute and factorial cardiac scope was not significant (Figure 5.3).

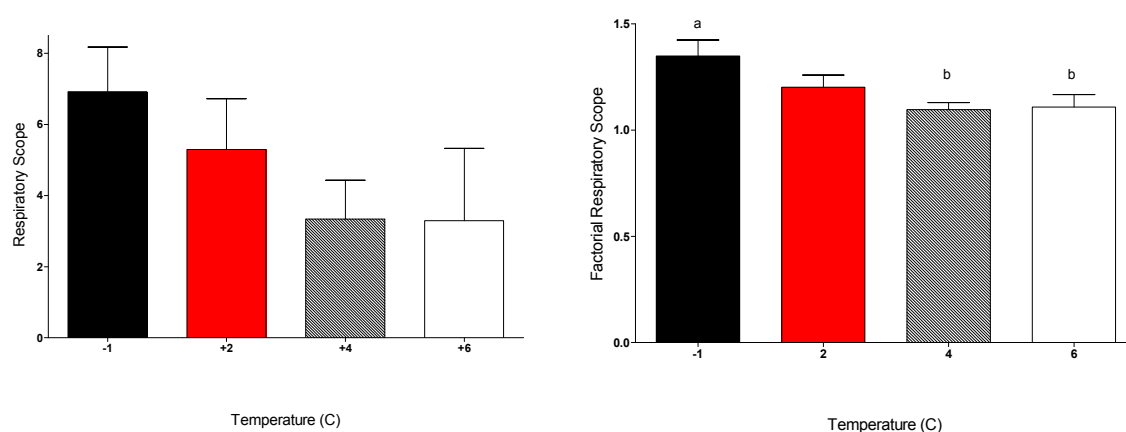
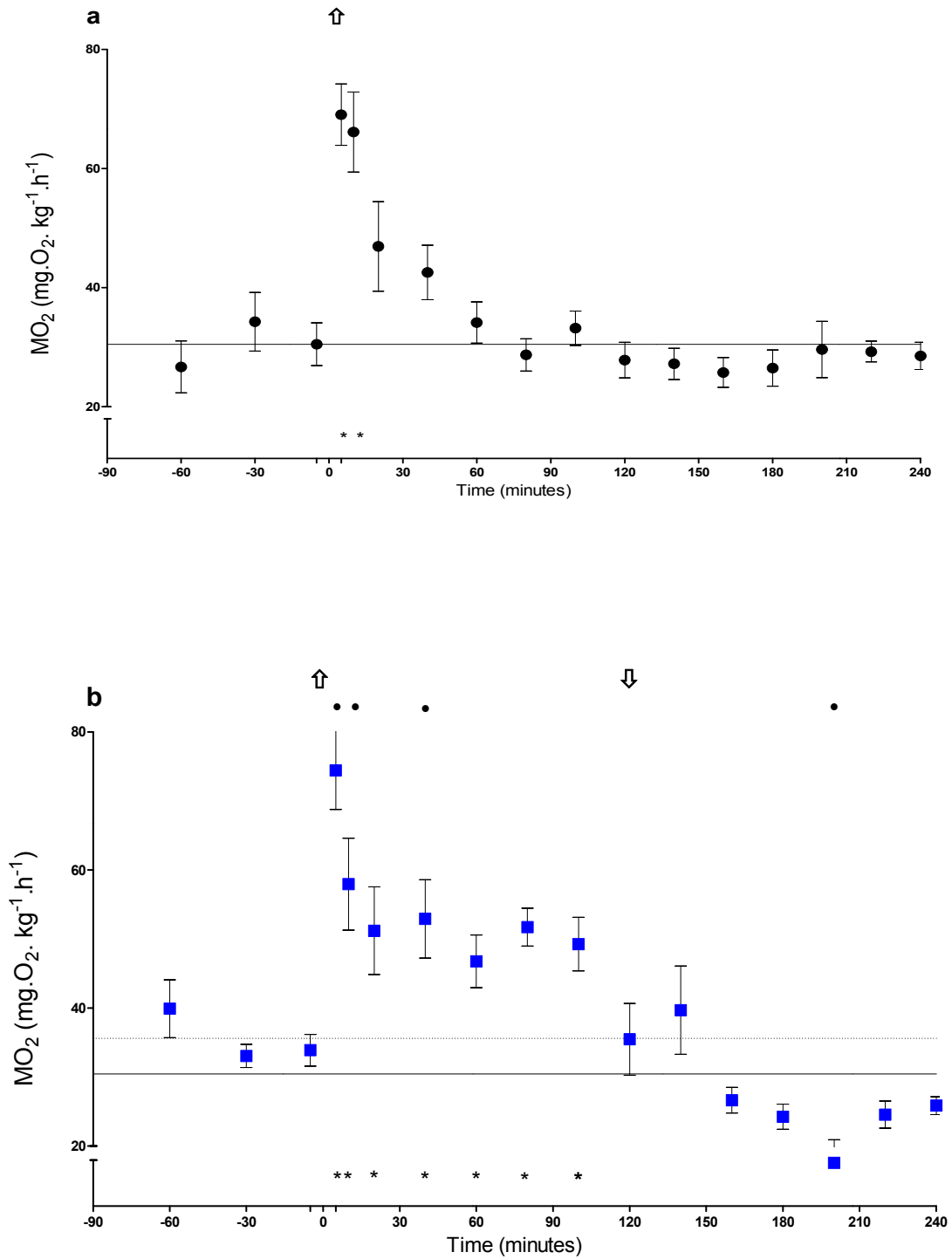


Figure 5.4: Respiratory Scope of *T. bernacchii* at different acute temperature exposures after exercise. a) Absolute respiratory scope (breaths per minute) b) Factorial respiratory Scope. Differing letters denotes statistically significant differences, $p < 0.05$ (n=8).

The respiratory scope decreased over +1, +2 and +4°C and plateaued between +4 and +6°C. Absolute respiratory scope was 6.91 ± 1.26 , 5.29 ± 1.42 , 3.33 ± 1.03 and 3.29 ± 2.03 at -1, +2, +4 and +6°C respectively (Figure 5.4a). Factorial respiratory scope was 1.35 ± 0.08 , 1.2 ± 0.06 , 1.09 ± 0.03 and 1.11 ± 0.06 at -1, +2, +4 and +6°C respectively ($F_{3,28} = 4$, $p = 0.017$). Post – hoc Tukey’s Multiple Comparison Test showed -1°C was significantly higher than the +4 and +6°C treatment (Figure 5.4b)

5.3.4 Recovery of *T. bernacchii* from Exercise

5.3.4.1 Recovery Metabolic Rate



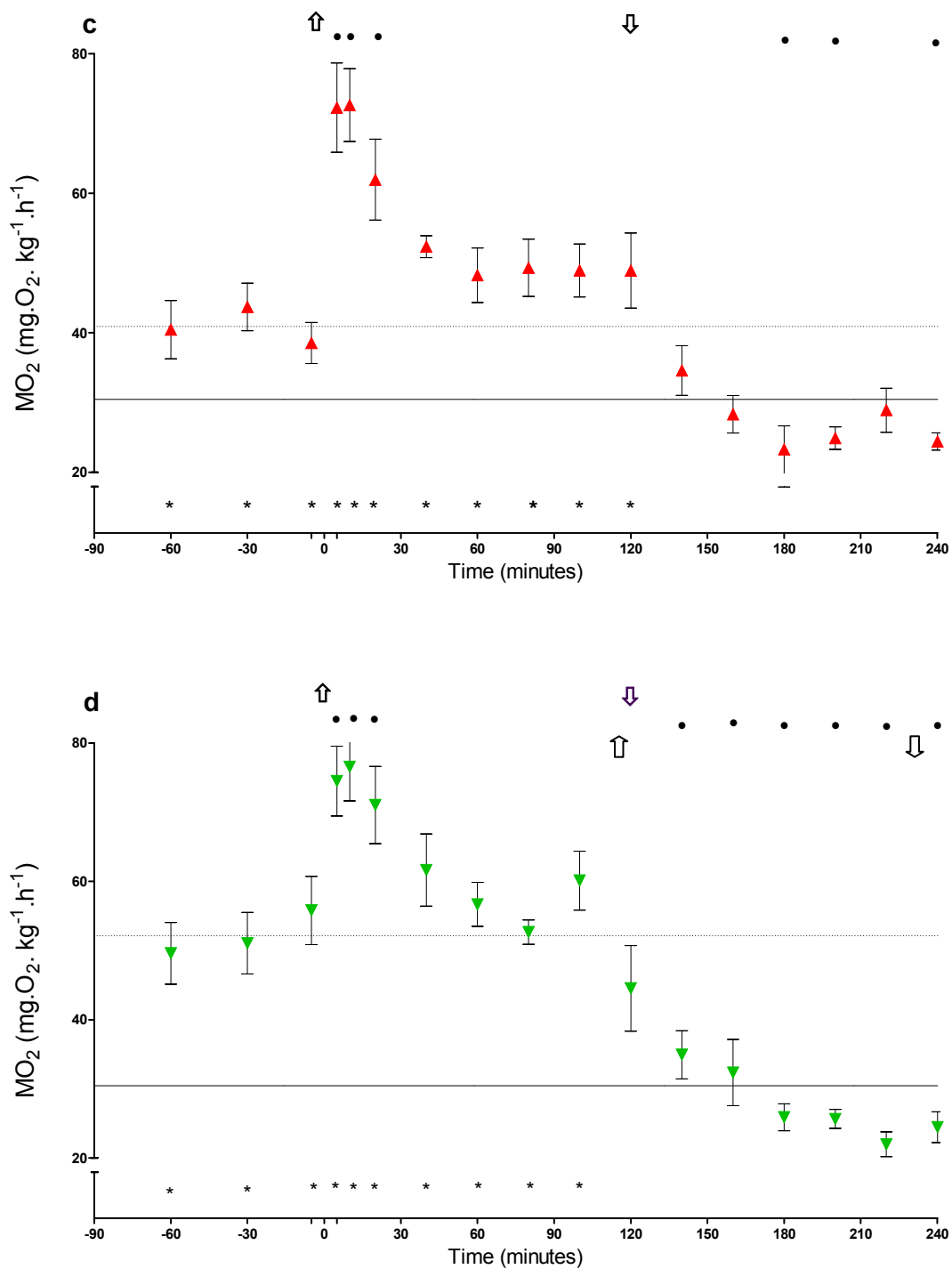


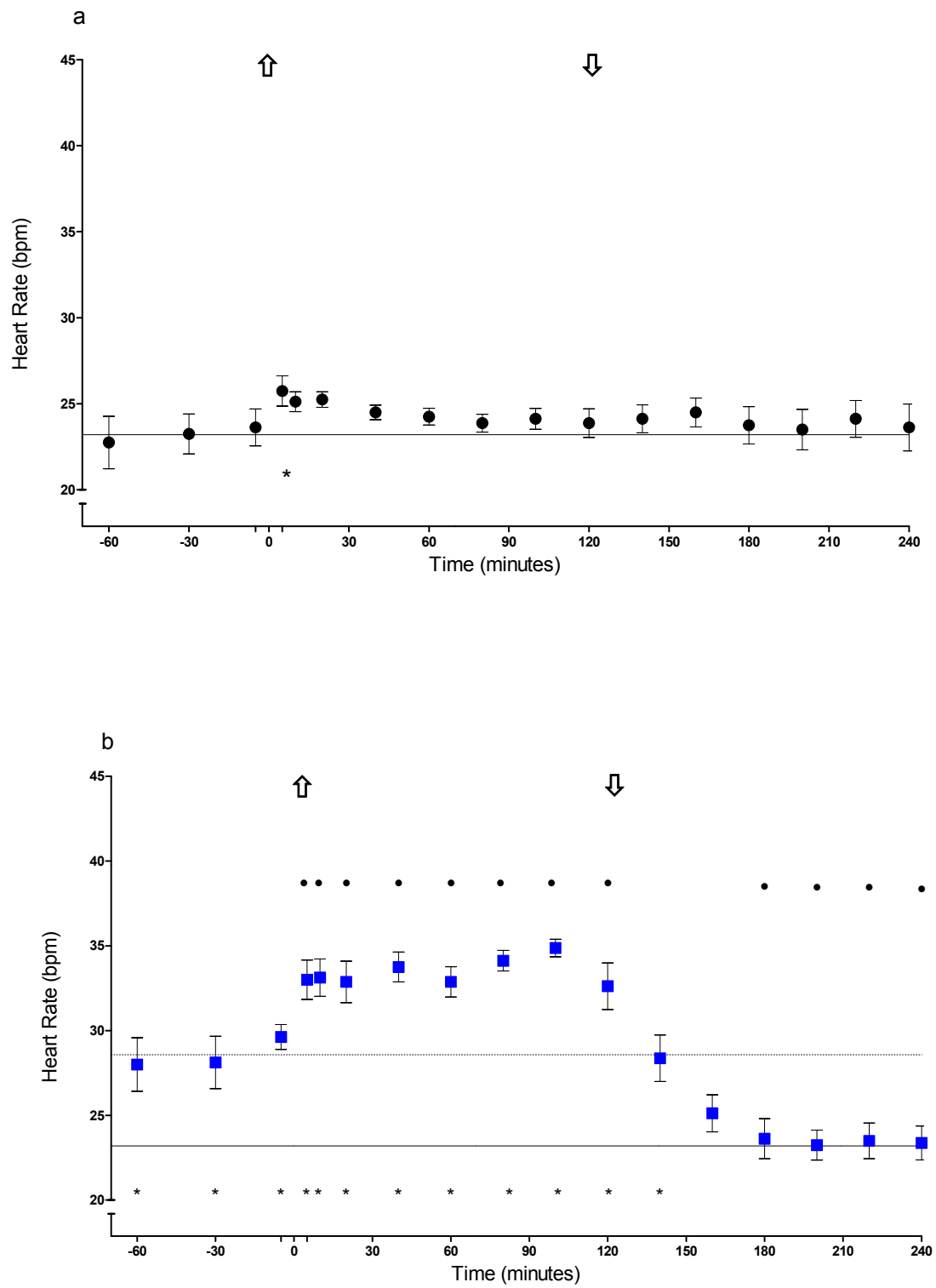
Figure 5.5: The metabolic rate (mg.O₂. kg⁻¹. h⁻¹) of *T. bernacchii* before and after exercise. a: -1 °C b: +2°C c: +4°C d: +6°C. Horizontal solid line represents the control resting metabolic rate, broken horizontal line represents the resting metabolic rate at the treatment temperature ↑ represents time of exercise, ↓ represents temperature change of water to control -1°C. * denotes statistical difference from control resting metabolic rate, ● represents statistical difference from temperature treatment resting metabolic rate ($p < 0.005$) ($n = 8$).

The metabolic rate (MO_2) of *T. bernacchii* following exercise was characterised at all temperature treatments by a sharp increase in MO_2 (Figure 5.5). At the two lowest temperature treatments, -1°C and $+2^\circ\text{C}$ $MO_{2\text{ max}}$ occurred directly after exercise (5 minutes) ($F_{14, 98} = 11.78$, $p < 0.0001$; $F_{15, 105} = 15$, $p < 0.0001$) (Figure 5.5a and b), and at 10 minutes in the $+4^\circ\text{C}$ and $+6^\circ\text{C}$ temperature treatments ($F_{15, 105} = 21.53$, $p < 0.0001$; $F_{15, 105} = 24.58$, $p < 0.0001$) (Figure 5.5 c and d).

In the control treatment MO_2 had returned to levels not significantly different from $MO_{2\text{ resting}}$ within 20 minutes of recovery (Figure 5.5a). At all other temperatures MO_2 lowered slightly from $MO_{2\text{ max}}$ but remained elevated and plateaued. Following introduction of -1°C water, two hours into a four hour recovery period, MO_2 rapidly declined and mirrored -1°C MO_2 three hours through recovery or one hour following temperature reduction (Figure 5.5b, c and d).

Two- Way repeated measures ANOVA analysis showed that time, temperature and the interaction had significant effects on MO_2 recovery following exercise ($F_{48} = 2.34$, $F_{16} = 56.65$ and $F_3 = 10.64$). After 120 minutes of recovery, when water was flushed to -1°C , there was no statistical difference between temperature treatments and all mirrored -1°C metabolic rates, suggesting full MO_2 recovery.

5.3.4.2 Recovery Heart Rate



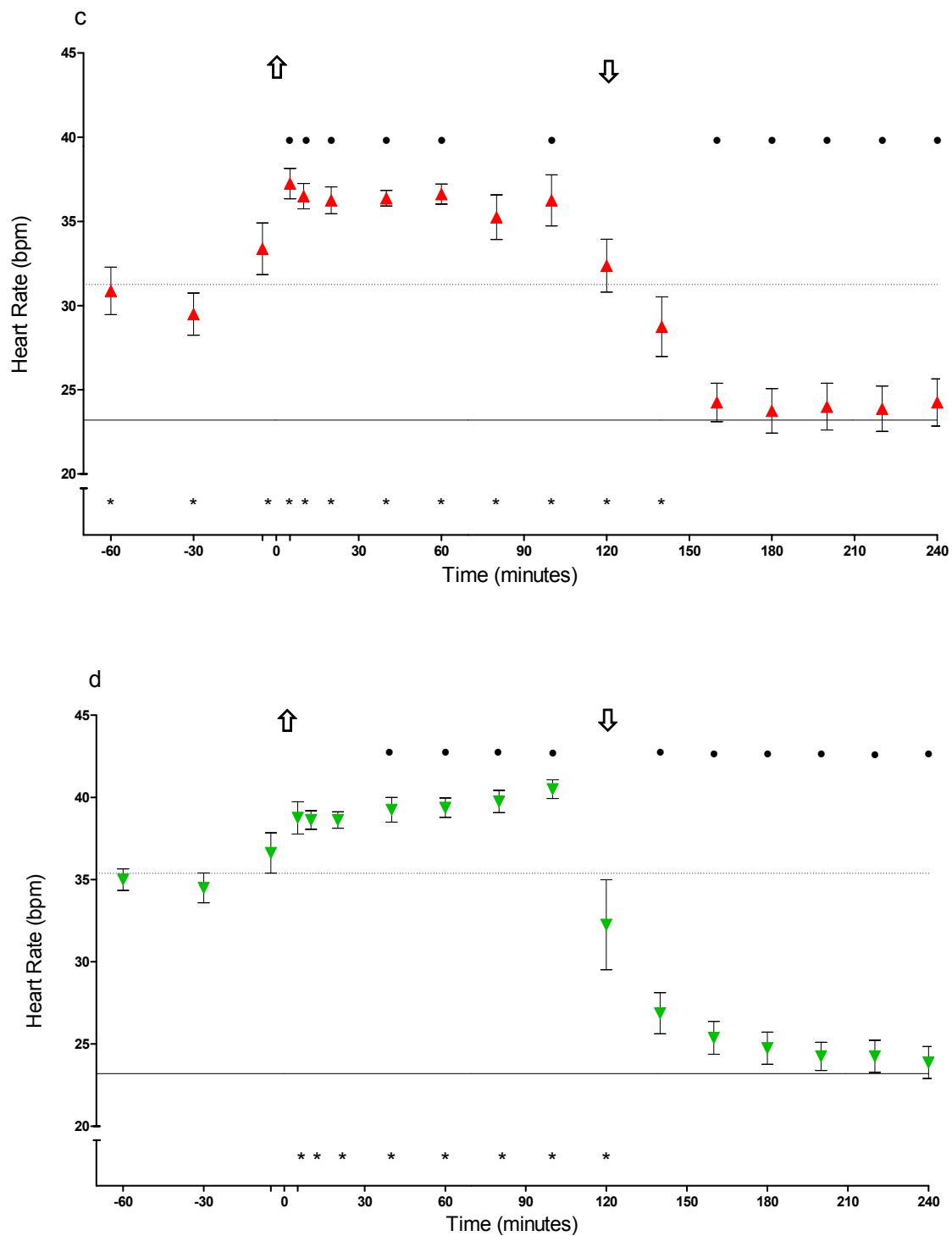


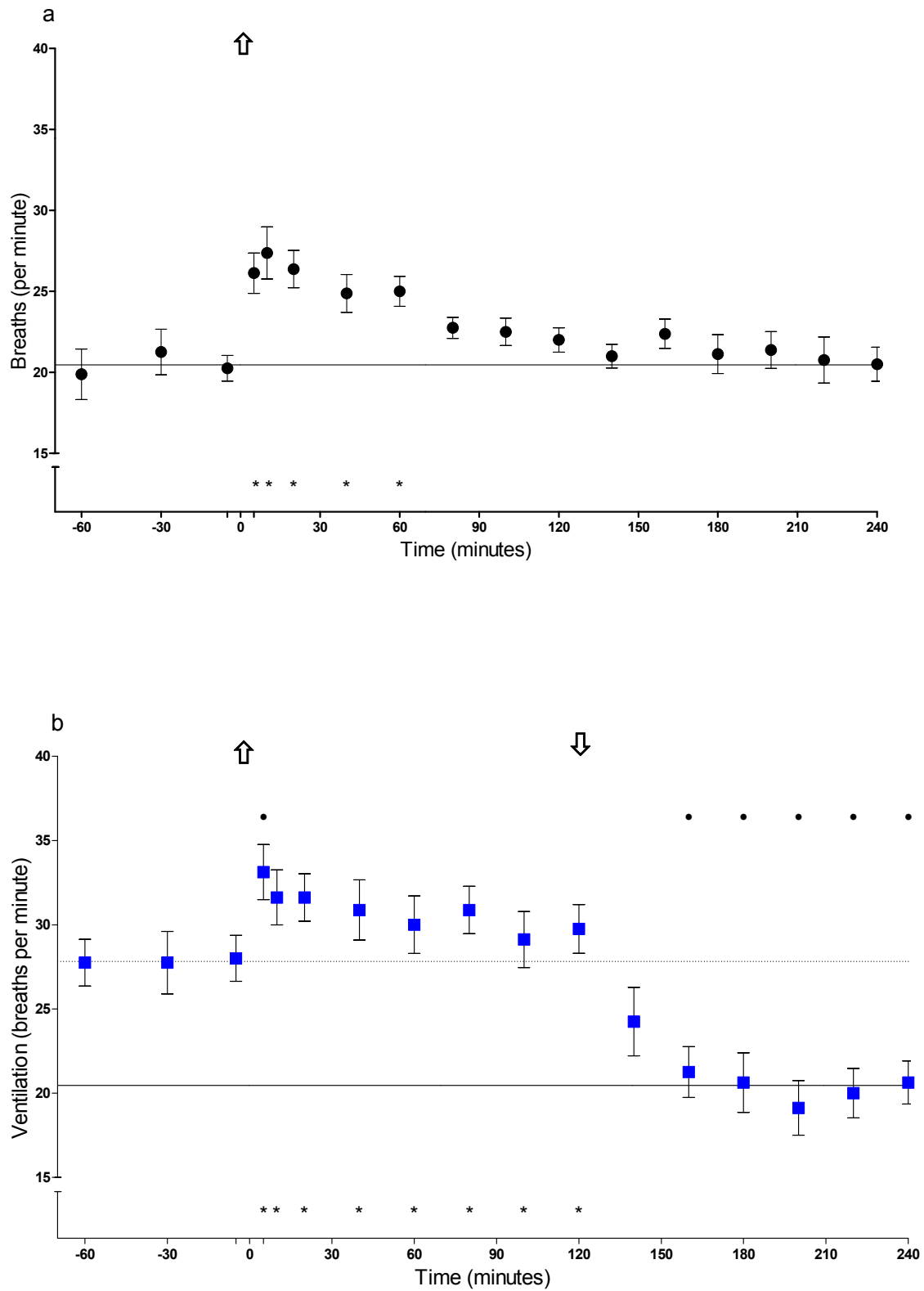
Figure 5.6: The heart rate (beats per minute) of *T. bernacchii* before and after exercise. a: -1 °C b: +2°C c: +4°C d: +6°C. Horizontal solid line represents the control resting heart rate; broken horizontal line represents the resting heart rate of the treatment temperature ↑ represents time of exercise ↓ represents temperature change of water to control -1°C. * denotes statistical difference from control resting heart rate, ● represents statistical difference from temperature treatment heart rate ($p < 0.005$) ($n = 8$).

One way repeated measures ANOVA analysis of the control treatment f_H was not significant, post- hoc analysis showed f_H was only different from $f_{H \text{ resting}}$ one minute after exercise (Figure 5.6a).

In all other temperature treatments, immediately following exercise, f_H was characterised by a sharp increase, followed by a plateau or slight increase. Directly following flushing of the respirometer to -1°C at 120 minutes, f_H in all treatments quickly declined then stabilised around the control $f_{H \text{ resting}}$. All treatments were significantly different from the relevant temperature $f_{H \text{ resting}}$ for essentially the entirety of recovery, reaching similar values only as values dropped from above to below $f_{H \text{ resting}}$ after water was lowered to -1°C (Figure 5.6 b, c and d), In these treatments ANOVA analysis was significant, $F_{15,105} = 16$, $p < 0.0001$, $F_{15,105} = 29$, $p < 0.0001$ and $F_{15,105} = 62.21$, $p < 0.0001$ at +2, +4 and $+6^\circ\text{C}$ respectively.

Two- Way repeated measures ANOVA analysis showed that time, temperature and the interaction had significant effects on f_H recovery following exercise ($F_{16} = 77.05$, $F_3 = 38.04$ and $F_{48} = 9.38$, $p < 0.0001$). After 120 minutes of recovery, when water was flushed to -1°C no time points were statistical different between temperature treatments and all mirrored -1°C f_H , suggesting full f_H recovery.

5.3.4.3 Recovery Ventilation Rate



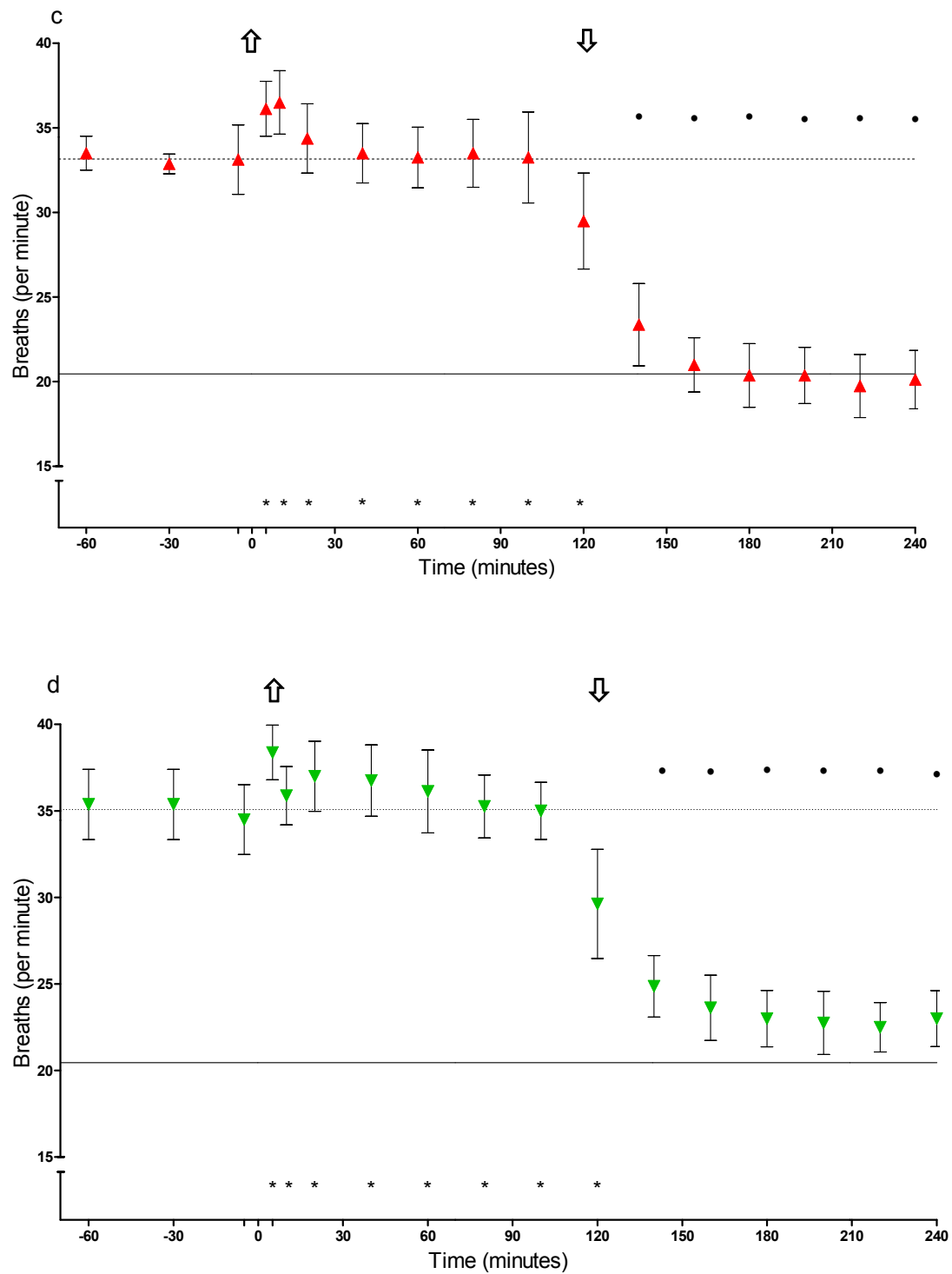


Figure 5.7: The ventilation rate (breaths per minute) of *T. bernacchii* before and after exercise. a: -1 °C b: +2°C c: +4°C d: +6°C. Horizontal solid line represents the control resting ventilation rate, broken horizontal line represents the resting ventilation rate of the treatment temperature \Uparrow represents time of exercise, \Downarrow represents temperature change of water to control -1°C. * denotes statistical difference from control resting ventilation rate, • represents statistical difference from temperature treatment ventilation rate ($p < 0.005$) ($n = 8$).

f_V at all treatments was characterised by a sharp increase directly following exercise, (Figure 5.7). The control treatment f_V peaked 10 minutes after exercise and had returned to $f_{V \text{ resting}}$ after 60 minutes ($F_{14, 98} = 9.251$, $p < 0.0001$) $f_{V \text{ max}}$ was 45% higher than $f_{V \text{ resting}}$ (Figure 5.7a).

At all other temperatures, directly following exercise f_V showed a slight increase from the relevant temperature treatment $f_{V \text{ resting}}$, however this increase was only significant in the +2°C treatment (Figure 5.7b). f_V remained elevated and plateaued following exercise until 120 minutes, when water was flushed to -1°C, at which point all f_V began dropping. By 140 minutes all f_V had lowered to control $f_{V \text{ resting}}$ levels, although the +6°C remained slightly elevated, hovering around 23 bpm (Figure 5.7 b, c and d).

Two - Way repeated measures ANOVA analysis showed that time, temperature and the interaction had significant effects on f_V recovery following exercise ($F_{16} = 70.03$, $F_3 = 7.984$ and $F_{48} = 5.361$, $p < 0.0001$). After 120 minutes of recovery, when water was flushed to -1°C no time points were statistically different between temperature treatments and all mirrored -1°C f_V , suggesting full f_V recovery.

5.4 Discussion

The thermal tolerance of marine fishes is predicted to be controlled by the aerobic scope of the animal (Nilsson et al. 2009) and reduction in aerobic scope is predicted to be the key physiological mechanism governing the response of fishes to increases in ocean temperature (Rummer et al. 2014). Due to the ‘thermal specialisation paradigm’ which predicts a loss of thermal flexibility as a trade off for specialisation to the thermally stable waters (Seebacher et al. 2005), the majority of Antarctic teleosts inhabiting the cold waters of the Southern Ocean function only over a narrow range of temperatures (Wilson et al. 2002). Climate change mediated seawater temperature increase is predicted to affect individual organisms with flow through effects altering population structure and ecosystem function (Pörtner and Knust 2007). Behavioural responses such as geographical relocation to colder water (Sunday et al. 2012) is not possible for species such as *Trematomus bernacchii* that inhabit the southernmost waters of the Southern Ocean as further movement is restricted by the coastline of the Antarctic landmass. For Antarctic fishes rising ocean temperature may not be immediately lethal as some species can survive significant periods of time at elevated temperatures. For example Robinson and Davison (2008a,b) showed *Pagothenia*

borchgrevinki survive and acclimate following prolonged exposure to +4°C. However, the acclimation ability of *T. bernacchii* is limited and the success varies throughout the literature (Chapter 3; Chapter 4; Robinson 2008; Jayasundara et al. 2013). As a reduction in aerobic scope may redirect energy away from essential processes such as reproduction, feeding and growth, warming temperatures may significantly compromise physiological processes in this species (Robinson and Davison 2008a; Rummer et al. 2014).

5.4.1 Metabolic, Heart and Ventilation Rates Pre Exercise

In the present study the resting metabolic, heart and ventilation rate, of *T. bernacchii* at -1°C, the temperature the species inhabits in its natural habitat was well within values found previously in the literature. Traditionally, resting metabolic rate of *T. bernacchii* has been measured between 12.8 and $54.24 \pm 3.59 \text{ mg.O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (Wells et al. 1987; Ware 1999; Steffenson 2005; Robinson 2008; Enzor et al. 2013; Chapter 3; Chapter 4), the present study control -1°C resting metabolic rate of $30.46 \pm 2.47 \text{ mg.O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ sits in the middle of that range (Figure 5.1a). The resting heart rate, $23.2 \pm 0.7 \text{ bpm}$ (Figure 5.1b), sits above 10.5 ± 0.9 (Axelsson et al. 1992), however this is likely due to disparity in methodology as heart rate in Axelsson et al. (1992) was measured on anaesthetised animals. In the present study the resting -1°C heart rate mirrors Macdonald (1987), $23.5 \pm 0.42 \text{ bpm}$, Davison et al. (1995), 21.9 ± 0.4 , 21.6 ± 0.4 , $19.5 \pm 0.7 \text{ bpm}$ and Jayasundara et al. (2013), $21.6 \pm 1.4 \text{ bpm}$. Earlier investigation places the resting ventilation rate of *T. bernacchii* between 15.7 ± 3.4 and $21.3 \pm 3 \text{ bpm}$ (Forster et al. 1998; Campbell et al. 2009; Jayasundara et al. 2013; Chapter 3; Chapter 4), the $20.46 \pm 1.15 \text{ bpm}$ resting ventilation rate in the present study again mirrored previous findings (Figure 5.1c).

In polar fish, metabolic resting rate, like many biological rate functions, responds predictably to increased temperature. Acute rises in temperature are typically accompanied by an increase in metabolic rate and a Q_{10} value of approximately two is characteristic for Antarctic fish (Gehrke and Fielder 1988; Macdonald 1998; Clarke and Johnston 1999; Rummer et al. 2014). This metabolic increase has been postulated to be driven by the temperature driven kinetic changes in the cell, the UTD (the universal dependence of metabolism) or alternatively a result of passive protein leakage across the interior mitochondrial membrane (for review see Clarke and Fraser 2004). Regardless of the mechanism increased oxygen is necessary to fuel increases in cellular activity (Robinson and Davison 2008a). For example, the oxygen consumption of the spangled perch *Leiopotherapon unicolor* increased sixfold over 30°C

temperature range (Gehrke and Fielder 1988), and in two species of cardinal fish, *Ostorhinchus cyanosoma* and *Ostorhinchus doederleini* following a 4°C temperature increase, resting metabolic rate doubled (Nilsson et al. 2009). In the Antarctic notothenioid *P. borchgrevinki* a 7°C increase in water temperature resulted in a near doubling of resting metabolic rate (Lowe and Davison 2006). In the current experiments resting metabolic rate changes compare well with previous findings; following acute temperature exposure from -1 to +6°C $Q_{10} = 2.16$ and a 71% increase in resting metabolic rate occurred (Figure 5.1a).

Cardiac function is strongly influenced by temperature with acute increase typically leading to a rise in heart rate (Farrell 2002; Robinson et al. 2010). This was true of the present study with the resting heart rate of *T. bernacchii* increasing 50% between -1 and +6°C, $Q_{10} = 1.83$ (Figure 5.1b). Cardiac output increase may be the direct result of thermal effects on biological rates; additionally, due to the reduced carrying capacity of oxygen at increased temperature cardiac output may increase to maintain constant tissue oxygen supply (Franklin et al. 2007). As temperature increases so too does stress placed on the heart as cardiac output must elevate to provide sufficient oxygen delivery to the tissues. Resting ventilation rate of *T. bernacchii* has been shown to be temperature dependent; increasing with acute temperature exposure (Jayasundara et al. 2013; Chapter 4). Forster et al. (1998) reported an 18 fold increase in resting ventilation rate in *T. bernacchii* over an 11°C temperature increase. This ventilatory response is characteristic of teleosts, ventilation initially increases with temperature to meet increased oxygen demand (Perry and Wood 1989), followed by a decline in rate as thermal maximum is approached (Jayasundara et al. 2013). In the present study, the rate of ventilation increase declined, resting ventilation $Q_{10} = 2.79$, between -1 and +2°C, compared to $Q_{10} = 1.3$ between +4 and +6°C. However, at the upper temperature, +6°C, resting ventilation still increased (Figure 5.1c). This suggests +6°C approaches but does not exceed the thermal maximum for resting ventilation of *T. bernacchii*; this is further supported by Jayasundara et al. (2013) who observed a decline in resting ventilation rate at +7°C in this species. However, as stroke volume was not measured in this study it is not possible to conclude if the ventilation increase decline occurred as a ventilation maximum was approached or if *T. bernacchii* switched ventilation strategy, increasing stroke volume as temperature increased to enhance oxygen delivery; an energetically advantageous strategy employed to reduce the energetic costs of pumping water across the gills (Perry and Wood 1989; Wilson et al. 2002; Graham 2006).

The resting values obtained in the current experiments suggest the increase in all resting rates was a Q_{10} effect, however as *T. bernacchii* have a very narrow thermal range with an upper lethal limit of +6°C (Somero and DeVries 1967) it is likely thermal stress was a contributing factor.

5.4.2 Maximal Rate and Metabolic Scope

Typically maximum metabolic rate of fish is measured when an animal is at a point of maximal sustained activity (McBryan et al. 2013), however, as *T. bernacchii* will not exercise in a swimming tunnel (Axelsson et al. 1992; Davison et al. 1994), the rudimentary method of chasing fish with a net was employed. This technique has been used previously to exercise this species (Davison et al. 1994; Campbell et al. 2009), and as fast start burst swimming and acceleration is often more critical than sustained swimming in benthic ambush fish (Davison 2005) this technique was deemed appropriate. In inactive species such as *T. bernacchii*, which inhabit small territories with little movement between home bases (Davison 2005; Campbell et al. 2009), maximum metabolic rate can be determined using the maximum oxygen uptake during the initial phase of recovery (McBryan et al. 2013). In the current study, as fish required relocation from the exercise tank to the respirometers as well as reconnection to E.C.G leads, the highest oxygen uptake, heart and ventilation rate immediately following exercise were used to constitute maximum rates.

As with biological resting rates, acute temperature rise generally increases maximal metabolic rate and cardiac output until a maximum, established by functional or structural constraints, is reached (Farrell 2002; Franklin et al. 2007). However, reduction in aerobic and cardiac scope occurs if maximal rate increase is lower than resting rate increase at any given temperature (Farrell 2002; Peck et al. 2004; MacBryan et al. 2013; Seth et al. 2013). Nilsson et al. (2009) found in the cardinal fish *O. cyansoma* aerobic scope declined more than 310% over a 4°C temperature increase. The authors contributed the decline to resting metabolic rate increase with no corresponding maximum metabolic rate increase. Among polar ectotherms, testing of Antarctic invertebrate species showed the bivalve mollusc *Laternula elliptica* and the limpet *Nacella concinna* experienced 50% failure in the essential biological activities righting and reburying when animals were exposed to 3°C; the scallop *Adamussium colbecki* showed even greater constraint, with 100% failure in swimming ability occurring by 2°C. Failure in all three species was attributed to an aerobic scope model, with loss of aerobic capacity credited as the cause of loss of biological function (Peck et al. 2004). Furthermore,

Peck et al. (2007) (as cited in Peck et al. 2008) demonstrated the temperature constraint was due to oxygen supply as burying in *L. elliptica* success was enhanced or diminished with increased or decreased oxygen levels respectively. Antarctic fish appear to have a larger thermal range than invertebrates inhabiting the same water (Peck et al. 2008); however small increases in temperature do result in reduction of scope. Swimming is energetically expensive, maximum metabolic rate is significantly higher than resting metabolic rate, frequently in excess of 10 times higher and the effect of acute temperature on active and resting rates can differ (Robinson and Davison 2008b). The absolute aerobic scope of *P. borchgrevinki* has been reported at 189 mg. O₂ kg⁻¹ h⁻¹ at 0°C, comparable to tropical and temperate fish species; however, over a 6°C temperature increase factorial scope declined from 6.8 to 3.0 (Lowe and Davison 2006). In the present study the aerobic scope of *T. bernacchii* at -1°C was 38.58 ± 5.64 mg. O₂ kg⁻¹ h⁻¹, considerably lower than that of *P. borchgrevinki* previously reported. The reason for this may be threefold; firstly, exercise protocol varied between studies. Due to the rudimentary net method, the metabolic rate of *T. bernacchii* in the present study while raised may not have been maximal. Secondly, the reduction may be due to differences in lifestyle. *P. borchgrevinki* are unusual among nototheniids in that they are an active pelagic fish, in comparison, *T. bernacchii* is a sluggish benthic species, reduction in aerobic scope most likely reflects these lifestyle differences (Davison et al. 1994; Robinson 2008). This is further supported by comparing the aerobic scope of a fish of similar lifestyle. The Arctic staghorn sculpin (*Gymnocanthus tricuspis*) a benthic species found only in Arctic waters had a slightly higher though similar aerobic scope of 57 ± 5 mg. O₂ kg⁻¹ h⁻¹ when tested at 4°C, the temperature the authors speculated was that species optimum (Seth et al. 2013). And thirdly, in some species of benthic sluggish fish swimming does not elicit the maximum metabolic response; this can be reserved for other processes such as specific dynamic action (Robinson 2008). However, this is not likely in this species as the maximum metabolic rate at -1°C following exercise was 87 % greater than following a feeding event at the same temperature (Figure 3.2 and 4.1a).

Metabolic scope of *T. bernacchii* was independent of temperature until 4°C at which point scope became constrained as temperature increased (Figure 5.2a and b). This was due to the increase of resting metabolic rate being much more pronounced than maximal metabolic rate (Figure 5.1a); resting metabolic rate increased 71%, Q₁₀ = 2.16 from -1 to +6°C, in comparison, maximum metabolic rate increased 10%, Q₁₀ = 1.3. The reduction in aerobic scope fits well with the OCLTT, which suggests an optimal temperature above which

processes above maintenance processes such as growth and reproduction are not sustainable (Pörtner 2001; Pörtner and Knust 2007; Pörtner and Farrell 2008; Seth et al. 2013; Rummer et al. 2014). *T. bernacchii* inhabit -1.9°C water, therefore -1°C may represent the optimal thermal range for this species (Chapter 3). Aerobic scope at $+6^{\circ}\text{C}$ reduced to 24.41 ± 4.92 mg. $\text{O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ suggesting that at $+6^{\circ}\text{C}$, energy may be utilised to maintain elevated basal metabolic processes with little metabolic energy available for processes above maintenance functions (Figure 5.2a and b). This is further supported by the inability of *T. bernacchii* to acclimate and in some studies survive prolonged periods in $+4^{\circ}\text{C}$ water, and the upper lethal temperature of $+6^{\circ}\text{C}$ in this species (Somero and DeVries 1967; Robinson 2008; Enzor et al. 2013; Jayasundara et al. 2013).

5.4.3 Cardiac and Respiratory Scope

During swimming fish increase cardiac output by an increase in heart rate and stroke volume, increasing the delivery and supply of oxygen and metabolites to the working tissue (Franklin et al. 2007). The proportional contribution to cardiac output is species specific, in Antarctic fish increase is mediated primarily through increases in heart rate as the notothenioid cardiovascular system is characterised by an already high stroke volume allowing high cardiac output even with a low heart rate (Axelsson et al. 1992; Robinson 2008). In the present study the cardiac scope at -1°C was the lowest of any temperature treatment (Figure 5.3a), heart rate may not have required a large increase as oxygen delivery may have been sufficient to fuel burst swimming, a function associated with a small number of physiological and biochemical steps (Wilson et al. 2001). Furthermore, Davison et al. (1994) showed exercise in comparison to hypoxia and increased temperature caused only a slight change in haematocrit, haemoglobin concentration and plasma chloride levels in *T. bernacchii*. However, the small cardiac scope at -1°C in the present study adds to previous research suggesting heart rate in Antarctic notothenioids is highly constrained. The maximum heart rate of *P. borchgrevinki* following an exhaustive exercise event at -1°C was 27.6 ± 0.7 bpm, a factorial scope of only 1.44 (Franklin et al. 2007). Similarly, Axelsson et al. (1992) showed when cholinergic and adrenergic inputs were blocked the intrinsic heart rate of *T. bernacchii* was only 21.6 bpm.

As with resting heart rate, acute temperature exposure typically results in an increase in maximum heart rate and if the thermal dependence of maximal rate is lower than that of the resting rate, cardiac scope will be reduced (Franklin et al. 2007). In the present study cardiac

output was not measured, however the cholinergic system has been shown to strongly regulate the heart rate of *T. bernacchii*, exerting approximately 80% tonus on resting heart rate (Axelsson et al. 1992; Forster et al. 1998; Egginton et al 2006; Robinson 2008). Forster et al. (1998) showed at 10°C, heart rate in this species more than doubled suggesting cholinergic control of the heart was lost at high temperature. In the present study the increase in heart rate across all temperatures suggests a loss of cholinergic tonus on the pacemaker of the heart until heart rate reaches a maximum reducing scope at elevated temperature (further discussed below in recovery) (Figure 5.3). Respiratory scope of *T. bernacchii* was thermally dependent between -1 and +2°C, although both resting and maximum rates increased; the reduction of scope was due to the maximum rate increasing at a slower rate, resting ventilation -1 to 2°C $Q_{10} = 2.79$, maximum ventilation -1 to +2°C $Q_{10} = 2.21$. At +4 and +6°C respiratory scope decreased such that there was no statistical difference between resting and maximum ventilation and theoretically no scope available for elevated oxygen requirements occurred during exercise (Figure 5.1c). This reduction in respiratory scope may represent a thermally induced maximal ventilation rate, at which point oxygen uptake across the gill may not be sufficient. This suggests if *T. bernacchii* are unable to decrease metabolic rate (oxygen demand) at elevated temperatures the respiratory system may not sufficiently meet increased oxygen demand, requiring a switch to anaerobic metabolism. This finding supports previous findings in the literature; +6°C is the upper lethal temperature in this species (Somero and DeVries 1967), while Jayasundara et al. (2013) found no acclimation of the respiratory system in *T. bernacchii* during a 14 day exposure to 4.5°C. Moreover, Robinson (2008) reported only one fish survived a 28 day exposure to +4°C with oxygen deficiency attributed as the likely cause of thermal death. The present study suggests, due to an elevation in oxygen demand and a reduction of respiratory scope at elevated temperatures, the rate of diffusion between the external medium and the blood may become limiting and insufficient to maintain aerobic processes. If exposure to increased temperature is prolonged, due to unsustainable anaerobic metabolism and associated by products, organism death can occur (Mark et al. 2002)

5.4.4 Recovery from Exercise

Following exercise the metabolic, heart and ventilation rates of *T. bernacchii* were elevated, to what extent dependent on time and temperature exposure (Figure 5.5, 5.6 and 5.7). In fish metabolic rate remains elevated to meet the energetic demand of reinstating cellular and

metabolic homeostasis and heart and ventilation rates remain elevated to supply oxygen to power elevated metabolic rate. The timeframe of recovery is dependent on both the degree of the metabolic disturbance and rate at which clearance occurs, both of which are species specific (Robinson 2008b). In *T. bernacchii* at environmental temperatures recovery appears to occur rapidly. Metabolic and heart rate in the -1°C fish peaked at five minutes (the first measurement following exercise) while ventilation peaked at ten minutes (Figure 5.5a, 5.6a and 5.7a). All rates dropped rapidly with both metabolic and heart rate not significantly higher than resting rates after 20 minutes of recovery (Figure 5.5a and 5.6a). This supports previous findings of fast recovery following episodes of metabolic increase such as exercise in *T. bernacchii*. Davison et al. (1994) suggests carangiform (burst) swimming does not result in high levels of haematological changes, such as major increases in haematocrit or haemoglobin, normally expected during episodes of metabolic increase. Moreover, lactate levels following exercise remain moderately low in this species and are quickly metabolised (Davison et al. 1988; Davison et al. 1995; Lowe and Davison 2006; Robinson 2008).

At elevated temperatures, metabolic, heart and ventilation rates were temperature dependent. Initially during recovery metabolic rate declined slightly but remained elevated. Two hours into recovery, directly following the introduction of -1.9°C water metabolic rate returned to the control resting rate, suggesting rapid recovery from acute thermal stress (Figure 5.5b, c and d). Similar to metabolic rate, ventilation in the +2°C treatment increased above resting levels, remaining elevated, however at +4 and +6°C, ventilation failed to increase (Figure 5.7b, c and d). This suggests that at +4°C and +6°C *T. bernacchii*, due to Q_{10} increases on resting metabolic rate (discussed above, 5.4.1) and thermal stress, have no respiratory scope available. Return of ventilation rate to 19bpm occurred within 40 minutes of return to -1.9°C in all treatments bar the +6°C which lowered quickly but hovered around 22bpm suggesting a longer timeframe is required for ventilation recovery following acute exposure to +6°C. Heart rate remained elevated at levels significantly higher than the given treatment's resting rate. Previous research has shown acute temperature exposure affects cholinergic modulation of the heart in *T. bernacchii* (above; Forster et al 1998). The present study suggests, during acute exposure to elevated temperature following an exhaustive exercise event, cholinergic tonus is greatly reduced. A reduction in water temperature is necessary to enable *T. bernacchii* to reinstate cholinergic control on the pacemaker of the heart at which point restoration occurs rapidly resulting in a prompt reduction in heart rate (Figure 5.6b, c and d).

5.5 Summary

The rate of warming in the Southern Ocean matches all other ocean regions. Water around the Antarctic Peninsula has increased by approximately 1°C in the last half century (Meredith and King 2005) and the trend is predicted to continue by at least 2°C within the next century (Somero 2010). Stenothermic species such as *T. bernacchii* are likely to be impacted by climate change due to the negative effects of increased temperature on aerobic scope and the corresponding consequences on life histories and population and community structures (Rummer et al. 2014).

The present study supports the “OCLTT” as the aerobic and respiratory scope of *T. bernacchii* was optimal at -1°C in accordance with the water temperatures this species has inhabited for several million years (Eastman 1993). As with previous research (Davison et al. 1994), exercise elicited a small response in *T. bernacchii*, comparative to another Antarctic notothenioid *P. borchgrevinki* the absolute scope was greatly reduced. This is likely due to difference in lifestyle, *T. bernacchii* is a sluggish benthic fish and a large aerobic scope may not be necessary to accommodate this species lifestyle. Temperature had a larger impact on the metabolic, cardiac and respiratory scope, all declined as temperature increased and in the case of ventilation was absent at +4 and +6°C, the upper lethal limit for this species (Somero and DeVries 1967). Recovery of all parameters, metabolic, heart and ventilation rate was temperature dependent and upon return to -1°C rapid.

T. bernacchii have limited acclimation ability (Chapter 3 and 4), therefore exposure to increases in water temperature may be detrimental and in this species a thermally induced reduction in aerobic scope may affect individual physiological processes, behaviour and ultimately survival.

CHAPTER SIX

General Conclusion

6.1 Thesis Objectives

6.1.1 Acclimation Ability of *Trematomus bernacchii*

Antarctic marine ectotherms have long been considered thermally inflexible; this has been attributed to the specialisation of these organisms to the thermally stable water of the Southern Ocean (Peck et al. 2014). Huey and Hertz (1984) postulated a theory termed “Jack of all temperature, master of none”, suggesting organisms inhabiting thermally fluctuating environments possess acclimation ability, while those in thermally stable environments do not. Seebacher et al. (2005) suggested that phenotypic thermal inflexibility, such as that observed in Antarctic notothenioids, is due to a trade off in which thermal specialisation incurs a loss of thermal flexibility. Research of several Antarctic organisms seems to support this theory; for example La Terza et al. (2001) (Antarctic ciliate *Euplotes focardii*), Robinson 2008 (Striped rockcod *Trematomus hansonii*; Sharp-spined notothenia *Trematomus pennellii*); Peck et al (2009) (brittle star *Ophiotus victoriae*). However some organisms are the ‘exception to the rule’ and in the case of Antarctic notothenioids, *Pagothenia borchgrevinki* has shown thermal flexibility, successfully acclimating to a 5°C temperature increase (Robinson and Davison 2008b). The success of *Trematomus bernacchii* to acclimate to +4°C is variable according to the literature with mortality or no information on survival numbers reported (Hoffman et al. 2000; Ream et al. 2003; Podrabsky and Somero 2006; Robinson 2008; Bilyk and DeVries 2011; Enzor et al. 2013), therefore *T. bernacchii* does not join *P. borchgrevinki* as an exception to the thermal specialisation paradigm. However the present study shows clearly, under the right conditions *T. bernacchii* are able to acclimate to a 4°C increase and make important physiological adjustments.

Limitations imposed by a short research season resulted in fish being placed in the acclimation tank at the wet lab facility, Scott Base, Antarctica approximately 7 days

following capture. Previous research and the findings in this study suggest this may not be sufficient to allow recovery from capture and acclimation to laboratory condition, in the present study only 5% of *T. bernacchii* placed in +3°C survived the 28 day acclimation period. However, survival rate of fish flown to the University of Canterbury and housed for several months prior to the acclimation was 100%. Adjustment of resting oxygen consumption following 28 days of acclimation to +3°C was the most significant physiological modification. Acute exposure to +3°C resulted in expected Q_{10} elevation of resting metabolic rate, however during the 28 day acclimation period, metabolic adjustments occurred and perfect compensation of resting metabolic rate was observed. Investigation of physiological challenges on acclimated fish was only possible when investigating specific dynamic action as all other experiments occurred at Scott Base: Antarctica.

6.1.2 Specific Dynamic Action

This research was novel as it quantified the previously undefined metabolic cost of feeding, (SDA response) in *T. bernacchii* at environmental temperature, outside its natural thermal range, following an acute temperature increase and following a 28 acclimation period. The response of *T. bernacchii* held at environmental temperature was typical of polar organisms, characterised by a small metabolic increase continuing over a prolonged period of time, in this study 72 hours. All parameters, the factorial scope and the SDA coefficient fell within normal ranges reported in the literature for fish tested in a natural thermal range. Acute temperature exposure to +3°C resulted in an elevated resting metabolic rate, masking the small SDA response typical of Antarctic notothenioids. Successful acclimation of fish in this treatment resulted in *T. bernacchii* making physiological adjustments. The acclimation treatment showed not only perfect compensation in resting metabolic rate, but demonstrates the effect temperature has on the rate of biological processes. The SDA response peaked faster in the acclimated treatment, nine hours vs. 24, following the feeding event, duration also occurred faster, 24 vs. 72 hours in the acclimated treatment compared to ambient -1°C temperature treatment. The implications of this for the species are yet unknown. However, these findings serve as a valuable foundation for further investigation of the associated parameters, such as assimilation of nutrients and growth of the species.

6.1.3 Hypoxia and the Effect of Acute Temperature Exposure on *Trematomus bernacchii*

The Southern Ocean due to its thermal stability, has contained high levels of oxygen for several million years, therefore *T. bernacchii* are unlikely to have presently or historically encountered hypoxic conditions. The present study clearly shows this species has retained physiological responses to reduced oxygen however the response is limited compared to those teleosts that inhabit variable oxygen environments. The P_{crit} of -1°C *T. bernacchii* was resolved at 95.14mmHg. Resting metabolic rate values were calculated under what is considered normoxic conditions (150 and 120mmHg). However, *T. bernacchii* showed immediate sensitivity to slight decreases in oxygen tension, particularly in the control treatment and therefore the -1°C P_{crit} may have been overestimated. At $+2$, $+4$ and $+6^{\circ}\text{C}$ P_{crit} was 69.73, 93.67 and 102.21 mmHg respectively, as temperature exposure increased Q_{10} related effects on biological rates acted to increase oxygen demand, counteracting metabolic suppression, the strategy normally employed by teleosts during a hypoxic challenge. At all temperatures tested *T. bernacchii* showed almost immediate metabolic increase, a conventional response to moderate reductions in environmental oxygen tension, likely in part due to a metabolically costly increase in ventilation; again a common strategy to increase the total volume of oxygen at the respiratory surface. However in this species at the two highest temperatures tested, ventilation increase was absent. Oxygen supply may not have been sufficient to meet increased oxygen demand at elevated temperatures, resulting in P_{crit} occurring at higher oxygen tension as temperature increased. The most significant physiological adjustment demonstrated by *T. bernacchii* to a hypoxic challenge was the presence of a bradycardia previously unreported in this species. The bradycardia was significant and consistent at all temperatures tested, with heart rate of *T. bernacchii* reducing by 25% between 150 and 50mmHg regardless of the treatment temperature. The experiment illustrates both the retention of physiological responses of *T. bernacchii* to hypoxic events in conjunction with resilience to acute temperature challenges as recovery from both was immediate and rapid.

6.1.4 The Effect of Temperature on the Aerobic Scope of *Trematomus bernacchii*

The “OCLTT” (the oxygen and capacity limited thermal tolerance theory) postulates aerobic scope diminishes at temperatures in excess of thermal optima and disappears at critical temperatures (Pörtner 2001; Pörtner and Knust 2007; Pörtner and Farrell 2008). In the present study both aerobic and respiratory scope were independent of temperature up to +4°C, after which aerobic scope diminished and respiratory scope was absent. Thermal dependence of scope was due to a faster rate of change in resting rates, in particular in the case of metabolic rate where maximum rates showed no significant increase. Evidence suggests the heart rate in Antarctic notothenioids is highly constrained at environmental temperatures; the present study further supports the literature as cardiac scope was lowest at -1°C. Above ambient temperatures cardiac scope was greater. This was likely due to a temperature induced loss of cholinergic tonus on the heart following an exercise event; reinstatement of which was temperature dependent, as following return to ambient temperature recovery was rapid. Additionally, recovery of metabolism and respiratory systems was temperature dependent and rapid upon return to environmental temperatures. This study follows the theory of the “OCLTT” and suggests -1°C is within the optimum temperature range for *T. bernacchii*, in agreement with the water temperature this species has inhabited for several million years. Moreover, the lack of respiratory scope at higher temperature fits with previous research as the success of *T. bernacchii* to acclimate to +4°C is variable and the upper lethal limit for this species sits at +6°C.

6.2 Methodological Considerations

Antarctica, due to its extreme location and environment presents its own set of methodological challenges. The length of a research season is finite, in the case of this study seven weeks over two consecutive summer seasons, totalling 14 weeks. Accessibility to fish source sites relies on the presence and thickness of ephemeral sea ice and weather conditions, in addition all scientific equipment must be transported to the field or Scott Base wet laboratory facilities. These limitations restrict replicate numbers and place time constraints on all aspects of experiment procedures. In the present study this was evident during the acclimation protocol. To ensure completion of experiments within the research season only seven days were available as a recovery period from capture prior to commencement of acclimation to +3°C, within 31 days 100% mortality occurred. Conversely, animals returned

to New Zealand and allocated several months to acclimate to laboratory conditions experienced 100% success of acclimation to the same temperature (covered above). Arguably this limitation is not without merit as it adds to the body of knowledge contributing toward acclimation protocol.

6.3 Future Investigation

The present study is novel in that several physiological processes and responses previously undefined in *T. bernacchii* have been quantified; therefore much of the findings can be used as a baseline for future studies. Biochemical analysis, including plasma sodium, chloride, glucose and lactate would be a future avenue of research to determine if the changes observed in oxygen consumption and cardiovascular function were evident at a molecular level. In regard to the SDA response of *T. bernacchii* future investigation opportunity is vast. Examination of ammonia excretion using a quantitative spectrophotometric assay to characterise the $\text{NH}_3 - \text{N}$ excretion profile, would allow comparison to the SDA oxygen consumption profile established in this study. Additionally, characterising gut evacuation time and gastrointestinal blood flow dynamics via surgically implanted blood flow probes would also act to build a digestion profile in this species.

6.4 Environmental Implications

Assessing temperature change in Antarctica is complex; however data collected from Antarctic stations strongly suggest both air temperature on the continent and water temperature in the surrounding Southern Ocean is increasing. Warming is most dramatic at the West Antarctic Peninsula, air temperature has risen 3°C since beginning of recordings in 1951 and the sea surface temperature has warmed by more than 1°C in the last 50 years (Meredith and King 2005; Robinson 2008; Somero 2010; Peck et al. 2014). This increase is expected to continue; within the next century, warming around the coastal regions of Antarctica is predicted to increase by +2°C. Understanding and investigating the physiological processes underpinning Antarctic marine species sensitivity to thermal variation is crucial to identify the effects predicted climate change may have on individual species and overall community structure (Somero 2010; Morley et al. 2012) and the literature suggests many Antarctic marine populations will not survive the level of predicted sea temperature increase (Peck 2002; Peck et al. 2009; Clarke et al. 2012). However this research

demonstrated the ability of *T. bernacchii* to acclimate to +3°C, a temperature higher than current projected sea temperature increase, and therefore provides some optimism for this species in a changing global climate.

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