Cod in Hot Water:

**The Thermal Physiology of Blue cod (*Parapercis colias*),
Black Cod (*Paranotothenia angustata*) and Emerald
Rock Cod (*Trematomus bernacchii*)**

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Abstract

Climate change is believed to be altering the Earth's climate, and driving ocean systems into conditions which could fundamentally and irreversibly modify ecology systems, in particular ectothermic marine ecosystems are at great risk. By understanding the relationship between temperature and rates we gain insight into species thermoregulatory behaviour. This project was aimed at investigating what effects chronic and acute temperature changes will have on three species of fish species, blue cod an endemic New Zealand species, black cod a Sub-Antarctic species, and emerald cod an Antarctic species. Fish were exposed to chronic temperature changes for a period of four weeks to gain insight into their ability to acclimate to new temperatures. Blue cod were chronically exposed to 8°C, 12°C, 18°C, black cod were chronically exposed to 8°C, 12°C and emerald cod were exposed to 2°C. Ventilation rate, heart rate and oxygen consumption were measured during acute temperature changes to determine tolerance ranges and the limiting factors to thermal tolerance. Each species was exposed to a different range of acute temperatures depending on chronic exposure temperature. The results showed that blue cod proved to be the most thermally flexible species, showing partial to complete acclimation, with large shifts in tolerance range between acclimations. Black cod and emerald cod showed a shift in tolerance ranges but did not show strong acclimation. In conclusion ventilation rate and heart rate proved to be limiting factors to thermal tolerance. At higher acute exposure temperatures fish were pushed past their tolerance ranges and oxygen consumption reached levels so high that even if ventilatory and circulatory systems did not fail, it would only be a matter of time before mortality occurred. Further studies are needed to understand the mechanisms behind limitation of thermal tolerance to gain an in-depth understanding of how climate change will impact marine ecosystems.

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Introduction

1.1 Climate change

Climate change is believed to be changing the Earth's climate, and driving ocean systems into conditions which could fundamentally and irreversibly alter ecology systems (Hoegh-Guldberg and Bruno 2010). Rapid climate change is increasing with temperatures potentially increasing at rates that may exceed an organism's ability to adapt, this is recognised as an important issue that could potentially to lead to large scale collapses of ecosystem structure (Clarke 2003, Doney et al. 2012). Earth's atmosphere and oceans have been gradually warming over the past 50+ years due to greenhouse gas emissions, in turn as the atmosphere warms due to the effects of CO_2, so does the ocean (Doney et al. 2012). Terrestrial temperatures are rising, in Antarctica rapid environmental warming being reported over the past 30-60 years (Harringon et al 2010, Walther et al. 2002).

We are currently living in a period with the highest ever recorded CO_2 concentration in Earth's atmosphere, the last time atmospheric CO_2 levels were this high, both the North and South poles were completely free of ice cover causing significant sea level rise (Miller et al. 2005). If all polar ice was to melt in the Arctic sea level would rise by 12 m (Hoegh-Guldberg and Bruno 2010, Serreze and Barry 1996). Climate change can drive changes in both abiotic and biotic interactions in ecosystems (Doney et al. 12). It is widely accepted that marine biogeography is largely shaped by direct effects of temperature as due to temperature being one aspect to the complex mix of physical and biotic factors that determines the niche of an animal (Clarke 2003). Due to temperature playing such an important role this warming could have detrimental effects on all kinds

of life in many different ways. Physical, chemical, and biological changes to environments as a result of climate change have the potential to alter physiological functioning, behaviour, and productivity of organisms (Doney et al. 2012). Therefore an appreciation of behavioural and physiological responses to temperature change is critical in understanding the survival of animals in areas likely to be strongly affected by climate change.

Physical changes are already emerging around the world from Polar Regions to the tropics, and have been for some time, glaciers are retreating and each year sea ice cover at the poles consists of younger sea ice (Aggrio & Thomas 2004, Clarke et al. 2007). Huge icebergs are breaking off Antarctic ice sheets and the Antarctic Peninsula has warmed by 1.5 °C which is causing changes in flora and fauna (Vaughan and Drake 1996, Walther et al. 2002). Penguins are finding themselves in ice free areas and more plants are starting to grow on the Peninsula due to warmer climates (Walther et al. 2002). If CO_2 emissions are not reduced these changes will become greater in scale and number.

Changes in physiology and distribution of terrestrial and aquatic organisms have observed over the past 20 years (Davis et at. 1998). Aquatic ectothermic organisms will likely be hit the hardest by climate change as their oxygen consumption is largely dependent on the temperature of their environment due to their inability to regulate internal temperatures (Clarke 1993, Killen et al. 2010). Although terrestrial temperatures are increasing at a more rapid rate, many terrestrial organisms have the ability to regulate their body heat whereas the majority of aquatic animals do not. It is important to understand the effects of temperature change on organisms in lab conditions so it can be related back to an ecosystem level.

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1.1.1 Warming of oceans

The oceans of the world play a crucial role in regulating climate as they span 70% of the Earth's total surface (Hoegh-Guldberg and Bruno 2010, Pörtner 2008). They are tremendous in volume, and host a huge variety of life with marine ecosystems being centrally important to the biology of the planet (Hoegh-Guldberg and Bruno 2010). Climate change is starting to change these oceans with serious consequence (Hoegh-Guldberg and Bruno 2010). Vast amounts of CO_2 are being readily absorbed (30 percent) causing acidification and sea levels are rising due to melting of the polar caps (Gibson et al. 2011, Harley et al. 2006, Hoegh-Gulberg et al. 2000). Substantial increases in ocean temperatures were first recorded in the 1950s, if warming continues to increase it is predicted that the Arctic will be ice free some time during the mid-twenty-first century (Doney et al. 2012). Ocean currents, water depth and salinity are also important in shaping species distribution and survival (Pörtner 2008). These factors are all important in controlling the distribution, physiological performance and behaviour of marine organisms, meaning changes in these could have detrimental effects on organisms living in the oceans in many different ways (Hoegh-Guldberg et al. 2007, Pörtner 2008, Pörtner and Knust 2007).

Rising sea levels, due to melting of polar ice caps (Hoegh-Guldberg and Bruno 2010) will cause a shift in habitats and affect overall ecosystem make up as more species migrate to new areas. Another side effect of warming is the increase in coral bleaching frequency and intensity in tropical areas (Walther et al 2002). Recent variation in prevalence and severity of disease in outbreaks in marine ecosystems is thought to be related to climate change (Harvell et al. 2009). At an organisms level temperature will

have the most detrimental effect due to its large influence of physiology, at whole

organism level down to cellular and molecular levels, to distribution and survival

(Doney et al. 2012). Individual species responses may also impact other species as they

are linked directly and indirectly through various biological and behaviour interactions

of other species within the community leading to community level effects (Doney et al.

2012). As environmental temperature has a major influence on physiological

performance of ectotherms the climatic shifts expected to occur with climate change are

of major concern (Hawkins 1995).

1.1.2 South Pacific and Southern Oceans

The Pacific Ocean is the largest on the Earth, it is broken down into the South Pacific

and North Pacific Ocean (Karner et al. 2001). The South Pacific section spans from the

Southern Ocean to the equator and is home to many linear island chains of volcanic

origin, New Zealand being one of these (Hauri and Hart 1993). New Zealand is

completely surrounded by the Pacific Ocean, the area of the Pacific Ocean to the west

between New Zealand and Australia is called the Tasman Sea. Warming of the eastern

South Pacific Ocean has been observed (Schaffer et al. 2000).

The Southern Ocean comprises all the waters 60° south and beyond, within the

Southern Ocean the Antarctic Circumpolar Current (ACC) exists (Johnson and Bryden

1989, Rintoul et al. 1988). This current evolved after the separation of Antarctica from

Australia and South America causing a loss of warm water supply to Antarctica

(Johnson and Bryden 1988). The formation of the ACC his has traditionally been seen

as the initiation of cooling of the Southern Ocean, giving rise to the permanent ice sheet

coverage (Pörtner et al 2004, Pörtner et al 2007). Antarctica used to have a similar

ocean temperature to New Zealand at about 15°C, now the waters surrounding

Antarctica are usually below 0°C due to the ACC. The ACC is a strong eastward flow

which connects each of the world's oceans (figure 1.1). As all oceans are connected any

large changes to one, will gradually affect the others.
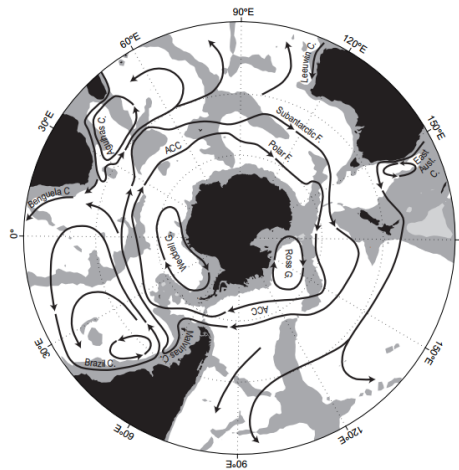
Figure 1.1: (Rintoul et al. 2010) ACC connecting to all the world's oceans. Map shows

current and patterns of the Southern ocean and the southern areas of the worlds' oceans.

1.2 Effects of temperature on organisms

Ecological factors, such as temperature, will directly or indirectly act on an organism's

physiology affecting growth, locomotion, reproduction and energy demands as the

overall cost of life varies significantly with temperature (Bushnell and Jones 1994,

Claireaux et al. 1995, Clarke 2003, Clarke and Johnston 1999, Doney et al. 2012,

Harley et al. 2006, Hochacka and Somero 2002), especially for aquatic ectothermic

organisms as many marine organisms live close to their tolerance ranges (Clarke 1993,

Hughes et al. 2003, Killen et al. 2010, Parmesan 1996, Somero 2002). Temperature's

direct effects on biological processes are due to its influence on kinetic energy, which is

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responsible for determining the rate of reactions (Claireaux et al. 1995, Clarke and

Fraser 2004, Clarke and Johnston 1999, Fry 1958, Hoegh-Guldberg and Bruno 2010,

Jobling 1981, Schurmann and Steffensen 1997). These changes occur in all kinds of

mechanisms, and can sometimes be linked, such as increase in oxygen consumption and

respiratory demand as more oxygen is needed to fuel metabolic processes. Physiological

processes limit thermal tolerance as temperature can have an overall impact on

physiology, population distribution, community composition, phenology/biodiversity

(Pörtner 2001). The effects of temperatures, and species critical temperatures differ

between species and populations, in particular depending on if a species if eurythermal

or stenothermal (Pörtner 2001).

1.2.1. Physiology

The physiological and biochemical factors of temperature-tolerance limits and the

setting of these limits have been subject to much study for 50+ years now (Somero and

DeVries 1967). Physiology is the study of the normal function of living organism and

how the molecules, cells and metabolic processes interact to sustain life. Temperature

has a profound influence on physiology, in particular in ectotherms. Ectotherms are

adapted to depend upon maintenance of the temperature windows of their natural

habitat (Pörtner 2001). Increase in temperature is likely to be the most detrimental effect

of climate change due to its strong influence on an organism's physiology. When an

organism is faced with a change in temperature, they attempt to maintain physiological

homeostasis to counteract changes occurring at the cellular level (Clarke 2003).

The biggest effect temperature has on organism's physiology is in terms of ventilation

rate, heart rate and most importantly oxygen consumption due to the increase in kinetic

energy leading to faster reaction rates, therefore higher oxygen demand (Clarke and Fraser 2004). An increased reaction rate affects many key biochemical processes within a cell such as, enzyme production and/or reactivity, protein variability and ATP production (Clarke 2003, Somero 1995). The understanding of physiological and biochemical mechanisms is crucial to understanding whole organism function and ecosystem impacts in a changing climate (Pörtner 2008).

1.2.2 Ventilation rate and heart rate

Ventilation rate and heart rate are indicative of oxygen demand (Johnston and Dunn 1987). Ventilation rate and heart rate are commonly measured to gain an understanding of an animal's ability to meet oxygen demand. Together ventilatory and circulatory systems are key processes to supplying an organism with the oxygen it needs to fuel its biochemical processes. In some species ventilation rate and heart are tightly linked, this is due to these processes working together to supply oxygen. Oxygen is taken in over the gills in fish via diffusion, oxygen is exchanged to oxygen carrying proteins within the blood across a concentration gradient, and blood is circulated to the desired organ (Holeton 1970). Circulation rate needs to be maintained at a certain level to ensure there is a concentration gradient for movement of oxygen to the oxygen carrier proteins. If the demand for oxygen changes, either increases or decreases, then ventilation rate and heart rate will change to maintain supply of oxygen to a level required to maintain the concentration gradient to allow transfer.

Periods at which fish are struggling to meet oxygen demand can be seen when measuring ventilation rate and heart rate as fish will start breathing very fast and very deeply linked with a rise in heart rate. Limited functional capacity of these oxygen

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supply systems have been found to characterise the borders of an organism's thermal tolerance window as inadequate O_2 supply will affect the long-term fate of organisms (Frederich and Pörtner 2000).

1.2.3 Oxygen consumption

Oxygen consumption varies with temperature due to the kinetic energy of biochemical processes at the cellular level being influenced by temperature (Claireaux et al. 1995, Clarke 2003, Jobling 1981, Schurmann and Steffensen 1997). As temperature rises, so does chemical reactions due to the number of molecules whose activity exceeds the threshold increases, causing an increase in metabolic processes and therefore an increase in oxygen demand (Gon and Heemstra 1990). Oxygen consumption A.K.A metabolic rate has been determined as the most convenient and relevant measure of metabolic response to temperature. The utilisation of oxygen by mitochondria to generate ATP is crucial to the functioning of an organism (Bushnell and Jones 1994). Metabolism is responsible for fuelling an organism with the energy it needs to maintain homeostasis, enable growth, reproduction, movement and all other functions (Clarke 2003, Killen et al. 2011). This process can be measured as a rate, oxygen consumption, which is the measure of the oxygen needed to fuel essential metabolic processes within the cell (Clarke 2003, Hulbert and Else 2000, Killen et al. 2010). Oxygen consumption can be influenced by external factors, such as temperature and oxygen availability; and reflects how organisms respond to these external physiological challenges (Clarke 2003). There are different types of oxygen consumption, with standard oxygen consumption being the most common measure. Standard oxygen consumption reflects

the minimum oxygen demand required for sustaining life (Hulbert and Else 2000,

Schurmann and Steffensen 1997).

The common way to measure oxygen consumption of an organism is via the use of

respirometry (Steffensen 1989). Respirometry allows you to obtain an estimate of

metabolism under controllable conditions, which makes it a favoured experimental

technique amongst physiologists. It allows you to measure resting oxygen consumptions

of an organism either in conditions similar to its environment or you can introduce

stressors, such as temperature change or exercise. Closed box respirometry allows you

to measure the amount of oxygen in the environment in proportion to its metabolic

demands (Cech 1985. Clarke 2003, Hopkins and Cech1990). By looking at resting

oxygen consumption changes across different environmental conditions, i.e.

temperature, you can gain insight into the organisms' ability to withstand environmental

changes (Clarke 2003).

1.2.4 Thermal range

Temperature influences aerobic scope, the measure of demand for ATP (Clarke 2003).

Temperature has a direct effect on oxygen consumption that can be expected to not only

reduce the ability to perform activates but also to compromise growth, reproduction and

other behaviours (Campbell et al. 2005, Clarke 2003, Pörtner et al. 2007). When aerobic

scope is exceeded anaerobic metabolism is relied upon to meet oxygen demand, this is

time limited and if organisms continued to be exposed to high stressors their fate could

be at risk. The ranges in which animals can survive is known as tolerance range or

thermal limitation and can be described by upper and lower limits, these are known as

pejus temperatures and range (Pörtner 2001).

The width of this pejus range reflects the amplitude of temperature fluctuations in the habitat of a species (Pörtner et al. 2000). The concept of pejus, pejus meaning 'getting worse', and range states that animals are characterised by an oxygen and capacity limited thermal tolerance (Pörtner et al. 2000). Pejus range refers to a tolerance range in which in organism can meet oxygen demand, the first level of thermal intolerance occurs in fully oxygenated waters through the onset of a mismatch of oxygen supply and demand (Pörtner 2001). Shelford's "law of tolerance" (1931) was defined according to the range of tolerance to abiotic factors like temperature. It takes into account optimal tolerance in the middle with high and low pejus ranges on either side encompassing physiological range (figure 1.2). It possible to determine optimum range by using this model and considering physiological break points, i.e. when circulatory and ventilatory systems fail (Frederich and Pörtner 2000). This can arise due to temperature change, and temperature's influence on physiological processes. With continued cooling or warming organisms can be pushed outside their aerobic scope and reach their critical threshold temperatures (T_C) at which energy is insufficient so anaerobic metabolism begins (Pörtner 2001).

In aquatic animals a drop in aerobic scope characterises the onset of thermal limitation, the drop is due to the limited capacity of circulatory and ventilatory systems. It is thought that thermal tolerance is determined by oxygen and capacity level effects which provide understanding into the mechanisms shaping limited performance (Pörtner 2009). When an organism is pushed outside its tolerance range anaerobic metabolism, metabolism that doesn't involve the use of oxygen, sets in due to a mismatch between oxygen supply and demand (Frederich and Pörtner 2000, Pörtner 2009). Extended exposure to temperatures outside physiological tolerance range will eventually lead to death unless thermal acclimation occurs.

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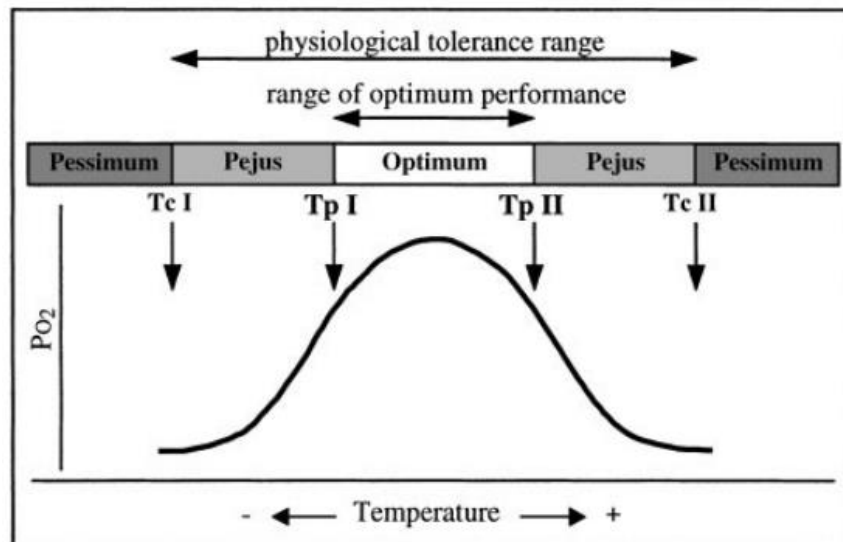
Figure 1.2: (Frederich and Pörtner 2000) Model of temperature tolerance. T_p (pejus

temperatures) indicate optimum range. T_c (critical temperatures) indicate physiological

tolerance range, beyond these points there is the onset of anaerobic metabolism.

Tolerance ranges can be assessed by observing the rate of change at the lower and upper

ends of an organism's tolerance window.

1.2.5 The Q_{10} effect

The relationship between temperature and oxygen consumption is assumed to be

exponential in nature due to the phenomena known as the Q_{10} effect (Killen at al. 2010).

The Q_{10} effect relates to the change in rate of a biological or chemical system in

response to a 10°C increase in temperature (Doney et al. 2012, Killen at al. 2010). The

standard rule of Q_{10} is that for every 10°C increase in temperature reactions rates will

also increase, usually 2-3 for most physiological processes under normal conditions

(Brockington and Clarke 2001, Clarke and Fraser 2004, Hochacka and Somero 2002).

Q_{10} has no units as it is a factor by which rates change, for example a certain species of

fish may have a Q_{10} of 1.7, meaning that for an increase of temperature by 10°C the

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change of rate is equal to 1.7. This makes resting oxygen consumption far more

expensive at warmer temperatures than at lower temperatures (Clarke 2003). Prolonged

exposure to changing temperatures may cause fish to struggle to meet oxygen demand

due to temperature influencing rates with Q_{10} values dropping off as rates become

limited. During a change in temperature organisms may struggle to obtain enough

oxygen from their environment to fuel increased oxygen consumption as systems may

start to fail. When systems first begin to fail some organisms have inbuilt mechanisms

which allow them to regulate these systems and compensate against temperature effects.

1.2.6 Temperature compensation/acclimation and metabolic cold adaptation

When faced with a temperature change some organisms have the ability to alter

homeostatic responses which serve to offset the effects of temperature on the chemistry

of the cells (Johnston and Dunn et al. 1987). These changes can involve alterations to

metabolism and, the free energy of ions and molecules (Johnston et al. 1991).

Individuals can reorganise physiological functions in response to exposure to new

environmental temperatures and reduce the initial rise in oxygen consumption to

previous standard levels (Precht 1955, Robinson and Davison 2008). The process of

compensation is the maintenance of physiological rates in the face of temperature

change, and is thus a form of homeostasis (Clarke 1993).

Long term adjustment to environmental temperature causes fundamental differences in

response to temperature change among individuals at various temperatures (Precht

1955). These temperatures need to be outside their normal environmental temperature

range or last for extended periods of time to see high level compensation / acclimation

responses (Precht 1955). Some species are more capable of acclimation due to being

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more eurythermal in nature than others (Bullock 1955, Claireaux et al. 2005). Organisms can only acclimate and shift thermal windows to a point due to limits and trade-offs. These trade-offs work at structural and functional levels making perfect acclimation rare, it is more common to see overcompensation, partial compensation and under compensation (Brodte et al. 2006, Precht 1955).

Organisms in cold climates, such as those in the Arctic and Antarctic can show metabolic cold adaptation. Many fish found in Polar Regions are perfectly compensated to handle low temperatures by increasing their resting oxygen consumption and negating the influence of temperature on rate (Robinson and Davison 2008). When compared to temperate fish at same exposure temperatures, those fish that show metabolic cod adaptation will have higher oxygen consumptions (figure 1.3). This metabolic cold adaptation (MCA) specialisation ensures that fish are good at surviving in these harsh climates, however when populations are locally adapted, they can sometimes find themselves over specialisation comes a lack of thermal flexibility, and species have limited tolerance to seemingly small changes in water temperatures (Bilyk and DeVries 2011, Clarke 2003, Doney et al. 2012, Hoegh-Guldberg and Bruno 2010, Pörtner et al. 2000, Robinson and Davison 2008, Somero et al. 1968).

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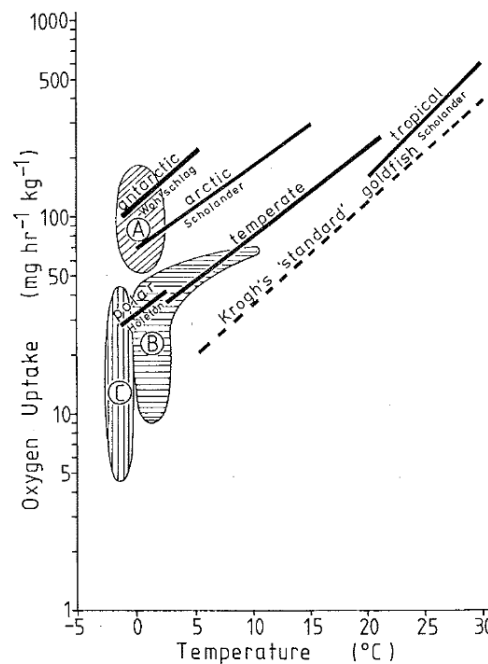
Figure 1.3: (Gon and Heemstra 1990) Comparison of oxygen consumption of Antarctic, Artic, temperature and tropical fish species. Shows metabolic cold adaptation in cold water species as they have higher oxygen consumptions than temperate fish at the same temperatures.

1.2.7 Distribution

In general there is a strong correlation between habitat temperature and oxygen consumption in ectothermic animal, with geographical distribution being strongly linked to thermal tolerances (Frederich and Pörtner 2000), suggesting when animals are faced with undesirable changes to their environments which exceed their tolerance range are likely to seek out new areas with favourable conditions (Hawkins 1995). Many species range and abundance are directly influenced by temperature (Clarke 2003, Doney et al. 2012, Hodkinson 1999). As current habitats become unfavourable due to temperature or other climate change effects some organisms are migrating and

exploiting new habitats. Climate change is already causing geographical shifts in distribution of both aquatic and terrestrial species (Clarke 2003, Ives 1995, Pörtner 2008). In particular poleward movements are being made as areas become too warm as far as 403km (Brodte et al. 2006, Perry et al. 2005). Many species first response to increased temperatures outside their tolerance range will be to migrate to cooler areas to return to favourable conditions, by either moving to higher latitudes, deeper depths or towards poles (Doney et al. 2012). Poleward movements have been recorded in plants, flying insects, marine birds and fish (Hughes 2000, Thyrring et al. 2015). As mentioned above temperature can have a detrimental effect of organisms' ability to meet metabolic demand, rather than stay in high energy demanding habitats which will likely result in reduced productivity, growth and survival, some species will place their energy into migration. As organisms migrate, changes in transportation of nutrients and organic matter will occur which has the potential to disrupt marine ecosystems (Doney et al. 2012).

Future shifts in species distributions will depend on how fast the current habitat is changing due to climate change and how good and fast species are at spreading to new areas (Pörtner 2008). There is a possibility that widespread extinction could be a result in areas where dispersal capabilities are limited, along with suitable habitats to migrate to (Perry et al. 2005).

1.3 Study species

My project involves looking at the effects of warming on three different species of cod, found in the South Pacific and Southern Oceans. None of these species are a true gagid cod, they are all perciformes but belong to differing families. As they all have similar features and behaviours that have caused them to pick up the nicknames of cod. *Parapercis colias* (blue cod) is a eurythermal endemic fin-fish species to New Zealand found only in the South Pacific Ocean (Beentjes and Carbines 2005). *Paranotothenia angustata* (black cod) is a semi Sub-Antarctic species, are found in both the Pacific and Southern Oceans (Campbell and Egginton 2007). *Trematomus bernacchii* (emerald rock cod) is a stenothermal Antarctic notctheniod are endemic to the Southern Ocean (Fukuchi & Marchant 2006). It is important when comparing oxygen consumptions of fish from different latitudes that they are ecologically similar, all these fish are inshore species with habitats made up of similar constituents.

1.3.1 Parapercis colias (blue cod)

Blue cod is a bottom-dwelling, eurythermic benthic species endemic to New Zealand (Bentjee and Carbines 2005, Gebbie 2014). Despite the common name of blue cod, these fish are not true cod but are in fact members of the *Pinguipedidae* family (Carbines and McKenzie 2002, Diaz-Guisade 2014). Members of the *Pinguipedidae* family are found in the Indo-Pacific (Carbines and McKenzie 2002). Blue cod are most abundant around Southland and Chatham Islands, however they can be found in almost all coastal regions around New Zealand (Blackwell 1997). The furthest north blue cod are found is in Northland at temperatures reaching 22°C+ in Summer, to as far south as Snares Island with waters dropping below 8°C in mid-winter (Beentjes and Carbines

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2005, Carbines 1998, Carbines 1999, Cranfield et al. 2001, Gebbie 2014, Rapson 1956).

Blue cod is one of the most important commercial and recreational finfish species

throughout New Zealand, particularly in Southland (Beentjes and Carbines 2005,

Cranfield et al. 2001, Gebbie 2014, Govier 2001, Henderson 2009).

1.3.1.1 Habitat and distribution

Blue cod prefer complex heterogeneous habitats, made up of multiple different

components (Rapson 1956). In particular they like jagged bedrock, reef edges, and

shingle/gravel areas, rubble, mud and sand bottoms interspersed with broken shells, and

silt up to depths of 150 m+ (Davidson et al. 2001). Distribution of blue cod is

influenced by many factors including; age, habitat and food availability along with tidal

cycles (Carbines 1999, Cole et al. 2000). Blue cod is found from shore to shelf edge

along the entire coastline of New Zealand (Cranfield et al. 2001. Davidson et al. 2001).

It is suggested that blue cod's distribution changes with age. Adult blue cod are found in

deeper waters, whereas smaller juvenile cod are commonly found in shallow inshore

reef areas (Choat and Ayling 1987).

It is common for members of *Pinguipedidae* to show territorial tendencies, this holds

true for the blue cod which tend to hold large and rather loose territories (Carbines

1998, Mutch 1983). It is quite often that a large adult's territory will overlap with that of

smaller juvenile blue cod but due to differing diets and the lack of sexual competition

they maintain a peaceful relationship.

1.3.1.2 Size and age

Maximum size for blue cod is over 60 cm in length with a weight of four kg (Carbines 2004, Cole et al. 2001, Cole et al. 2003). In Southern regions blue cod on average are larger than in Northern regions due to cooler waters (Beentjes and Carbines 2005, Diaz-Guisade et al. 2012). This is suggested to be a result of Southern blue cod having a faster growth rate (Rapson 1956). A difference in abundance and size structure between inshore and offshore populations has been seen in many different locations (Beentjes and Carbines 2005).

Age dependent colour change is common in blue cod. Juveniles between 10-15 cm are predominantly white with broad brown or pale grey in colour bands along each side (figure 1.4), whereas large blue cod, 20 cm and above and are more distinctly blue in colour with green/grey coloured bands on each side (figure 1.5). Size at maturity also varies with location (Gebbie 2014).

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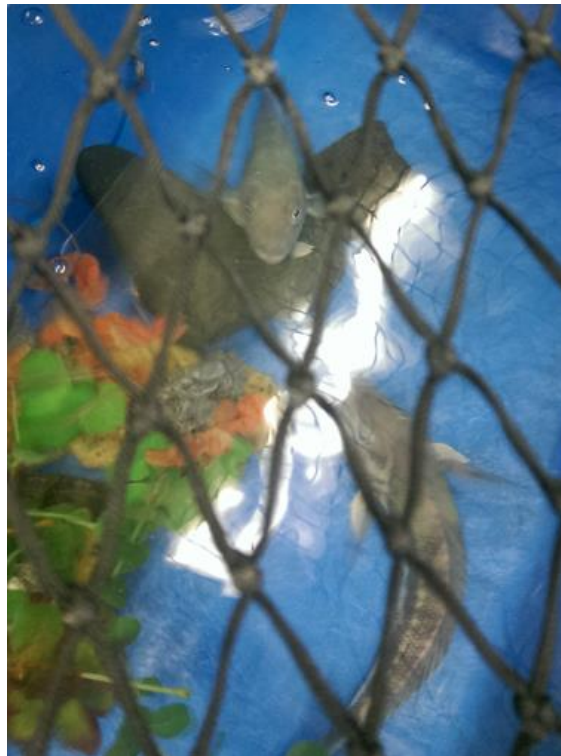
Figure 1.4*:* Juvenile blue cod, brown in colouring, less than 20 cm in length.

Figure 1.5: Adult blue cod, blue and green in colour, 20 cm +.

1.3.1.3 Diet

Blue cod show opportunistic carnivore tendencies and eat almost anything that comes

their way (Bentjee and Carbines 2005, Gebbie 2014). By examining stomach contents

Rapson managed to determine blue cod main food sources. In general they feed on

plankton and nekton, and small fish, as well as being known to eat other blue cod

(Gebbie 2014, Jiang et al. 2002, Rapson 1956). On examination of stomach contents the

likes of pilchards, sprats, octopus and crustaceans were found. Diet is seen to vary with

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age, with juveniles dependent on amphipods, young adults mainly feeding on

crustaceans and benthic invertebrates and adults predominantly feeding on other fish

(Mutch 1983).

1.3.1.4 Importance

Due to blue cod's nationwide distribution they are a key player in New Zealand's marine

ecosystems and fisheries. Blue cod has been a food source of interest dating back to pre-

European times when Maori depended on blue cod as a stable part of their diet (Batsone

and Sharp 1999, Cranfield et al. 2001, Govier 2001). Blue cod is still important to

Maori recreational fishing culture and go by the Maori names rawaru and pakirikiri

(Diaz-Guisade 2014, Gebbie 2014). Over-fishing is currently an issue for certain

populations of blue cod around New Zealand. Both commercial and recreational

fisherman reported declines in numbers and size of blue cod (Diaz-Guisado 2014,).

Marlborough Sounds in particular saw a huge decrease in 2007. Extra fishing laws were

introduced and there was an increase in abundance and size of the blue cod population

in Marlborough Sounds (Mace and Johnstone 1983). Currently there are eight separate

blue cod fisheries/management areas established around New Zealand. Blue is a hardy,

robust species that lack a swim bladder which makes them an excellent study species as

they can survive capture, transfer and experimentation.

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1.3.2 Paranotothenia angustata (black cod)

Paranotothenia angustata (black Cod) is a semi Sub-Antarctic, demersal fish species found in waters around the lower South Island and the Sub-Antarctic Islands (Campbell et al. 2004, Campbell and Egginton 2007, Tetens and Wells 1984). Due to the harsh cold environment that is the Southern Ocean many organisms living within this have evolved ways to allow survival in sub- zero waters. These include but are not limited to; changes in buoyancy, haematology, enzyme and lipid make up, the production of anti-freeze proteins and metabolic cold adaptation (MCA). During the last ice age, around 10 million years ago, black cod are suspected to have crossed the polar front and migrated towards the Sub-Antarctic and New Zealand (Fago et al. 1992).

They are now known as a non-Antarctic notothenioid but still possess very similar physiological and behavioural traits with their Antarctic relatives, such as the presence of genes for antifreeze glycoproteins (Eastman 1988, Fago et al. 1992). As true for most nototheniods, black cod live a relatively inactive life only spending 10 percent of the time swimming exhibiting benthic tendencies and spending most of its time propped up on their pelvic fins on the ocean floor (Campbell et al. 2005). Black cod appear dark in colouring, mainly black with green and yellow scales and a white under belly (figure 3.3). Commonly black cod is only found within a narrow temperature range from 5°C to 10°C (Paul et al 1993). Once waters reach above 10°C black cod tend become harder to find, the lowest temperature black cod has been recorded at is during a successful acclimation to 4.5°C (Tetens and Wells 1984). There is a lot unknown about the black cod which makes them a very interesting species to work with. Black cod is a good comparison between Antarctic and temperate fish as they possess similar qualities to Antarctic fish but live in waters similar to those of temperate fish.

1.3.2.1 Habitat and distribution

Black cod prefer similar habitats to that of the blue cod however they are found in
slightly different locations due to temperature. The furthest North black cod is found is
around Banks Peninsula, and as far South as the Sub-Antarctic Islands. Black cod are
also found around nearby islands such as South Shetland, Elephant Island and South
Orkney Islands (Gon and Heemstra 1990). Recent warming of the oceans has been
Banks Peninsula bottom temperatures during the middle of winter still reading around
10.5°C, it has become a lot harder to catch black cod. The same goes for Otago
Harbour; black cod were often caught, whereas now it is becoming harder to catch
larger numbers. This suggests that black cod may be migrating further South back
towards the Antarctic. Black cod tend to live in shallow areas, 15-30 m deep, but are
also common in deeper waters around the Sub-Antarctic islands (Eastman 1988,
Kingsford et al. 1989, Kingsford and Choat 1989).

 Black cod become quite aggressive towards other black cod when they feel their
territory has been breached. This can provide issues for housing black cod for
experiments as they each need their own separate space.

1.3.2.2 Size and age

Black cod is a large and negatively buoyant species (Campbell et al. 2005, Paul et al.
1993), with the majority of black cod are over 35 cm in length when caught (Gon and
Heemstra 1990). Black cod have oblong body shape, with large heads/mouths and a
slim line tail. Little is known about the age of black cod but due to their relatively large

size and being nototheniods, it is suggested they are normally about 15-30 years old at

capture when over 35 cm in length (Paul et al. 1993).
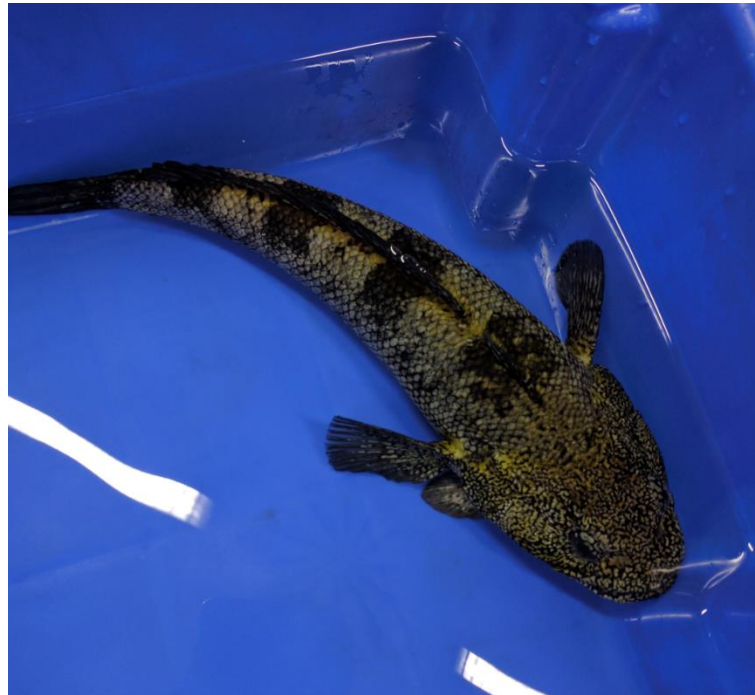
Figure 1.6*:* Black cod, 45cm long, large head and streamline tail.

1.3.2.3 Diet

Black cod is a sedentary bottom feeder (Fago et al. 1992, Tetens and Wells 1984). A

study of black cod populations in Snares Islands (Fenwick 1973) often saw the fish

sitting on the ocean floor waiting for prey to swim past and then swarm up from the

bottom to catch their prey, behaviour common amongst ambush predators. Prey was

found to vary from crustacea, to paua and smaller fish.

<u>1.3.2.4 Importance</u>

 Like the blue cod, black cod also lack a swim bladder which enables them to survive capture (Campbell et al 2005, Fago et al. 1992). These fish are an ideal species to link between Antarctica and temperate habitats as they possess qualities of Antarctic fish but live in more temperate waters. Black cod are often used as a comparison species due to their bottom dwelling lifestyle and is similarly buoyant to Antarctic fish species.

1.3.3 Trematomus bernacchii (emerald rock cod)

Emerald rock cod is a red-blooded, stenothermic, benthic ambush predator species found in the Southern Ocean, particularly in McMurdo Sound (Carginale et al. 1998, Egginton & Rankin 1998, Zimmerman & Hubold 1998). Belonging to the nototheniidae family, emerald cod is one of the many endemic fish species that have been isolated and evolved in the cold waters of Antarctica for millions of years (Eastman 1993, Hofmann et al. 2000, Montgomery & Macdonald 1998, Tagliafierro et al. 1998, Zimmerman & Hubold 1998). Emerald cod are usually pale brown or pinkish/brown in colour with about four dark bars on dorsal part of the body (figure 1.6) (Gon and Heemstra 1990). Many notothenioids, emerald cod included, possess the ability to offset the effects of low temperatures on their oxygen consumptions by the production of specialised enzymes (Hoffman et al. 2000). Living in these cold, thermally stable environments may cause the loss of heat tolerances and the ability to acclimate to short term temperatures changes due to specialisation (Hoffman et al. 2000).

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1.3.3.1 Habitat and distribution

Emerald cod is a bottom dwelling key species to the Ross Sea making up 90% of the abundance and biomass of fish fauna at Terra Nova Bay (Gon and Heemstra 1990, Hoffman et al. 2000). Emerald cod is widely distributed and usually found in near shore and first slope waters around the continental edge of Antarctica; most commonly in the upper 200 m, especially at depths of 20-30 m, but can live as shallow as the surface and as deep as 700 m (DeWitt 1971, Fukuchi & Marchant 2006, Gon and Heemstra 1990). These fish are only found in the extremely cold and thermally stable waters around Antarctica. Many Antarctic fish hold specialisations to survive in these extreme habitats. Due to their habitats being very thermally stable, emerald cod do not experience regular fluctuating temperatures, unlike those waters the blue and black cod live in (Hoffman et al. 2000). Short term temperatures changes are very undesirable to emerald cod as they are not usually exposed to those pressures, emerald rock cod have an upper lethal limit of around 6°C (Gon and Heemstra 1990).

1.3.3.2 Size and age

Adult emerald cod are darker in colour, predominantly in the dorsal area (Gon and Heemstra 1990). On average females live for ten or more years and reach lengths of 35 cm; whereas males live for five or more years and are 28 cm at maximum length (Gon and Heemstra 1990, Wohlschlag 1961). Males reach maturity first at a length of 14.5 cm, whereas females reach sexual maturity at a length of 17.5 cm (Gon and Heemstra 1990). In general notothenioids grow slower when compared with temperate species (Kock 1985). Spawning takes place throughout October to January with some local

variation (Dearborn 1965). Aquarium based observations indicate that emerald rock cod stop feeding a month before spawning (Sakakibara et al. 1989).

1.3.3.3 Diet

Emerald rock cod is a primarily benthic feeder, but like the blue and black cod is an opportunistic carnivores and will fed on pelagic prey when available (Gon and Heemstra 1990). They feed on a wide variety of prey ranging from amphipods and isopods to nemertine worms and sometimes fish eggs, in winter months Emerald rock cod tend to eat less. (Fukuchi & Marchant 2006).

1.3.3.4 Importance

Emerald cod are dependent on the foodweb of McMurdo Sound. If there were to be any changes to this food web due to climate change and warming of the ocean this could have a dire effect on these fish. Emerald cod is known to show metabolic compensation, this involves emerald rock cod increasing its standard oxygen consumption to counteract the effect of low temperatures (Somero et al. 1968). Specialisation like this will make it harder for emerald cod survive temperature change as they lack the plasticity to acclimatise to changing environments (Wilson et al. 2001). Emerald cod, along with the black cod, belongs to the notothenioid family, this making it a good comparison species for this study, in particular due to its metabolic cold adaptation as it will supply an increased oxygen consumption at cold temperatures to compare to when acclimating blue and black cod to cooler temperatures.

Figure 1.7: Emerald rock cod, 16 cm long.

1.4 Aims and hypothesis

The main objective of this project is to examine the ventilatory, circulatory and
metabolic physiology of blue cod, black cod and emerald cod and their ability to
withstand temperature change. To investigate, I will be answering the following specific
questions: What effects will a chronic rise (and fall) in temperature have on resting
ventilation rate, heart rate and standard oxygen consumption? What effects will an acute
rise (and fall) in temperature have on ventilation rate, heart rate and standard oxygen
consumption? What are the Q_{10} coefficients for these rates across acute temperature
exposures? What are the upper and lower thermal limits for these three species, and how
does their ability to tolerate temperature changes differ from one another?

I hypothesise that with an increase in temperature there will be an increase in ventilation
rate, heart rate and standard oxygen consumption. During chronic temperature

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exposures I hypothesise that blue cod will acclimate best due to their eurythermal natural habitats, whereas black cod and emerald cod will not acclimate as well due to their natural environments being more thermally stable (stenothermic in nature). This will also be apparent when looking at acute temperature effects with blue cod being able to tolerate a wider range of temperatures than black and emerald cod. I predict emerald rock cod will show metabolic cold adaptation and that black cod will show elevated rates at cooler temperatures but unlike emerald rock cod will not show full metabolic cold adaptation. I predict that Q_{10} coefficients will be largest near the upper and lower exposure temperatures as these fish are pushed outside of their tolerance zones. Large drop offs in rates will likely be seen at lowest and highest exposure temperatures as fish begin to struggle at temperatures outside their thermal range.

1.5 Importance of study

Physical and chemical changes of the ocean will have strong direct or indirect effects on the physiology of many marine organisms. It is important to understand the strength of these effects as they could cause population, community and ecosystem level effects. Understanding what a marine organism's thermal limit is before they reach suboptimal physiological performance, resulting in likely death, is of high importance. Without knowledge on how species themselves will respond to warming it is hard to understand the effects at an ecosystem level. Respiratory demand measurements contribute to our understanding of temperature adaptation as they have a direct insight into energetic costs and what it costs to run metabolic systems when acclimated to a particular temperature (Clarke 2003).

This thesis will provide a structured and detailed account of my studies and findings with chapters focusing on my experiment methods, results and discussion, ending with a

conclusion on the impact temperature change will have on these fish species and in

general fish of the South Pacific and Southern Oceans.

Chapter 2

Methods

All the fish used in this project were caught through the University of Canterbury over several trips in several different locations. These fish were housed in the Biological Sciences Aquarium at UC and experiments were carried out on these fish under standard lab conditions.

2.1 Capture and captivity

2.1.1 Fishing location

All blue cod and black cod fishing was conducted by the University of Canterbury on the School of Biological Sciences boats Rapaki and Koaro. One location was used to catch black cod, Otanerito Bay, Akaroa (figure 2.1). Blue cod were caught from many different areas within Akaroa; Akaroa Harbour, Titoki Bay, Little Tikao Bay, Sleepy Bay, Otanerito Bay, Pompeys pillar and around reefs further out from cliffs and bay areas (figure 2.1), with some caught at Motonau in North Canterbury. All emerald cod fishing was conducted via the help of Antarctic New Zealand through a hole in the sea ice in Antarctica. Emerald cod fishing was completed over a summer season at Cape Evans located on Ross Island, Antarctica.

Figure 2.1: Map of Akaroa image from: http://maps.google.com. Green circle (◯)

represents the most visited area for both black cod and blue cod. White Star (✶)

represents blue cod fishing locations. Red Cross (☆) represents Marine Protected

Areas.

2.1.2 Traps and bait

Steel traps 91 m x 91 m covered in fishing net proved to be most effective (figure 2.2).

Smaller collapsible steel traps were also used. Both the larger and smaller traps had one

way entrances to prevent fish from escaping. Two different ways of securing bait within

the traps were used. In larger traps, small cylindrical containers were filled with bait,

closed and attached to the top of the trap, in smaller traps onion bags proved to work

well. Bait consisted of fresh mussels, crabs, paua guts along with a pre made mixture of

ground up paua and mussels. Paua guts proved to be the best bait as each trap loaded

with this contained more fish. The traps were attached to 15-30 m of rope to allow the

traps to sit on the reef/ocean floor, also attached to this rope were a buoy and a flag so

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the traps could easily be seen by other boats and us for collection. Hook and line were
used to fish for emerald rock cod baited with fluorescent lures.

Figure 2.2: Steel 91 m x 91 m trap used for fishing. All traps had closable lids, plastic
tube in the centre to hold bait and orange fingers in the entrances to traps to stop fish
swimming out once inside. All traps were attached to 15 m+ lengths of rope, a flag and
a buoy.

2.1.3 Catching blue cod

Blue cod fishing trips were usually day trips. Once the desired location on the water was
reached traps would be freshly baited and carefully lowered overboard bottom down
onto rough bottomed areas, as indicated by the sonar reader on the boat, at depths of 15-
30 m. After an hour we returned to the first trap and a hook was used to latch onto the
rope to aid in bringing the trap onboard, the winch on the boat was used if needed. Once
the trap was pulled on board all fish were removed from the trap, with desired fish being
placed into a chilly bin filled with seawater, and the unwanted fish returned. If only a

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few fish were caught during the first haul traps would be re-baited and re-distributed.

Once the desired numbers of fish were caught we returned to University. During the

drive back to UC, fish were kept in chilly bins with slicker pads to keep water cool and

bubblers to keep the water oxygenated. The temperature of the water during capture was

12 - 15 °C.

2.1.4 Catching black cod

Black cod fishing trips were always overnight trips as night time fishing proved to be

the most successful for catching these fish. Once the destination was reached traps

would be freshly baited, when a shallow and reef filled area within our desired location

was reached the traps were lowered overboard, bottom down. Traps were then left

overnight and we returned the next morning usually around 7am to retrieve the traps.

Once the trap was pulled on-board all fish were removed from the trap with desired fish

being placed into a chilly bin on board filled with water (figure 2.4) and the unwanted

fish returned. Once the desired numbers of fish were caught we returned to University.

Slicker pads were placed in chilly bins with the fish, along with bubblers, to keep water

temperatures low and water oxygenated on the return trip to the University. The

temperature of the water during capture was 10.5°C.

2.1.5 Catching emerald cod

All fishing was carried out during a summer season in Antarctica at Cape Evans, Ross

Island. A 10 cm hole was melted in the sea ice via the use of a heated melting coil.

Fishing rods were used to catch these fish. Hook and lines were lowered 10-20 m,

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baited with fish and/or fluorescent lures. Rods were reeled in when a bite was felt,

emerald cod on the line were quickly transferred from the hook into a chilly bin fill of

fresh sea water. This transfer of fish from the ocean to the chilly bin needed to be done

extremely fast due to below zero air temperatures. Fish were then transported back to

Scott Base. Fish were transported from Scott Base to a dedicated Antarctic aquarium

system at the University of Canterbury. The temperature of the water at the time of

capture was -1.5 °C.

2.2 Captivity

2.2.1 Aquarium housing

Black and blue cod were initially housed in the Biological Sciences flow-through

Aquarium with sea water maintained at 12.5°C and on 12h/12h light/dark cycle with a

thirty minute sunrise/sunset in Dolav (1000) fish tanks (figure 2.3). Black cod were

housed individually, whereas blue cod were housed four fish to a tank. Rocks, cinder

blocks and fake plants were placed in tanks to try and provide similar habitat to the

wild. These decorations also provided refuge for fish in tanks housing more than one

fish. Nets were placed over the top of all blue cod tanks as these fish were found to

jump out of tanks otherwise. All fish were left for thirty days or more for acclimation

and recovery after fishing before experimental use. All fish were fed every second day

(unless fish were fasting before experimentation) a varied diet of thawed frozen

mussels, shrimp and octopus, fresh mussels, crab meat and Paua guts. Paua guts

appeared to be the favourite among the black cod. Emerald rock cod were kept in a

separate temperate controlled PC2 Antarctic Fish aquarium. Fish were kept at

temperatures ranging from 2°C. Each fish was kept in a separate 300 L tank, three tanks

had an average temperature of 1.5°C, and the other four tanks had an average of 2°C.

Emerald cod were fed a diet of fresh mussels. All tanks contained biological filters.

Figure 2.3: Dolav 1000 tank in Biological Sciences flow-through Aquarium. This particular tank contained black cod.

2.2.2 Acclimations

For warm acclimations fish stayed in the Biological Sciences flow-through Aquarium with sea water held constant to desired temperatures (12°C and 18°C). For cold acclimations (8°C) fish were moved to a controlled temperature room as the flow-through Aquarium sea water could not be maintained lower than 12°C. Fish were initially moved into 12°C water and left twenty-four hours. Water temperature was taken down by one degree per day over the space of a week until 8°C was reached. This was done to reduce stress and to avoid death upon exposure to a large temperature change. Full water changes were done regularly for fish in temperature control rooms as there was no flow-through cycle. A large tank near the back of the room was kept filled with fresh seawater from the tap at 15°C and left to cool to desired temperature for

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water change. Emerald cod were long term acclimated to 2°C in a separate temperature controlled PC2 aquarium.

2.3 Experimental

2.3.1 Closed-box respirometry

Respirometry is a common method used by physiologists to measure oxygen consumption of aquatic animals. Closed-boxed respirometry allows the continuous sampling of oxygen levels within the respirometer. This information can then used to calculate physiological measurements, such as metabolic rate.

Respirometry experiments were conducted on black cod, blue cod and emerald cod. All experiments were carried out in temperature controlled rooms, with the use of a water bath and heater to attain the desired temperatures for experimentation. Black cod were acclimated to 8°C and 12°C, blue cod were acclimated to 8°C, 12°C and 18°C; all these acclimations lasted a month or longer. Fish acclimated to 8°C fish were exposed to acute temperature changes ranging from 4°C to 16°C, black cod were taken as low as 4 °C whereas blue cod were only taken to 6°C due to the stress they experienced at this temperature. Emerald cod was the only species acclimated to 2°C; they were exposed to acute temperature changes ranging from 0°C to 8°C. All acute temperature exposures lasted for 90 minutes, 60 minutes for acclimation and 30 minutes for experimentation.
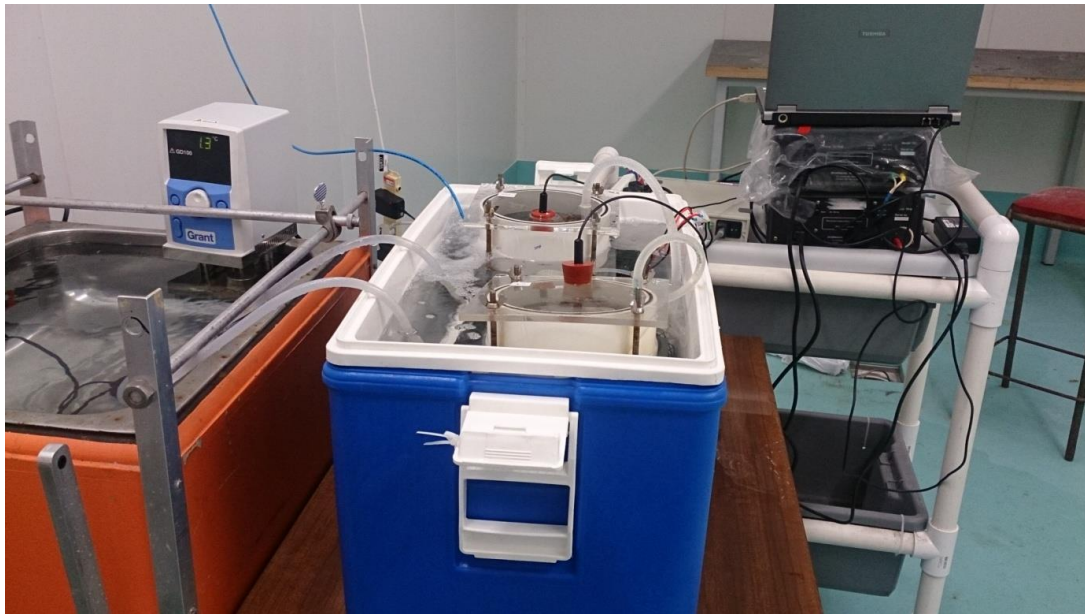
Figure 2.4: Experimental set up. Water bath on the left, respirometers in the middle, recording equipment at the rear.

2.3.2 Experimental set up

The set up for respiratory measurements consisted of a water bath, closed-box respirometer, oxygen measuring equipment (either Strathkelvin oxygen electrodes or FireSting), PowerLab and laptop (figures 2.5, 2.6). A water bath, with a heater and heat exchanger pads powered by pumps, was used to maintain the reservoir of water at the desired temperature and change temperature when needed. Three different sized closed box respirometers were used for the three different species. Black cod respirometers were rectangular in shape, holding 17.435 L of water. The blue cod respirometer was round, with greyed out sides and a volume of 14.732 L. Emerald cod respirometers were round, with greyed out sides and held 1.273 L of water. The oxygen electrodes were inserted into the respirometers and continuously sampled partial pressure (PO$_2$) which

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was recorded onto Powerlab in mmHg. A valve was used to control the influx of water into the respirometer (figure 2.5). During experiments, after calibration of the electrodes was complete, the valve was turned to close stopping water from flowing into the respirometer. Once an experiment was completed, the valve was turned to open to allow freshly oxygenated water flow into the respirometer so the fish could recover. After each day worth of experimentation that respirometer was cleaned to reduce bacteria growth (Dalla Via 1983).

All heart rate work was carried out in a respirometer. In addition to the respirometry equipment, an ADI animal bioamp and A.M systems teflon coated wire were used to record heart rate. The ADI animal bioamp box was connected up to PowerLab. A-M systems Teflon coated stainless steel 7 strand wire was attached to measure heart rate and ventilation rate via fish surgery techniques (see below). Filters were set for different channels on PowerLab to show Heart Rate and Ventilation Rate. Channel one was the raw data with a low-pass 50Hz filter to remove excess electronic noise. Channel two was set up with a band-pass filter to show heart rate; high frequency cut off set to 70Hz and low frequency cut off set to 10Hz. Channel three was set up for ventilation rate also with a band-pass filter; high frequency cut off set to 7Hz and low. Heart rate

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experiments were not conducted on emerald cod due to an unfortunate air-conditioning

malfunction which resulted in all the fish dying.
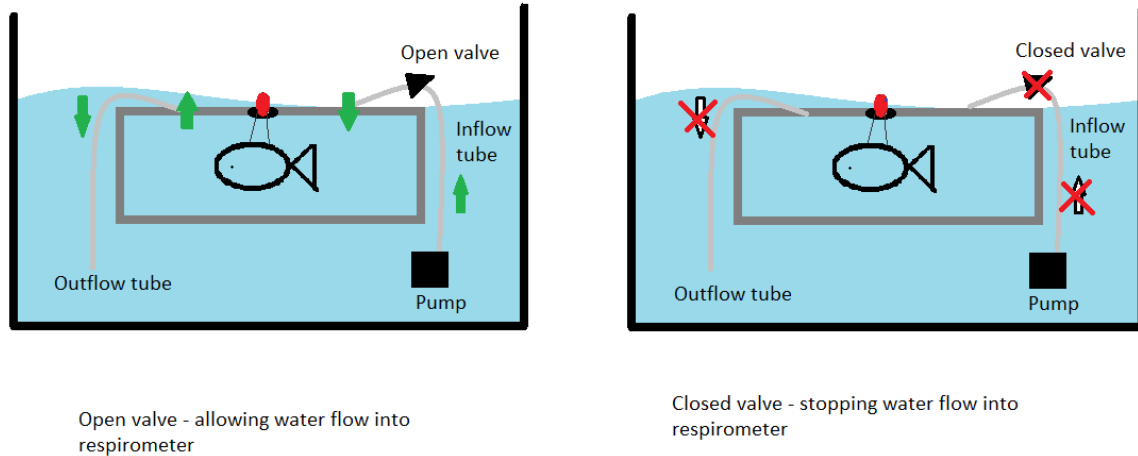
Figure 2.5*: Diagram depicting how the control valve works. When the valve is open

water is pumped into the respirometer. The water flows in from the tubing connected to

the pump and the respirometer lid and flows back out via another piece of tubing

attached to the lid to allow continuous flow through. When the valve is closed all

inflow and outflow of water ceases, causing the water inside the respirometer to be

sealed off for the duration of the experiment.

2.3.3 Calibration of Strathkelvin oxygen electrodes and Firesting

Oxygen electrodes were calibrated through PowerLab at the start of each day of

experiments and with each change in temperature, Firesting was calibrated through

Firesting software and PowerLab. The zero was achieved by using an oxygen free

sodium borate and sodium sulphite solution. The upper level was calibrated by taking

barometric pressure into account with the following equation.

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$$\mathbf{PO_{2(AS)}} = 0.2494\ (\mathbf{P_B\text{-}P_{WV}})$$

$\mathbf{PO_2}$ = partial pressure of oxygen (mmHg) in air saturated solution

$\mathbf{0.2094}$ = volumetric fraction of O_2 in the atmosphere

$\mathbf{P_B}$ = barometric pressure (mmHg)

$\mathbf{P_{WV}}$ = water vapour pressure at experiment temperature (mmHg)

2.3.4 Anaesthetic

Tricaine methanesulfonate (MS-222) was chosen as the anaesthetic for these

procedures. MS-222 is a commonly used fish anaesthetic due to its ability to readily

dissolve in water, and has been shown to allow fast recovery with minimal side effects

(Dziaman et al. 2005, Hill et al. 2004, Hill and Egginton 2010, Matsche 2011, Popovic

et al. 2012). Doses of 110mg/L were used for blue cod sedation and 95mg/ L were used

for black cod. These doses were chosen as they fully sedated fish within five minutes

exposure to reduce stress and handling. If needed, i.e if the surgery was going to be

long, fish were ventilated and kept sedated by flowing a low concentration 50 mg/L of

MS-222 across gills.

2.3.5 Surgery

The chosen fish for surgery was netted out of the holding tank in the Aquarium and

placed in a pre-determined concentration of MS-222. Within five minutes movement

and breathing would cease in the fish. The fish was removed from the anaesthetic and

placed on an operation sling. For particular long surgery sedation and ventilation was

maintained via a low pressure pump flowing low MS-222 concentration across the gills.

Two sutures were made on the fish to hold wires in place, one on the top by the front of

the dorsal fin, the other on the ventral surface of the fish located in-between the pelvic

fins. The ends of the ECG wires were stripped of their Teflon coating, bent to a 75°

angle, and hooked subcutaneously into the fish using a 25 gauge syringe needle. Wires

were positioned 2cm apart on a diagonal across the heart to provide the best voltage

readings. Wires were secured in place via the previously made sutures. Excess wire was

coiled into an Eppendorf Tube to stop the fish from becoming tangled in the wire during

recovery. Fish were left a minimum of 24 hours after surgery before conducting

experiments; this is standard protocol for use of anaesthetic in fish.

2.3.6 Respirometry experiments

Once the desired temperature was set and maintained a fish was placed in the

respirometer. The fish was left for twenty-four hours to recover from handling stress

and/or anaesthetic exposure. After twenty-four hours, the oxygen electrodes were

calibrated to the particular temperature that the experiment was undertaken at, all

bubbles (and fish excrement if any) were removed from the respirometer and the lid was

closed in place sealing the respirometer from outside air. Half an hour was given to

allow fish to recover from any stress brought about by bubble removal. Once the half

hour had passed the experiment was started. The valve was turned to closed, stopping

inflow of water into the respirometer. Recordings lasted for half an hour at each

temperature. During this time ventilation rate, heart rate and PO_2 were continuously

measured. Once the experimental time had passed the valve was turned to open so fresh

water could flush through the respirometer supplying the fish with freshly oxygenated

water. Temperature was then changed and fish was left for an hour to acclimate. After

the acclimation the experimental protocol was repeated.
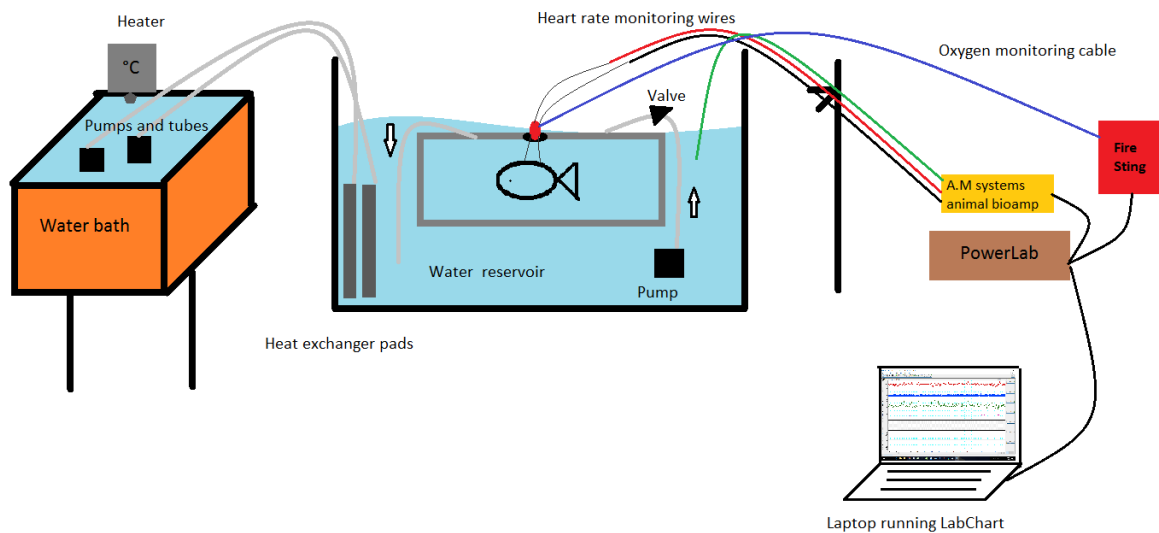
Figure 2.6: Diagram of experiment set up. On the far left is the water bath with heater

in place to heat and cool water to the desired temperatures. Pumps and heat exchanger

pads regulated the temperature of the water reservoir. The respirometer sits in the water

reservoir with inflow and outflow tubes. A pump and valve are connected to the inflow

tube to control influx of water. The fish is sealed off within the respirometer by a bung

which contains oxygen monitoring equipment (FireSting or Strathkelvin electrodes).

Heart rate wires are thread through the respirometer lid and attached to ADI animal

bioamp. FireSting and ADI animal bioamp are connected to the PowerLab which

displays the data on the laptop via the programme LabChart 7.

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2.4 Calculations and statistics

2.4.1 Metabolic rate calculation

Metabolic rates was be calculated by using the data measured in Respirometry experiments via the use of the following equation:

$$MO_2 \ (mg.O_2.kg^{-1}.h^{-1}) \ = \ (((\Delta PO_2 \ x \ C \ x \ V) \ / \ M)) \ x \ 31.99) \ x \ 3600)$$

ΔPO_2 = change in partial pressure of oxygen (mmHg) in air saturated solution (slope)

C = oxygen capacitance of seawater (μmol L^{-1} mmHg) - changes with temperature

V = volume of respirometer (L) - corrected for fish volume

M = mass of fish (g)

31.99 = molecular weight of oxygen

3600 = amount of seconds in an hour - conversion of per seconds from the slope into per hour for metabolic rate.

2.4.2 Q_{10} coefficients

Q_{10} coefficients provide insight into the effect of temperature on metabolism. The standard way to calculate Q_{10} is to expose fish to two different temperatures and calculate the change in rate. This coefficient is calculated using the following equation:

$$Q_{10} = (R_2/R_1) \ x \ (10x(T_2/T_1)$$

R_2 = Slope of metabolic rate at higher temperature

R_1 = Slope of metabolic rate at lower temperature

T_1 = Upper temperature

T_2 = Lower temperature

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10 = To account for 10°C if calculation does not involve a 10°C temperature change

2.4.3 Statistics and Data Storage

All data was stored in Excel or as a .txt, .csv files. Raw data was obtained from experimental read out on AD instruments LabChart 7 (7.3.7). All statistics was carried out on R-Studio (V. 0.99.902, 64 bit for Windows 10) or Excel (V. Microsoft Office 2013).

 Analysis of variance (ANOVA) with repeated measures was used to examine the statistical significance of data and the variation between groups. Data was analysed to view the difference between the control acclimation of 12°C, and experimental acclimations of 8 °C and 18 °C across acute temperature exposure. Tukey HSD tests were used to determine which data values were statistically significant. Data was tested for sphericity and homogeneity to ensure the data fit ANOVA parameters. Linear regression was used to model the effect of temperature on rates, these values can be found in table 2 and graphs are in appendices. A linear model was used, rather than exponential, as temperature range was small. All graphs were created in R-Studio or Excel with standard deviation and statistical significance as indicated by *.

2.4.4 Animal Ethics

Animal ethics was approved before any fishing or experiments were carried out on the fish. Animal ethics number is 2015/04R; this covered catching and experimentation on black cod, blue cod and emerald cod.

Chapter 3.

Results

Many fish species can modulate their biochemistry and physiology in response to environmental temperature change. The processes by which this occurs and the magnitude of the change differs between species. Ventilation rate, heart rate and oxygen consumption have been determined as the most convenient and relevant way to view these changes. By looking at these rates we can understand the effect temperature change, in particular climate change, may have on individual species. I viewed the effect of temperature on these rates in three different species of cod.

Physiological rates were determined for black cod, blue cod, and emerald cod individuals. Rates were measured continuously for half an hour during an acute temperature exposure respirometry experiment. Chronic and acute temperature experiments were conducted, 12°C was used as a control chronic exposure for black and blue cod.

Chronic and acute acclimation temperatures differed between species due to their differing natural habitats. Black cod are a cold water dwelling species living in environments ranging from sub-Antarctic waters, to the lower south of New Zealand (Paul et al. 1993). Due to their narrow temperature range this species was exposed to chronic temperature exposure at 8°C (n=3) and 12°C (n=4), with 12°C acting as the control temperature. After chronic exposure to 8°C fish were exposed to acute temperature changes ranging from 4-14°C. These fish were unable to survive exposure to temperatures lower than 4°C and higher than 14°C, stressed behaviour such as rapid movement and loss of the ability to keep upright was observed. Black cod acclimated to

12°C were acutely exposed to 6-18°C. These fish were unable to survive exposure to temperatures lower than 6°C and higher than 18°C, stressed behaviour such as rapid movement and loss of the ability to keep upright was observed, in one case one of the 8°C acclimated fish acutely exposed to 16 °C vomited and defecated.

Blue cod are found all around New Zealand at temperatures ranging from 8-22°C, tolerating a wider range of temperature than the black cod. The lowest blue cod were acclimated to was 8°C (n=4) with exposure to acute temperature changes ranging from 6-16°C. As a control acclimation blue cod were acclimated to 12°C (n=8) and were exposed to 6-20°C. Blue cod were also acclimated to a warmer temperature of 18°C (n = 4) and exposed to a larger range of acute temperatures 10-24°C.

Emerald rock cod are an Antarctic species, living in stable waters of around -1.8°C. The emerald rock cod used in this study were acclimated to 2°C during a year of captivity. Due to their cold water nature these fish were exposed to acute changes ranging from 0–8°C. When exposed to 8°C, only four fish were able to tolerance exposure. Species and temperature exposures is summarised in the table below, table 1.

Species	Acclimated temperature (°C)	Acute exposure temperature (°C)
Blue cod	8 n = 4	6-16 (n = 3 at 6 and 16)
	12 n = 10	8 - 20 (n = 3 at 8 and 20)
	18 n = 4	10 - 24 (n= 2 at 22 and 24)
Black cod	8 n = 3	4 - 14 (n = 2 at 14)
	12 n = 4	6 - 18 (n = 3 at 18)
Emerald rock cod	2 n = 6	0 - 8 (n = 4 at 8)

Table 1: Summary of species temperature exposures; all acute temperature exposures contained the same number of fish as chronic exposures, unless stated otherwise.

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Physiological measurement	Blue cod (8C)	Blue cod (12C)	Blue cod (18C)	Black cod (8C)	Black cod (12C)	Emerald rock (2C)
Heart rate	0.9291	0.8522	0.9638	0.9845	0.8703	-
Ventilation rate	0.8794	0.9852	0.9594	0.9729	0.9255	0.9746
Oxygen consumption	0.9556	0.9208	0.9349	0.9635	0.982	0.9411

Table 2: R_2 linear regression values - graphs in Appendices

3.1 Ventilation rate

Black cod of similar size 35–37 cm, 1400 g, blue cod of similar size 17–20 cm, 180 g, and emerald cod of similar size 12-16cm, 80 g, were exposed to acute temperature changes determined by their acclimation temperature. Ventilation rate measured of individuals for a period of half an hour after an hour temperature exposure time.

Exposure temp (°C)	Blue cod (8°C)	Blue cod (12°C)	Blue cod (18vC)	Black cod (8°C)	Black cod (12°C)	Emerald rock (2°C)
0	-	-	-	-	-	14 ± 1.6
2	-	-	-	-	-	16 ± 1.9
4	-	-	-	19 ± 1.6	-	18 ± 2.6
6	21 ± 1.5	23 ± 1.9	-	20 ± 2.5	33 ± 2.2	24 ± 5.3
8	24 ± 6.3	25 ± 2.9	-	24 ± 3.1	28 ± 5.7	27 ± 2.6
10	32 ± 8.9	26 ± 10.1	19 ± 1.3	26 ± 4.2	32 ± 4.5	-
12	37 ± 12.4	29 ± 9.0	20 ± 3.7	31 ± 4.9	37 ± 3.5	-
14	37 ± 11.1	34 ± 7.3	26 ± 3.0	38 ± 5.4	38 ± 5.0	-
16	47 ± 14.7	38 ± 11.6	33 ± 1.0	-	42 ± 4.2	-
18	49 ± 8.1	41 ± 7.4	38 ± 4.1	-	52 ± 5.9	-
20	-	45 ± 7.5	42 ± 5.5	-	-	-
22	-	-	45 ± 3.5	-	-	-
24	-	-	50 ± 6.3	-	-	-

Table 3: Ventilation rate across acute temperature exposure SEM.

Exposure temp (°C)	Blue cod (8°C)	Blue cod (12°C)	Blue cod (18°C)	Black cod (8°C)	Black cod (12°C)	Emerald rock (2°C)
0-2	-	-	-		-	2.3
2-4		-				1.9
4-6			-	1.3		1.8
6-8	1.9		-	2.5	1.2	4.2
8-10	4.2	1.5		1.5	0.4	1.8
10-12	2.1	1.7	1.3	2.4	1.9	-
12-14	1	2.2	3.7	2.8	1.1	-
14-16	3.3	1.7	3.3	-	1.6	-
16-18	1.2	1.5	2	-	2.9	-
18-20	-	1.6	1.6	-	-	-
20-22	-	-	1.4	-	-	-
22-24			1.7			
Overall	2	1.6	2	2	1.5	2.3

Table 4: Q_{10} values across acute temperature exposure.

3.1.1 Overall species variation

Ventilation rate was strongly influenced by acute temperature exposure. All species showed show an exponential increase in ventilation rate correlating to an increase in temperature. Figures 3.1, 3.2 and table 3 show with each temperature increase there is a corresponding increase in ventilation rate. In a few cases some fish were left out of experiment at certain temperatures as they could not tolerate the temperature exposure, refer to table 1 for n values. The lowest ventilation rate, 14 ± 1.6 for emerald rock cod, occurred at lower temperatures and the highest rate at higher temperatures, 52 ± 5.9 breaths min^{-1} in 12 °C black cod.
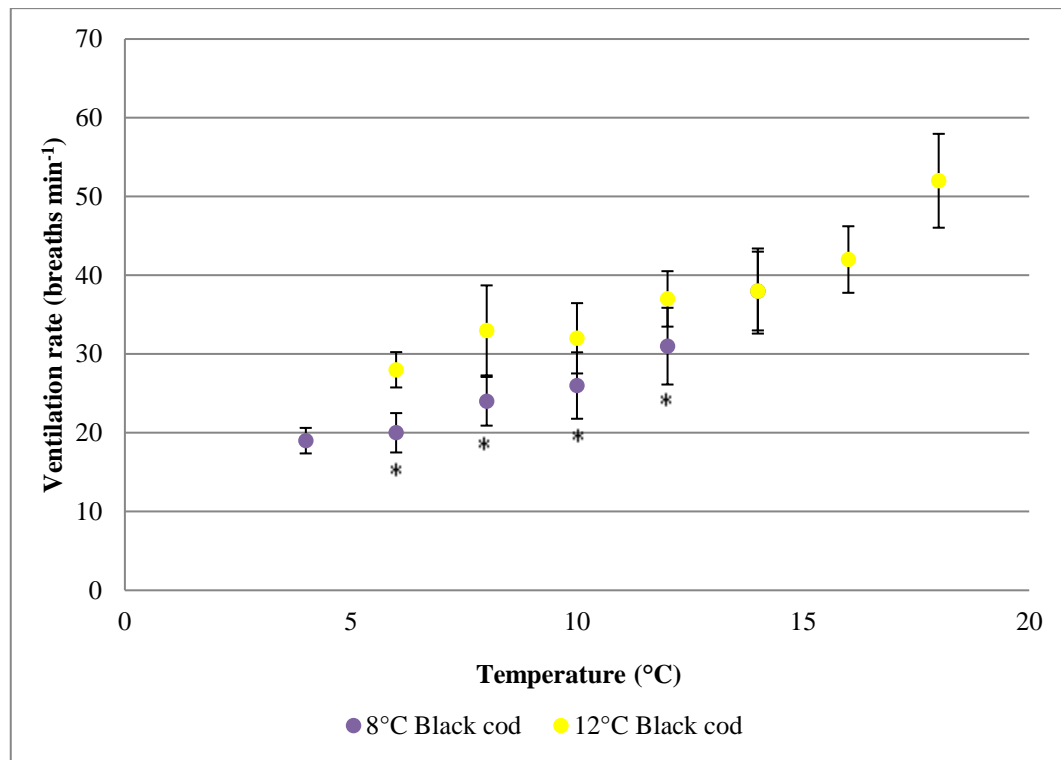
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Figure 3.1: Black cod 8°C and 12°C acclimated ventilation rate across acute

temperature changes ranging from 4-18°C. Significant differences in ventilation rates at

lower exposure temperatures. Mean values plotted with SEM, significant differences
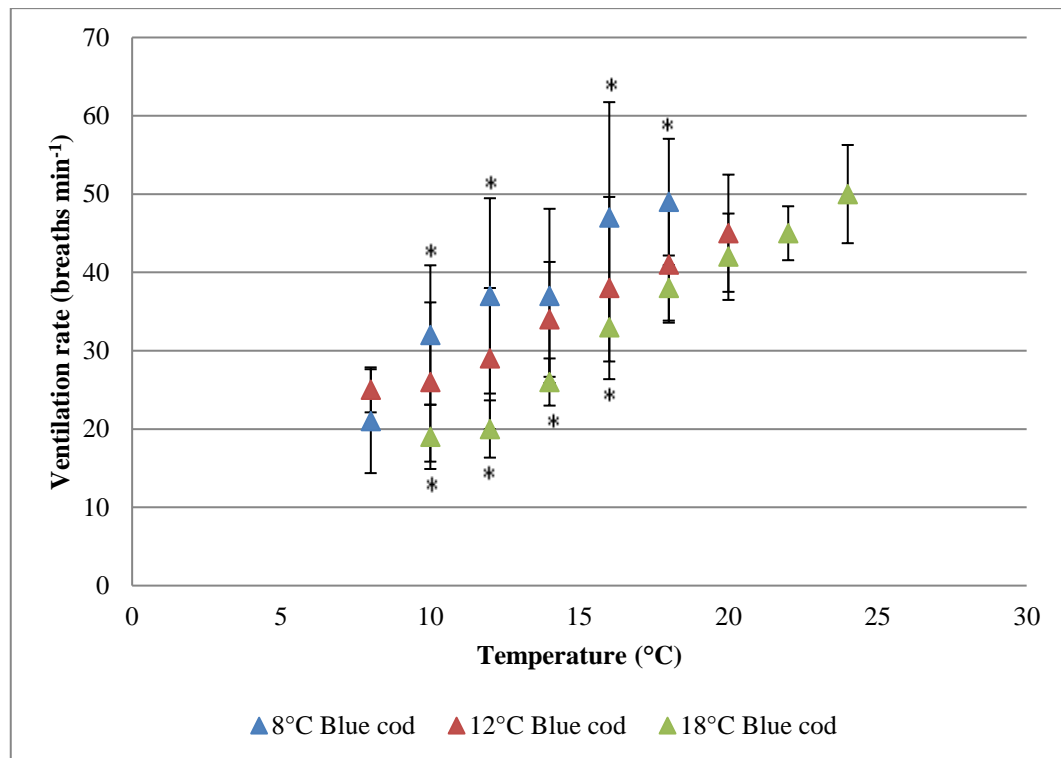
marked with *.

Figure 3.2: Blue cod 8°C, 12°C and 18°C ventilation rate across acute temperature changes ranging from 6-24°C. Significant change for 18°C acclimation and at most temperatures for 8°C acclimated fish. Mean values plotted with SEM, significant differences marked with *.

3.1.2 Within species variation

Acclimation temperature had an effect on the range of temperatures black cod could acutely be exposed to (table 3, fig 3.1). Warmer acclimated (12°C) fish lost the ability to survive in cold water (8°C and below), but were able to tolerate warm water. There is variation between ventilation rates, $p > 0.05$, 12°C acclimated has smaller increases in rate at lower temperatures (Q_{10} 1.2, 0.4, table 4) compared to 8°C which have a larger increase (Q_{10} 2.5, table 4). Both acclimations show a larger increase in rate at higher temperatures (Q_{10} 2.8, 2.9, table 4). Resting ventilation rate at acclimated temperature

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was very different for both acclimations, 24 ± 4.2 breaths min^{-1} for 8°C and 37 ± 3.5 breaths min^{-1} for 12°C suggesting the 8°C acclimated fish were not acclimated even though a significant difference was seen at lower rates.

As with black cod, acclimation influenced the range of acute temperatures that blue cod could be exposed to (table 3, figure 3.2). Resting ventilation rate was influenced by acclimation temperature (figure 3.2), showing a downwards shift with increase in acclimation temperature, $p > 0.05$. Resting heart rates 8°C and 12°C acclimated fish were within a similar range strengthening suggesting these fish show partial to complete compensation according to Precht's model (Precht 1955, Schmidt Nielsen 1979). At low exposure temperatures, those fish acclimated to 8°C and 18°C showed a rapid decrease in ventilation rate with a trailing off (Q_{10} of 4.2, table 4) strengthen the suggesting that systems were failing at lower temperatures. Emerald rock cod experienced large increase in rate at lower temperatures (Q_{10} 2.3, table 4) and higher exposure temperatures (Q_{10} 4.2, table 4) followed by a decrease (Q_{10} 1.8, table 4) suggesting these fish were also starting to experience failure.
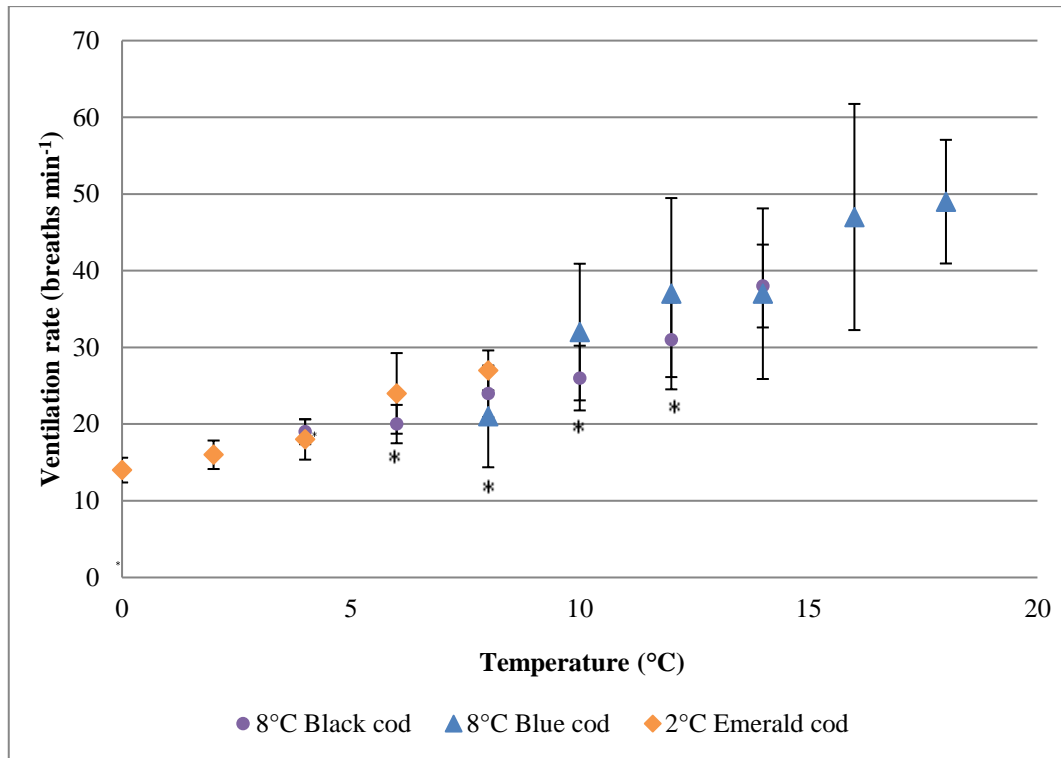
Figure 3.3: Comparison of cooler acclimations. Black cod and blue cod 8°C acclimated ventilation rate across acute temperature changes ranging from 4-16°C. Emerald rock cod 2°C acclimated ventilation rate differences across acute temperature changes ranging from 0-8°C. Mean values plotted with SEM, significant differences marked with *.
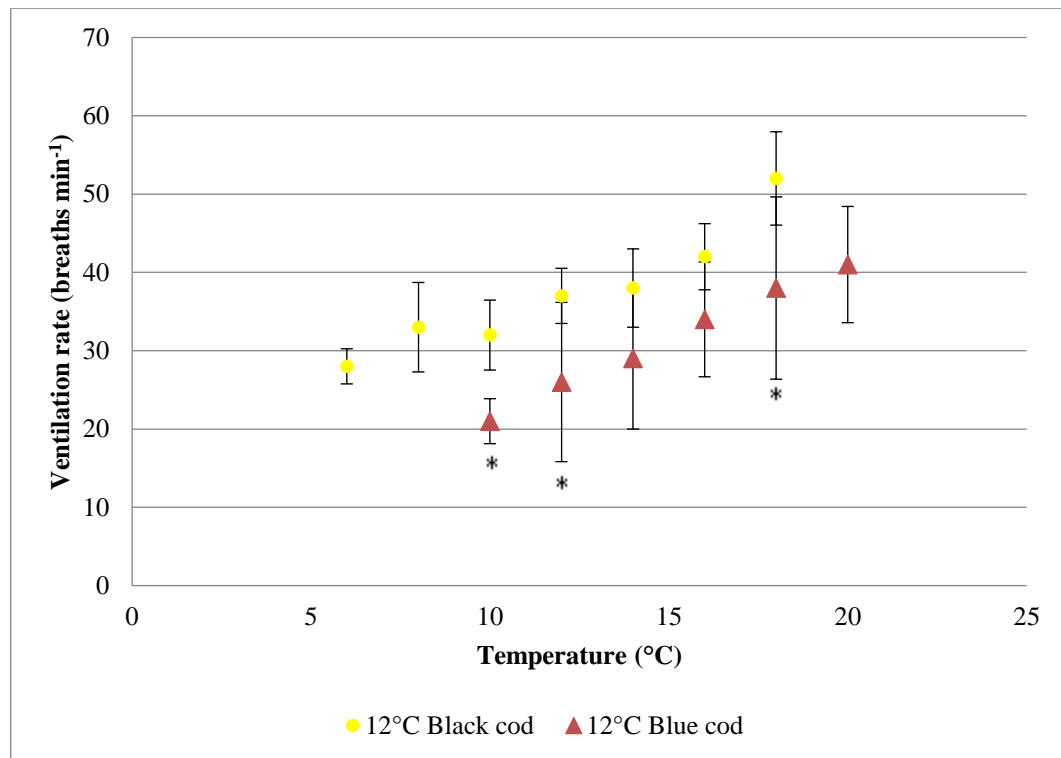
Figure 3.4: Comparison of warm acclimations, 12°C black cod and 12°C blue cod across acute temperature changes ranging from 6-24°C. Mean values plotted with SEM, significant differences marked with *.

3.1.3 Between species variation

All low chronic temperature acclimations showed a similar overall influence of temperature on ventilation rate (Q_{10} 2.0 ± 0.3), $p > 0.05$. Significant differences were seen in lower exposure temperatures between black and blue cod, $p > 0.05$. Blue cod experienced a larger drop off in rate (Q_{10} 4.2 to 1.9) at lower temperatures than black cod (Q_{10} 2.5 to 1.3). Blue and emerald rock cod struggled more at warmest exposure temperatures showing drop offs in rate, whereas black cod rate was still increasing suggesting these fish could potentially tolerate a greater increase in temperature (figure 3.4). Emerald rock cod maintained a higher rate, $p > 0.05$, than the other two species at

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the same exposure temperatures, this is due to these Antarctic fish showing metabolic cold adaptation.

Warmer acclimated (12°C) blue and black cod also showed a similar overall influence of temperatures on rates (Q_{10} 1.6 ± 0.4), p > 0.05. Black cod experienced a large fluctuation in rates, with a large increase at higher temperatures (Q_{10} 2.9) and a drop off at lower temperatures (Q_{10} 0.4), supporting that these fish were starting to fail. In comparison blue cod ventilation rate stayed relatively stable, and did not experience larger fluctuations suggesting blue cod ventilation rate was slightly less influenced by temperature (Q_{10} 1.6), however this is not a large enough difference to say this is the overall trend.

3.2 Heart Rate

Black cod of similar size 35–37 cm, averaging 1400 g were exposed to acute temperature changes determined by their acclimation temperature. Blue cod of similar size 17-20c m, average of 180 g, were exposed to acute temperature changes determined by their acclimation temperature. Heart rate was measured of individuals for a period of half an hour after an hour temperature exposure time. Heart rate was not determined for emerald cod.

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Exposure temp (°C)	Blue cod (8°C)	Blue cod (12°C)	Blue cod (18°C)	Black cod (8°C)	Black cod (12°C)
4	—	-	-	29 ± 1.6	-
6	30 ± 3.8	14 ± 2.8	-	30 ± 1.7	35 ± 1.9
8	32 ± 7.8	26 ± 1.5	—	32 ± 2.0	31 ± 4.4
10	37 ± 7.1	35 ± 1.0	25 ± 3.9	33 ± 2.5	36 ± 2.9
12	37 ± 6.5	39 ± 10.2	26 ± 6.6	38 ± 3.7	38 ± 6.0
14	50 ± 10.1	39 ± 5.1	29 ± 3.4	41 ± 4.1	44 ± 6.2
16	56 ± 10.9	45 ± 10.8	34 ± 3.9	-	48 ± 5.2
18	-	53 ± 7.1	38 ± 4.8	-	58 ± 2.6
20	-	57 ± 2.4	47 ± 5.1	-	-
22	-	-	45 ± 4.7	-	-
24	-	-	51 ± 5.3	-	-

Table 5: Mean heart rate across acute temperature exposure with SEM.

Exposure temp (°C)	Blue cod (8°C)	Blue cod (12°C)	Blue cod (18°C)	Black cod (8°C)	Black cod (12°C)
4-6	-	-	-	1.2	-
6-8	1.4	-	-	1.4	0.5
8-10	2.1	4.4	-	1.2	2.1
10-12	1	1.7	1.2	2	1.3
12-14	4.5	1	1.7	1.5	2.1
14-16	1.8	2	2.2	-	1.5
16-18	-	2.3	1.7	-	2.6
18-20	-	1.4	2.9	-	-
20-22	-	-	0.8	-	-
22-24	-	-	1.9	-	-
Overall	1.9	1.9	1.7	1.4	1.5

Table 6: Q_{10} (rate of change) values across acute temperature exposure.

3.2.1 Overall Variation

Heart rate was strongly influenced by acute temperature exposure for all species and

acclimations, with all species showing an increase with temperature, the strength of this

relationship is reflected in linear regression values (table 2). Figures 3.5, 3.6 and table 5

show with each temperature increase there is a corresponding increase in heart rate.

This is also reflected in linear regression values, table 5. In a few cases some fish were

left out of experiment at certain temperatures as they could not tolerate the temperature

exposure. Black cod acclimated to 12°C struggled with acute temperature exposure to

18°C, which resulted in only two fish being taken up to this temperature (figure 3.5).

Blue cod acclimated to 12°C struggled with acute temperature exposure to 6°C, which

resulted in only two fish being taken to this low temperature (figure 3.6). Blue cod

acclimated to 8°C struggled at 14°C resulting in only two fish being taken this high. As

expected the lowest heart rates, 14 ± 2.8 beats min^{-1} in 12°C blue cod, occurred at lower

temperatures, and the highest rates at the highest temperatures, 58 ± 2.6 beats min^{-1} in

12°C black cod.

Both acclimations show a lower increase in rate at higher temperatures (Q_{10} 2.8, 2.9,

table 6) in comparison to with ventilation rate (Q_{10} 1.5, table 6) indicating that

acclimation temperature had more of an effect on ventilation rate. This indicates

ventilation rate is more flexible than heart rate at warmer temperatures and is able to

exceed and maintain higher rates, suggesting that heart rate is the first physiological rate
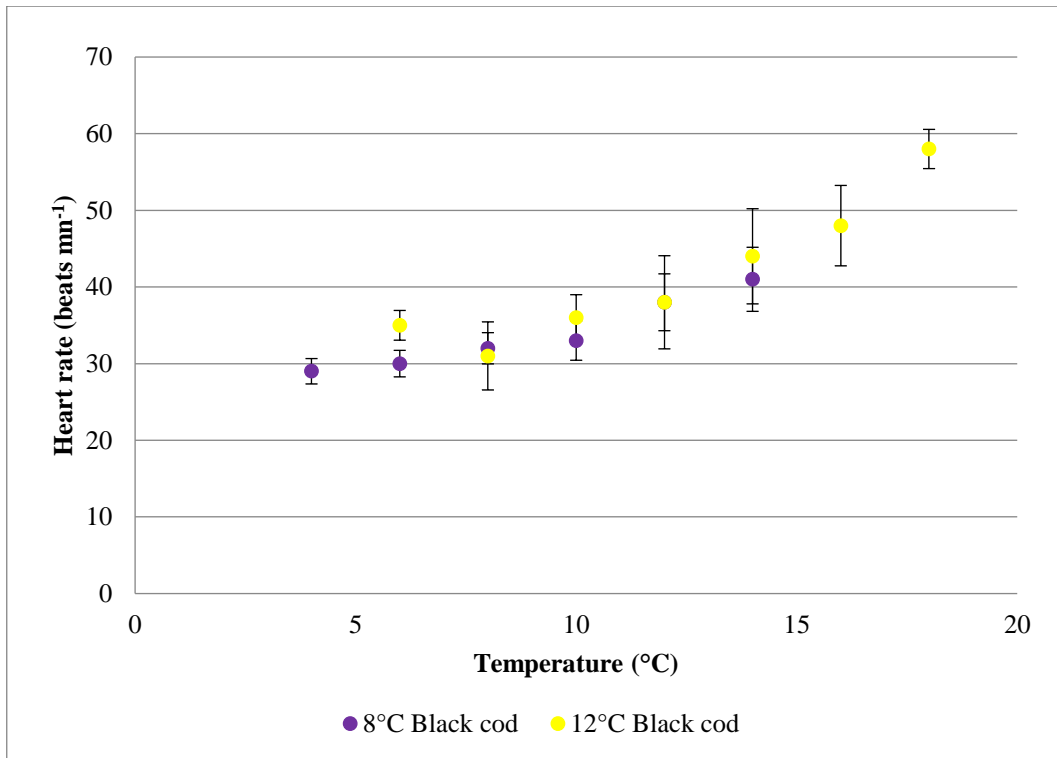
to fail.

Figure 3.5: Black cod 8°C and 12°C acclimated heart rate across acute temperature changes ranging from 4-18°C. Mean values plotted with SEM, significant differences marked with *.
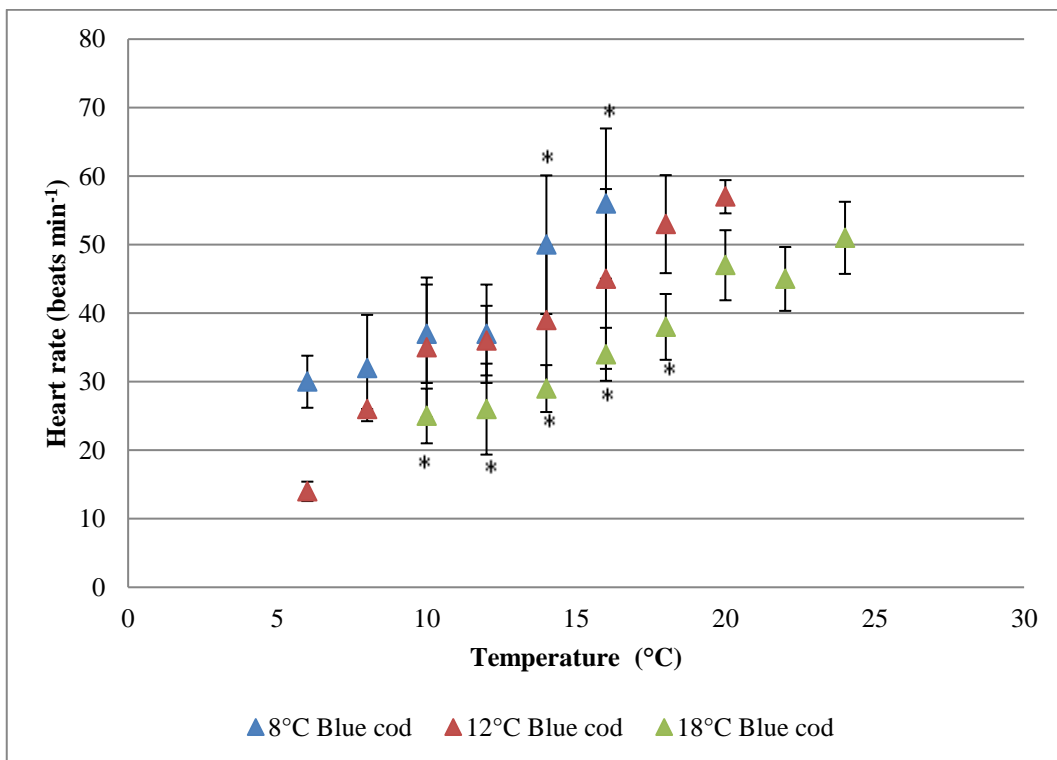
Figure 3.6: Blue cod 8°C, 12°C and 18°C heart rate across acute temperature changes ranging from 6-24°C. Significant change for 18°C acclimation and higher temperatures for 8°C acclimated fish. Mean values plotted with SEM, significant differences marked with *.
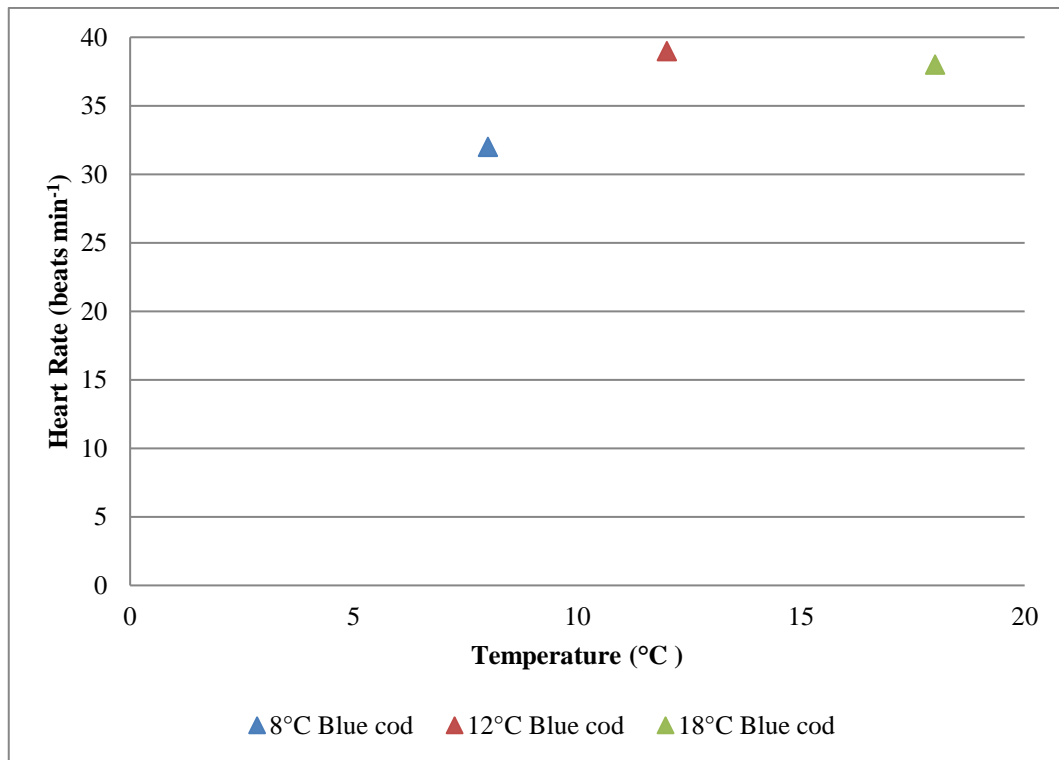

Figure 3.7: Blue cod 8°C, 12°C and 18°C resting heart rate at acclimation temperature.

3.2.2 Within species variations

Acclimation temperature had an effect on the range of temperatures that black cod could be acutely exposed to (table 4, figure 3.5). Warmer acclimated (12°C) fish lost the ability to survive in cold water (8°C and below), but were able to tolerate warm water. Acclimation temperature had no effect on resting heart rate at any acute exposure

temperature (P = 0.9). Black cod heart rate at its lowest was 29 ± 1.6 beats min^{-1} and at its highest 58 ± 2.6 beats min^{-1}.

As with black cod, acclimation influenced the range of acute temperatures that blue cod could be exposed to (table 5, figure 3.6).However unlike black cod, blue cod resting heart rate was influenced by acclimation temperature (figure 3.6), showing a downwards shift with increase in acclimation temperature, $p > 0.05$. A plot of resting heart rate against acclimation temperature (figure 3.7) showed partial to complete compensation according to Precht's model (Precht 1955, Schmidt Nielsen 1979). At high acute temperatures for 12°C and 18°C acclimated fish the increase in resting heart rate dropped off, as indicated by the small Q_{10} values (table 6), signalling that systems were failing. Interestingly this drop off of heart rate occurred at a higher temperature 22 °C for 18°C acclimated animals, 14°C for 8°C (table 6). At low exposure temperatures, those fish acclimated to 12°C showed a rapid decrease in heart rate (Q_{10} of 4.4, table 6) suggesting failing systems. Blue cod heart rate at its lowest was 14 ± 2.8 beats min^{-1}, and 57 ± 2.4 beats min^{-1} at its highest.
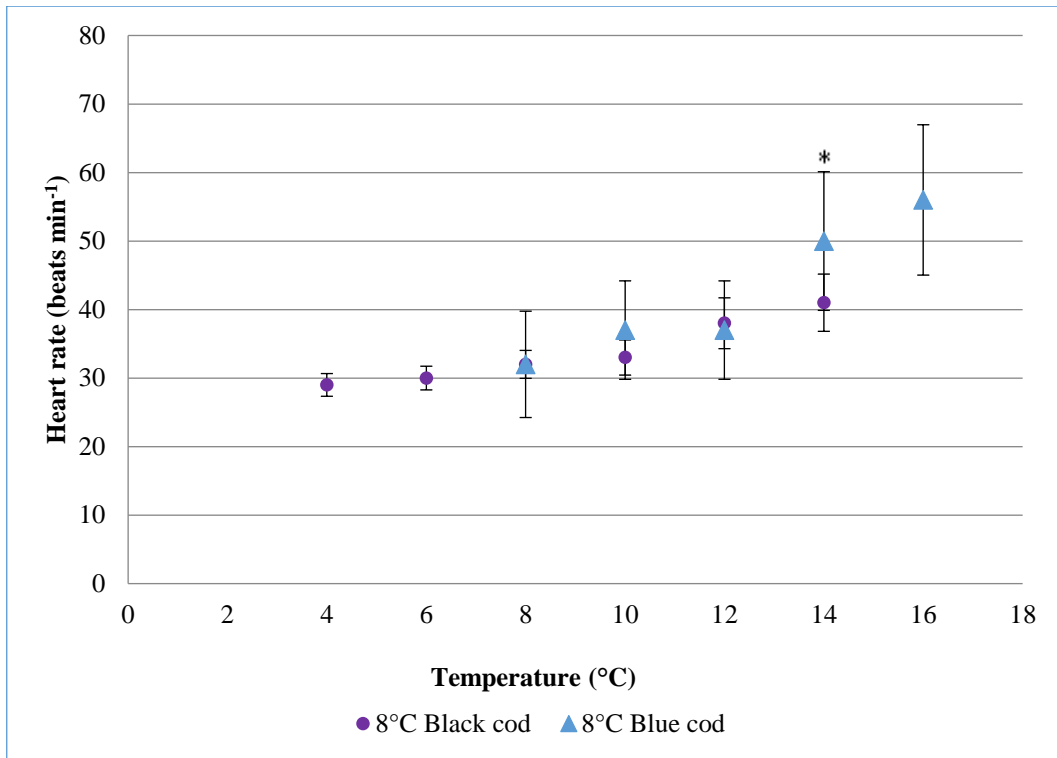
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Figure 3.8: Comparison on cooler acclimations. 8°C Black cod and blue cod heart rate differences across acute temperature changes ranging from 4-16°C. Mean values plotted with SEM, significant differences marked with *.
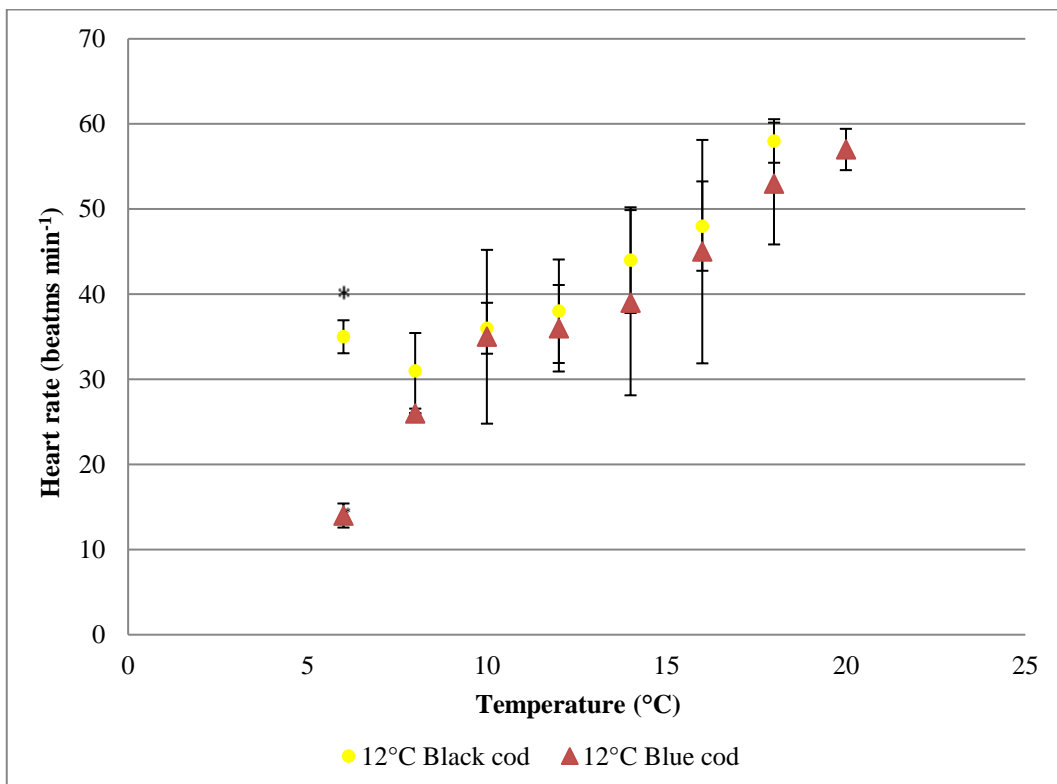
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Figure 3.9: Comparison of warm acclimations, 12°C black cod 12°C blue cod across acute temperature changes ranging from 6-24°C. Mean values plotted with SEM, significant differences marked with *.

*3.2.3 B*etween species variation

Both species acclimated to 8°C showed similar heart rates (figure 3.8), rates at acclimation temperature were the same for both species, 32 ± 7.8 beats min^{-1} (table 4). Blue cod acclimated to 8°C were able to tolerate warmer temperatures, whereas black cod were able to tolerate lower temperatures, this reflects the differences of their natural environments.

Overall back cod and blue cod maintained similar heart rates when acclimated to 12°C and exposed to the same acute temperatures, $p < 0.05$ (figure 3.9). However, at lower exposure temperatures the two species behaved differently, with black cod becoming active and blue cod becoming very sluggish causing a significantly higher rate for black cod than blue cod at the same acute exposure temperature $p > 0.05$, as reflected in figure 3.9. Resting heart rates at acclimation temperature, 12°C, were similar for both species, 38 ± 1.6 beats min-^{1} for black cod and 39 ± 1.7 beats min^{-1} for blue cod.

3.3 Oxygen consumption

Black cod of similar size 35-37cm, 1400 g, blue cod of similar size 17-20cm, 180 g, and emerald rock cod of similar size 12-16cm, 80 g, were exposed to acute temperature changes determined by their acclimation temperature. PO_2 was measured of individuals

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for a period of half an hour after an hour temperature exposure time, oxygen

consumption was then calculated.

Exposure temp (°C)	Blue cod (8°C)	Blue cod (12°C)	Blue cod (18°C)	Black cod (8°C)	Black cod (12°C)	Emerald rock (2°C)
0	-	-	-	-	-	10 ± 2.5
2	-	-	-	-	-	24 ± 2.4
4	-	-	-	11 ± 1.2	-	48 ± 4.6
6	14 ± 3	-	-	21 ± 6.1	28 ± 3.5	61 ± 9.2
8	18 ± 4.5	15 ± 1.5	-	28 ± 5.0	31 ± 4.9	88 ± 6.1
10	45 ± 25.3	28 ± 9.7	19 ± 5.7	38 ± 5.1	32 ± 0.8	-
12	68 ± 19.1	47 ± 18.6	28 ± 4.8	54 ± 12.8	36 ± 6.3	-
14	88 ± 19.7	86 ± 9.8	70 ± 28.1	63 ± 14.3	41 ± 11.8	-
16	158 ± 37.9	123 ± 8.3	111 ± 24.8	-	43 ± 9.3	-
18	-	161 ± 52.9	143 ± 12.6	-	50 ± 10.6	-
20	-	144 ± 51.0	190 ± 32.1	-	-	-
22	-	-	227 ± 0.0	-	-	-
24	-	-	292 ± 53.8	-	-	-

Table 7: Oxygen consumptions across acute temperature exposure, means with SEM

Exposure temp (°C)	Blue cod (8°C)	Blue cod (12°C)	Blue cod (18°C)	Black cod (8°C)	Black cod (12°C)	Emerald rock (2°C)
0-2	-	-	-	-	-	15.2
2-4	-	-	-	-	-	79.6
4-6	-	-	-	25.4	-	32
6-8	3.5	-	-	4.2	1.7	20
8-10	97.7	22.7	-	4.6	1.2	6.2
10-12	7.9	13.3	7	5.8	1.8	-
12-14	3.6	20.5	97.7	2.2	1.9	-
14-16	18.6	6	10	-	1.3	-
16-18	-	3.8	3.5	-	2.1	-
18-20	-	0.6	4.1	-	-	-
20-22	-	-	2.4	-	-	-
22-24	-	-	3.5	-	-	-
Overall	11.3	6.5	7.0	5.7	1.6	15.2

Table 8: Q_{10} coefficients values across acute temperature exposure.

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3.3.1 Overall variation

Oxygen consumption is strongly influenced by acute temperature exposure for all species and acclimations, all species show an increase with temperature. Figures 3.10, 3.11 and table 7 show with each temperature increase there is a corresponding increase in oxygen consumption. As expected the lowest rates, 10 ± 2.5 mg.O_2.kg^{-1}.h^{-1} in 12 °C blue cod, occurred at lower temperatures, and the highest rates at the highest temperatures, 292 ± 53.8 mg.O_2.kg^{-1}.h^{-1} in 12 °C black cod. Of interest Q_{10} value recorded from these experiments are extremely high at lower and upper exposure temperatures, with some 10 times the Q_{10} values seen in similar experiments, suggesting these fish were facing enormously high stress levels indicating they were reaching their lower and upper critical temperatures.
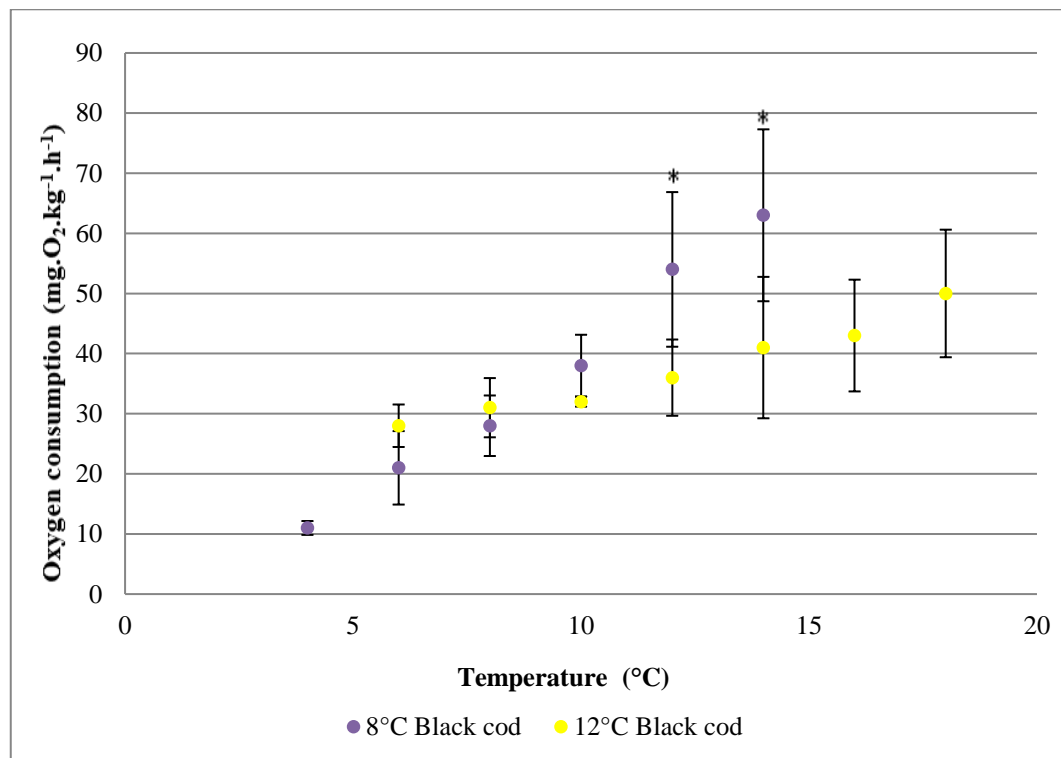
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Figure 3.10: Black cod 8°C and 12°C acclimated oxygen consumption across acute temperature changes ranging from 4-18 °C. Mean values plotted with SEM, significant differences marked with *.
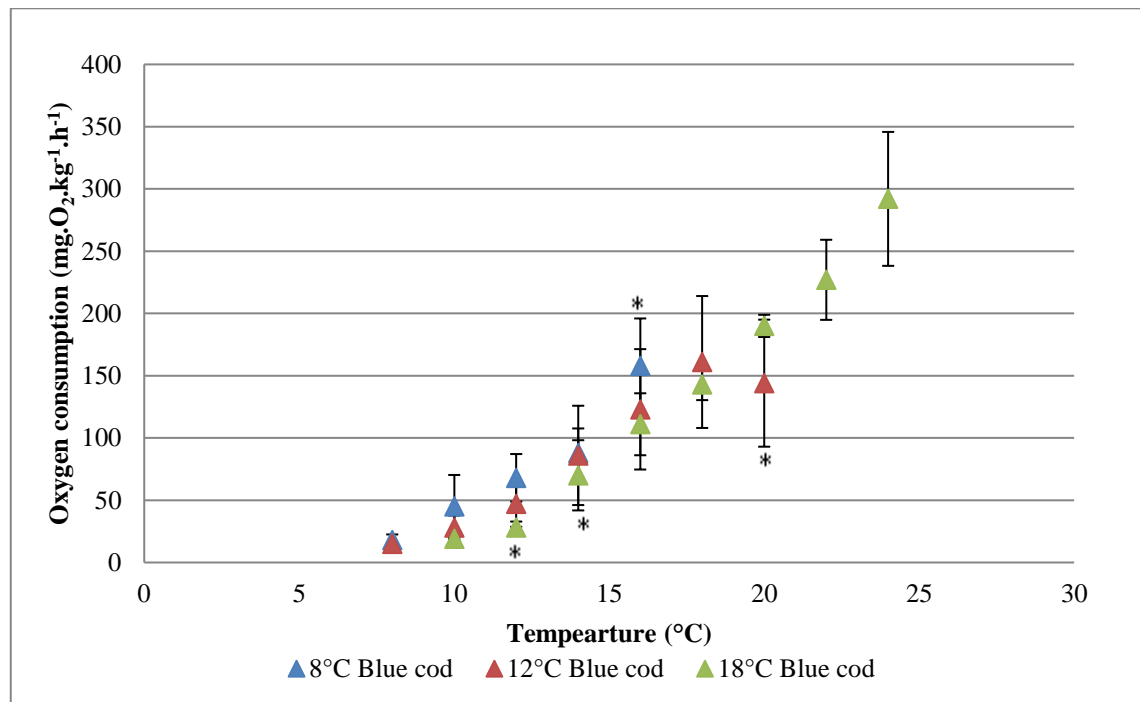

Figure 3.11: Blue cod 8°C, 12°C and 18 °C oxygen consumption across acute temperature changes ranging from 6 -24°C. Significant change for 18°C acclimation and at most temperatures for 8°C acclimated fish. Mean values plotted with SEM, significant differences marked with *.

3.3.2 Within species variation

Acclimation temperature had an effect on the range of temperatures that black cod could be acutely exposed to and influenced resting oxygen consumptions (table 7, fig 3.10).

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Black cod acclimated to lower temperature showed a larger influence of temperature on rate (Q_{10} 5.6, table 8) over acute exposure than those acclimated to a warmer temperature (Q_{10} 1.6, table 8) (figure 3.10). This indicates that 12°C acclimated fish oxygen consumption was less influenced by temperature and that these fish were able to better tolerate acute temperature exposures. Whereas cooler acclimation fish showed rapid drop off in rates at cooler exposure temperatures (Q_{10} 25.4, figure 3.12) and at higher exposure temperatures showed a reduction in the increase of rate (Q_{10} 5.8 to 2.2) suggesting these fish were chronically failing and longer exposure may have resulted in death. Black cod oxygen consumption at its lowest was 11 ± 1.2 mg.O_2.kg^{-1}.h^{-1}, and 63 ± 14.3 mg.O_2.kg^{-1}.h^{-1} at its highest.

As with black cod, acclimation influenced the range of acute temperatures that blue cod could be exposed to (table 7, figure 3.11). Again rate was influenced by acclimation temperature (figure 3.11), showing a downwards shift with increase in acclimation temperature, $p < 0.05$. For all acclimations the increase in resting oxygen consumption dropped off at higher temperatures, as indicated by the smaller Q_{10} values (table 8), signalling that systems were failing. At low exposure temperatures, all acclimations showed a rapid decrease in oxygen consumption as indicated by large Q_{10} values (table 8) again supporting that systems were failing. Blue cod oxygen consumption at its lowest was 14 ± 3 mg.O_2.kg^{-1}.h^{-1}, and 292 ± 53.8 mg.O_2.kg^{-1}.h^{-1} at its highest.
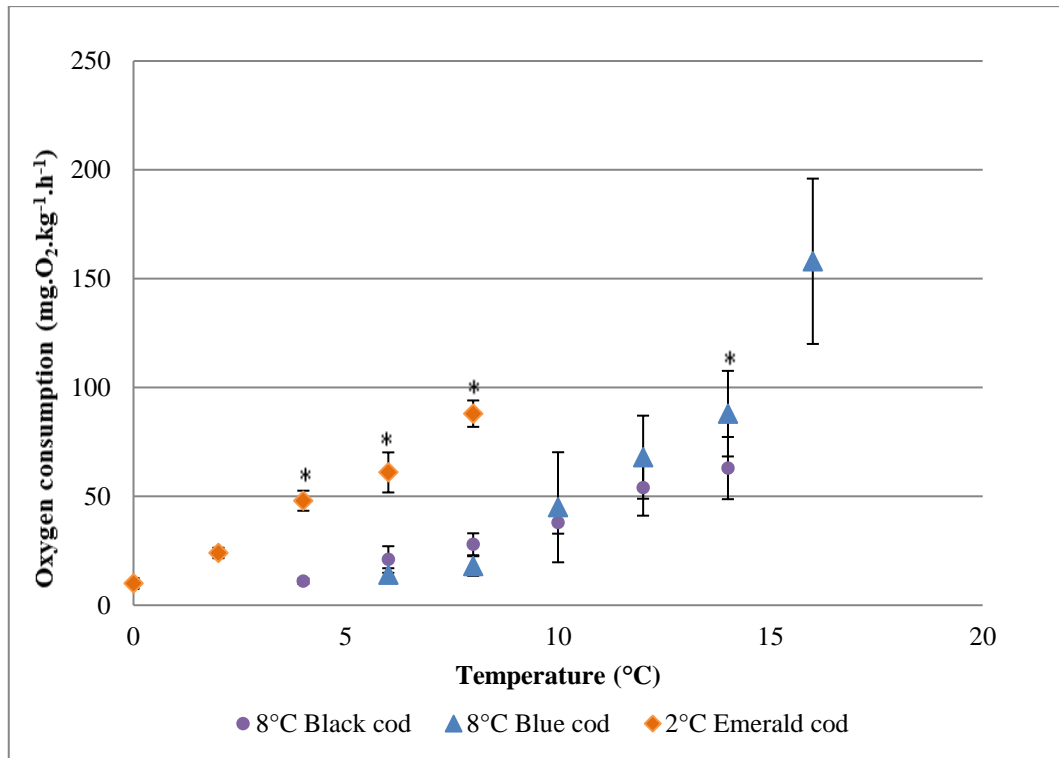
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Figure 3.12: Comparison on cooler acclimations. 8°C black cod and blue cod oxygen

consumption across acute temperature changes ranging from 4-16°C. Emerald rock cod

2°C acclimated oxygen consumption changes across acute temperature changes ranging

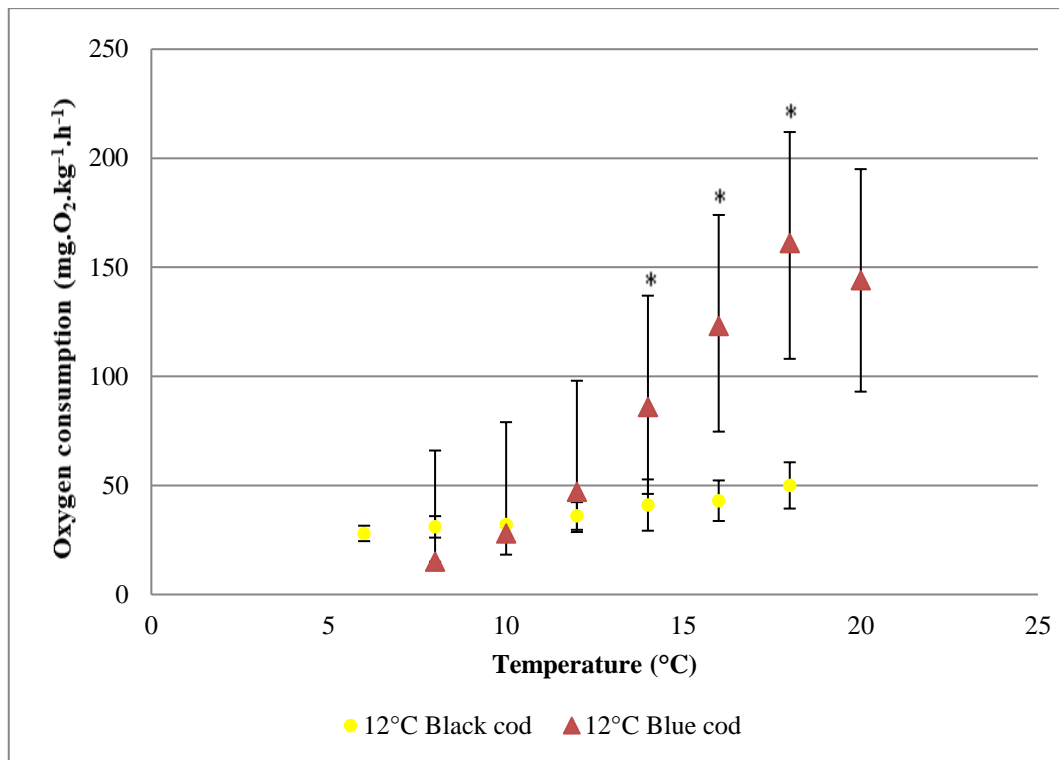from 0-8°C. Mean values plotted with SEM, significant differences marked with *.

Figure 3.13: Comparison of warm acclimations, 12°C Black cod and 12°C blue cod across acute temperature changes ranging from 6-24 °C. Mean values plotted with SEM, significant differences marked with *.

3.3.3 Between species variation

Both species acclimated to 8°C showed similar oxygen consumptions at lower temperatures (figure 3.12). Both species showed rapid drop off in rates at lower temperatures as indicated by large Q_{10} values (table 7), however rates at warmer temperatures varied, with black cod increase in rate dropping off (Q_{10} 5.8 to 2.2) whereas blue cod rate continued to increase (Q_{10} 18.6, figure 3.13). Emerald cod also showed a marginal drop off in increase at warmer temperatures, suggesting failure. Emerald rock cod showed higher resting oxygen consumptions at the same exposure temperatures as the other species perfectly showing metabolic cold adaptation.

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Interestingly black cod showed higher oxygen consumptions than blue cod at the same lower exposure temperatures.

Black cod and blue cod showed a significant difference in rates when acclimated to 12°C and exposed to the same acute temperatures, $p > 0.05$ (figure 3.13). Blue cod oxygen consumption showed a larger increase in resting heart rate drop off, as indicated by the small Q_{10} values (table 8), signalling that systems were failing. Whereas black cod showed a continued increase in rate at higher temperatures (Q_{10} 2.1). Again this supports that these fish may not have been pushed outside of their tolerance range, or they may be able to better tolerate acute temperature exposures.

Chapter 4.

Discussion

Respirometry experiments enabled me to determine the effect of chronic and acute temperature changes on ventilation rate, heart rate and standard oxygen consumption, and define tolerance range of three species of fish. It was evident that temperature had a strong influence on all rates. It is widely accepted that when faced with an increase in temperature most animals have inbuilt response mechanisms which cause an increase in oxygen demand due to the increased activity of metabolic processes (Bullock 1955, Campbell and Egginton 2007, Gräns et al. 2014), however processes differ between species and populations according to animal size, physiological status and thermal history (Glencross and Felsing 2006, Hawkins 1995).

Despite the strong influence of temperature on rate, chronic exposures only had a significant effect on blue cod rates, compared to black cod suggesting that black cod respond to chronic temperature exposure more alike Antarctic species (emerald rock cod) than temperate species (blue cod). Thus it seems blue cod are more capable of altering rates in response to long term temperature exposure. On the other hand acute temperatures changes affected all species, Q_{10} values represent this, and upper and lower limits were evident for the majority of species and acclimations.

4.1 Acclimation (chronic temperature exposure)

Temperature has a strong influence on all physiological rates and during long term exposure species find themselves needing to adjust their biochemical, physiological and behavioural processes (Clarke 2003, Gillooly et al. 2001, Johnston and Dunn 1987), however not all species are capable of doing this to high levels. It is known that species react differently to a given rate of change of temperature, and therefore can show different position, slope and steepness in rate:temperature curves (also reflected in Q_{10} values). This was seen during this project as blue cod showed great variability between chronic exposures, whereas black cod showed less.

4.1.1 Ventilation rate and heart rate

All species at all acclimations showed an increase in heart and ventilation rate with an increase in temperature, with drop offs in rate of change and levelling off at higher temperatures. The trend for both heart rate and ventilation rate was 8°C acclimated fish showing the higher rates, 12°C showing middle range rates and 18°C showing the overall lowest rates (figure 3.2). This downwards shift in rate is indicative of compensation according to Precht's model as with thermal acclimation it is possible to shift the position of areas of the rate:temperature curves, and alter tolerance range, as seen in figure 3.2 (Bullock 1955, Precht 1995). Black cod did not show much alteration of circulatory and ventilatory rates between acclimations, however they did show changes to tolerance range in acute exposure temperatures.

Blue cod have been able to alter their rates when exposed to chronic temperature changes. Hawkins (1995) states that "thermal compensation is complete if the rate of

production at this new steady state is similar to that preceding the temperatures change"
(Hawkins 1995). This was seen in blue cod which were able to maintain similar
preceding temperature resting rates (39 beats min^{-1}) at new acclimation temperatures
(32 beats min^{-1} at 8°C and 38 beats min^{-1} at 18 °C), due to compensating for the
differing influences of chronic temperature changes by homeostatic mechanisms of
various kinds (figure 3.7). This is likely due to the eurythermic behaviour of this species
as this species is distributed widely relative to environmental temperature around New
Zealand. As seen in various studies, for the past 70 years, eurythermal species are
typically temperate species and are capable of tolerating and compensating to many
different exposure temperatures with wide thermal tolerance ranges by increasing
altering mitochondrial levels (Bullock 1955, Pörtner et al. 2000, Pörtner 2002, Gräns et
al. 2014).

Black cod's response to warming differed to emerald cod, with heart rate was invariable
and ventilation rates varied across black cod acclimations, along with a change in the
temperature ranges the fish could be exposed to. Hoegh-Guldberg and Bruno (2010)
suggested that heart rate and ventilation rate in marine animals are linked but they do
not always match each other, sometimes ventilation rate can be more variable as
temperature has a stronger effect on ventilation rate. This was seen during these
experiments as ventilatory rates proved to be more variable in black cod during acute
temperature exposure, particularly at higher temperatures. Heart rate was very similar at
all exposure temperatures, whereas ventilation showed a small amount of variation
between the two acclimation temperatures, but in a temperature dependent manner
rather than a compensatory way suggesting that heart rate in black cod is very
invariable.

Species found in cooler waters tend to be more stenothermal in nature as cooler water climates are often more stable (Precht 1955, Johnston and Dunn 1987). Due to these fish permanently being exposed to lowered temperatures, many of them have already modified their physiological rates to enable prolonged survival at lower temperatures. This thermal specialisation is considered a trade-off for thermal flexibility, leaving these species at a disadvantage when faced with temperature changes (Pörtner et al. 2007). Unfortunately emerald rock cod were only acclimated to one temperature so this study only provides partial insight into their ability to acclimate.

Throughout experimentation emerald cod were less thermally flexible than black and blue cod. Emerald cod showed the largest Q_{10} values for change of rate in ventilation and oxygen consumption suggesting these fish were push far outside their tolerance range and were unable to compensate for temperature change. The rates observed for emerald rock cod are higher than those for black and blue cod at the same exposure temperatures showing these fish do display metabolic cod adaptation In particular Q_{10} values were high at warmer exposure temperatures with Q_{10} of 20. Fukuchi and Marchant (2006) suggested that emerald cod (at environmental temperature of -1.8°C) upper lethal limit was 6°C, in this study emerald cod were taken as high as 8°C when acclimated to 2°C. This suggests that even though these fish do show local specialised (MCA) they still retain some thermal flexibly and are able to shift their tolerance ranges and partially acclimate. Similar findings were made by Robinson and Davison (2008) in *Pagothenia borhcgrevinki*, showing that this species of fish was able to acclimate its resting oxygen consumption rates and ventilation frequency after a 5°C rise in temperature.

4.1.2 Oxygen consumption

Oxygen consumption followed the same trend as ventilation and heart rates, with all species and acclimations increasing with temperature. The trend blue cod showed both heart rate and ventilation rate was 8°C fish showing the higher rates, 12°C showing middle range rates and 18°C showing the overall lowest rates was also evident for oxygen consumption. Strengthening that these fish did partially to completely compensate for temperature difference, however variance in oxygen consumption rates were not as high as variation in ventilation or heart rates. It has been shown that heart rate and ventilation rate are closely linked and determine oxygen consumption (Holeton and Randall 1967).

Black cod oxygen consumption alike ventilation rate and heart rate showed little variability between acclimations, however oxygen consumption reflects a shift in tolerance ranges. This will be discussed in more detail below.

4.2 Tolerance ranges (acute temperature exposure)

Temperature has a strong influence on all physiological rates and during acute term exposure. Temperature exposures at lower and higher extremes causes significant increase in rates followed by a decline and levelling off of rates. Acute temperature exposure experiments allowed determination of species tolerance ranges and their ability to compensate to acute temperature changes.

Blue cod were exposed to the largest acute temperature range and black cod were exposed to a limited range. This different in ranges can be explained by temperature-dependent aerobic limits occuring earlier in larger animals

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than in smaller individuals (Pörtner 2004, Pörtner and Knust 2007). Black cod used in

this study were on average around 1400 g whereas blue cod were around 180 g.

4.2.1 Ventilation rate and heart rate

All species showed that ventilation rate and heart rate responded quickly to changes in

water temperature. As hypothesised blue cod, the eurythermal species, showed a wider

tolerance range than the stenothermic species, black cod and emerald cod, showed a

limited tolerance range. Similar findings have been found for eurythermal (Gräns et al.

2014) and stenothermal species (Claireaux et al. 1995). Interestingly heart and

ventilation rate appear to be limited in range, with heart rate being the first rate

measured to drop off at higher and lower temperature suggesting the insufficient supply

of oxygen around the body is the limiting factor to temperature tolerance in these

species). Oxygen delivery by ventilation and circulation is suggested to be the first line

of thermal sensitivity in relation to pejus range due to capacity limitations (Campbell

and Egginton 2007, Claireaux et al. 2005, Harley et al. 2006, Heath and Hughes 1973,

Pörtner 2001, Pörtner and Knust 2007).

Similar results were seen in spider crabs, Atlantic clams, Atlantic cod and rainbow

trout, when exposed to temperatures above 17 °C there was no further increase in

ventilation rate and rates levelled off significantly (Frederich and Pörtner 2000, Health

and Hughes 1973, Lanning et al. 2004, Mark et al. 2002, Pörtner et al. 1993, Pörtner

2000, Pörtner et al. 2004). These studies acknowledges the first line of thermal

sensitivity becomes apparent at the highest functional levels, that being circulation and

ventilation which show temperature-dependent changes in rate (Harley et al. 2006,

Pörtner 2002). In the study of eelpout and Atlantic cod (Lanning et al. 2004, Mark et al.

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2002, Pörtner et al. 2004) limitations were seen in circulatory capacity occurring before ventilatory limitations, this aligns with results of this study. In particular similar trends have been seen at warming temperatures and it been suggested the warming causes oxygen limitations not only by the forced rise in oxygen demand, but also by reducing oxygen solubility (Pörtner 2001, Pörtner and Knust 2007).

Over all acclimations blue cod survived exposure to a temperature range of 18°C, with fish at both high and low extremes showing signs of failure, suggesting loss of aerobic scope. This decrease in aerobic scope represents the point at which energy demands of ventilation and circulation become excessive, restricting increased oxygen supply to the tissues (Johnston and Dunn 1987). The trend of aerobic decline and higher and lower temperatures become common in temperature physiology literature and in 2001 Pörtner adopted Shelford's law of tolerance (Shelford 1931) and proposed the idea of 'pejus range' which was widely accepted.

In temperature experiments it is widely seen that when animals are pushed beyond pejus range ventilation and heart rate become more or less constant and independent of temperature, indicating capacity limitation (Pörtner 2001). This was clearly seen in these experiments as circulatory and ventilator rates either dropped off at high and lower temperatures or become less constant and independent of temperature suggesting failure.

Black cod heart rate did not change greatly over acute temperature exposures with irregularity seen at warmer temperatures suggesting heart rate is very invariable but becomes irregular at warmer temperatures. Campbell et al. 2005 carried out a study on black cod and long-term heart ranges changes. This study also gave insight into the limitation and variation in range of heart rate. Immediately after surgery fish showed a

rate of 46 beats min^{-1} which decreased to a resting rate of 34 beats min^{-1} after five days, and a varied rate of 5 beats min^{-1} during stress experiments suggesting there is little flexibility to black cod heart rate, which was also seen in this current study. Campbell and Egginton (2007) showed that black cod do lose cardio-respiratory coupling when faced with long term stress, and that this decoupling is followed by a change in oxygen consumption, suggesting the cardiac vagus influences metabolism (Campbell and Egginton 2007). Black cod heart rate when acclimated to 12°C did not vary much from when they were 8°C acclimated suggesting but heart rate managed largely uncharged following the trend seen in Campbell and Eggintons study (2007)

4.2.3 Oxygen consumption

The influencing relationship between temperature and oxygen consumption has been known for over 60 years, the concept was initially put forward by Hemmingsen (1950). The results in this study follow this relationship, metabolic sensitivity to environmental temperature changed with increases in positive exponential relation with initial acclimated rates of energy expenditure. During acute temperature experiments all species experienced steep increases in oxygen consumption with temperature with a levelling off at higher temperatures as fish were pushed outside their thermal tolerance range. Heath and Hughes (1973) saw the same response of oxygen consumption in rainbow trout.

 Increases in temperature have been documented causing sharp increases in oxygen consumption followed by a drop off in levels due so system failure. Oxygen consumption appeared to be the last rate to drop off, with some species experiencing no drop off in rate at all. Claireaux et al. (1995) showed similar effects to the ones seen in

this summer on Atlantic cod species, that the last limiting factor of temperature

tolerance is oxygen consumption.

A drop in oxygen consumption reflects the point at which oxygen supply can no longer

be compensated for by a rise in ventilation and circulation. Frederich and Pörtner (2008)

showed a similar response in spider crabs at temperatures beyond high pejus range,

ventilation and heart rates dropping off first, followed by the onset of a decrease in

oxygen consumption reflecting the rise in oxygen demand can no longer be meet as

ventilatory and circulatory systems are collapsing (Frederich and Pörtner 2008).

Ventilation rate increased progressively as oxygen consumptions increased causing a

higher oxygen demand, until a temperature was reach in which oxygen demand could

no longer be compensated for by ventilation increases and Q_{10} values drop (Frederich

and Pörtner 2000). This was seen in all species and acclimations apart from black cod

acclimated to 12°C. In particular this response was seen in 12°C blue cod, oxygen

consumption dropped at higher exposed temperatures, supporting that these fish were

pushed outside their tolerance range and systems were failing.

Of interest the Q_{10} value for oxygen consumption experiments are extremely high at

lower and upper exposure temperatures, before they level off. These higher Q_{10} are

typically only seen when fish are experiencing maximal aerobic activity during exercise

experiments (Gillooly et al. 2001), this indicates that these fish were severally stressed

and reaching their lower or upper critical temperatures.

4.2.2 Tolerance ranges

Limited oxygen availability and aerobic scope are crucial in limiting thermal tolerance

(Pörtner 2002). This tolerance is set at the level of organelles and their interactions with

the rest of the cell. Through acute exposure experiments tolerance ranges have been determined for these fish species by observing at which temperatures rates suddenly dropped off. Blue cod acclimated to 8°C were exposed to the narrowest temperature range of for the species, from 6°C to 16°C. Blue cod acclimated to warmer temperatures were able to tolerate wider temperature ranges, 14°C range. Overall the lowest temperature this species could survive exposure to was 6°C and the highest was 24°C.

Blue cod acclimated to 8°C range of optimum performance sat between a T_pI (lower pejus temperature) of 8°C and T_pII (higher pejus temperature) of 16°C, fish at either end of this range were able to survive so the lower and upper critical limits were likely not reached, however rates were starting to decline suggesting optimum range was exceeded. Blue cod acclimated to 10°C range of optimum performance sat between T_pI of 10°C and T_pII of 20°C, fish were exposed to temperatures as low as 6°C and heart rate was severally dropping at that those temperatures as reflected by small Q_{10} values (1.2 ± 0.4) indicating these fish were outside their optimum tolerance range. Fish acclimated to 18°C range of optimum performance sat between T_pI of 12°C and T_pII of 20°C, rate dropped off at higher temperatures and then increased again as fish was showing stressed behaviour thrashing about. Shifts of both low and high critical temperatures is particularly common in temperate species during acclimation to changing temperatures (Pörtner 2001).

Black cod have a narrower range than blue cod, 8°C fish range of optimum performance sat between T_pI of 6°C and T_pII of 16 °C, and 12°C fish range of optimum performance above T_pI of 8°C. The upper tolerance temperature for 12°C acclimated black cod was not found in throughout experimentation as no significant drop in rate was found,

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however stressed behaviour was observed suggesting these fish were near their upper

tolerance range.

Emerald rock have the narrowest range of all species with range of optimum

performance sitting between T_pI of 0°C and T_pII of 8°C. This is expected as these fish

are metabolically cold adapted making them very specialised. This species experienced

the highest range of rate (Q_{10} 15.2) suggesting these fish do still maintain a thermal

flexibility to a degree, but are not capable of full compensation to exposure temperature

due to over specialisation.

4.3 Limiting factors

Ectotherms are dramatically affected at all levels of thermal variation, thus temperature

changes pose a real challenge. The physiological mechanisms limiting cold and heat

tolerance have regained interest in the past 10 years due to the threat of climate change

and observed geographical shifts in distribution of ectothermic animals and plant

species (Etterson and Shaw 2001, Hughes 2000, Perry et al. 2005, Thyrring et al. 2015).

Perry et al. 2005 discovered several North Atlantic fishes have undergone shifts in their

mean depth distribution in response to warming (Perry et al. 2005). Black cod may be

undergoing poleward shifts are fish are populations as they are becoming increasingly

harder to catch, and you are needing to travel further south to successfully catch large

numbers of these fish.

Heart rate appeared to be the overall limiting factor for all species and acclimations as it

was the first rate to drop off. Pörtner et al. (1993) showed similar findings in Antarctic

clams. A collapse of circulatory systems was seen during long term recordings of

temperature stressed clams, this collapse was followed by a decrease in oxygen uptake and oxygen consumption. There is often a mismatch between these processes leading to loss of oxygen supply leading to collapse of physiological function (Pörtner 2002). Forgan and Forster (2010) showed that heart rate was the limiting factor in blue cod whereas ventilation and oxygen consumption proved to be more flexible.

All species in this study have responded differently to temperature change. Schiel et al. 2004 found that benthic species respond individualistically to climate change, this study supports this finding. This is significant as even if species populations or distribution is not altered by temperature change, or is altered to a lesser degree, the strength and occurrence of interspecific interactions might change as other species experience strong influences of temperature on distribution or physiological capability.

4.5 Significance of findings

Marine ecosystems are centrally important to the biology of the planet and rapid climate change is increasingly recognised as an important factor in marine evolutionary history and is capable of inducing catastrophic collapse of ecosystem structure (Clarke 2003, Hoegh-Guldberg and Bruno 2010).

The length of exposure, as well as temperature value, is also crucial in setting mortality of a species (Pörtner and Knust 2007). Throughout acute temperature period's fish were very quickly exposed to an increase in temperature (within an hour and a half). The responses from quickly changing acute temperature exposure show that if black cod and emerald cod were rapidly exposed to increased temperatures they would struggle, whereas blue cod would be able to tolerate changes.

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Adjustments to life at cooler waters prove to limit thermal flexibility. Some species, such as black cod, hold enough thermal flexibility to be able to tolerate chronic temperature changes even though they do not fully acclimate, whereas specialised species like rock cod would not survive a chronic warming of just 1 or 2 °C above ambient water temperatures.

Shifts in species distribution may be seen as a consequence of ocean warming, in particular pole-ward shifts of ectothermic animals (Clarke 2003, Harley et al. 2006, Pörtner and Knust 2007). This is due to their being a match between physiology of an organism and the thermal characteristic of its habitat, in turn species bordets being influenced by thermal physiology (Clarke 2003). When species are faced with temperatures ranges they cannot compensate long term for they will geographical change their distribution. Black cod showed they are unable to compensate for long term temperatures changes, as they ae becoming harder to catch in New Zealand waters this suggests they are shifting their distribution and heading to cooler waters down South in where they will not need to compensate for temperature changes.

4.6 Future studies

To gain an in depth insight the effect climate change will have on these species more studies are needed. In particular more temperature work is required on the black cod to determine upper tolerance range, it would be interesting to see how warm this fish can be taken as 18 °C did not seem to get the typical responses of a fish at this upper lethal limit. To access black cod's ability to acclimate more experiments where rates are measured over the month long acclimation would be of use.

To better access aerobic scope exercise studies should be carried out. By using the results gain from these studies, exercising the fish at their lower and upper tolerance ranges will provide insight into their aerobic scope for activity and the onset of anaerobic metabolism.

As ventilatory and circulatory systems seem to be the first line setting tolerance ranges in vitro circulatory and ventilatory experiments would help determine the exact limitations of these systems.

Tagging experiments of black cod would help determine what is happening to these populations and if they are migrating back South and how far they move in summer months. Further studies comparing black cod to other Antarctic nototheniod, such as *notothenia neglecta*, would determine how widely thermal tolerance has expanded in black cod since they migrated to New Zealand waters .

Gene level studies are of crucial importance for future work as having an understanding of genetic basis of physiological traits and with influence affecting temperature dependency will be needed to predict species and population level effects.

As compensation can occur on many different levels and within many different time frames, and the speed of temperature change having an effect on acclimation it would be good to look at the change in rate over the one month chronic temperature exposure. This would provide insight if to black cod are a species which initially shows compensation, as seen in acute exposure experiments, until they give in to the influence of temperature.

Chapter 5.

Concluding remarks

The physiological measures that set thermal window widths and depths defining thermal sensitivity is a key aspect to be considered in a climate change context. To be able to fully understand the impacts climate change and warming of the world's oceans will have on marine life an in-depth knowledge of species ability to withstand temperature change is needed. Studies such as this allow you to examine the ability of species to acclimate to new temperatures and alter their tolerance windows.

This study shows that these species responded to chronic and acute temperatures differently. The eurythermal species (blue cod) had a better ability to completely acclimate to new temperatures and become sluggish in behaviour at higher and lower temperatures, whereas the more stenothermic species (black cod and rock cod) did not acclimate but were able to shift their tolerance ranges and become very active at upper and lower exposure temperatures.

Circulatory and ventilatory systems proved to be the limiting factors of species thermal tolerance. These systems are the first line to become compromised and unmatched during temperature exposure experiments, resulting in an inability to meet oxygen demand. As thermal tolerance is crucial in setting an organisms niche shifts in tolerance and are high importance in climate change studies. When fish find themselves faced with changes outside of their tolerance range they are likely to re redistribute to areas more favourable. This distribution of species can have detrimental effects on local

ecosystems. If oceans continue to warm we are likely to see collapse of ecosystems as species migrate to new areas.

To have a complete understanding of climate change effects on marine ecosystems future studies need to look at active aerobic scope and exercising, tagging experiments for distribution analysis, gene level changes during acclimation and further studies into tolerance limitation.

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Linear regression graphs

Ventilation rate
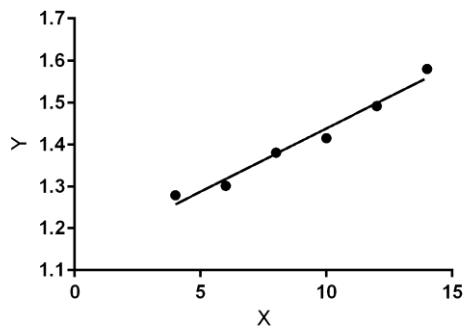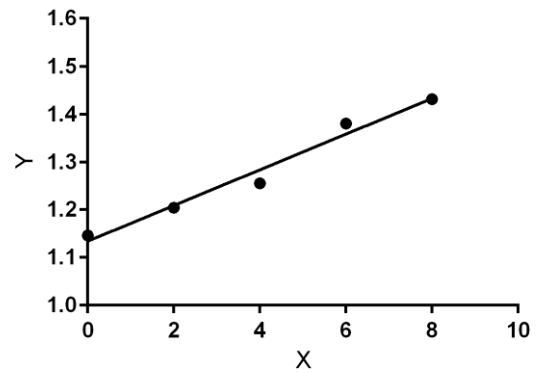

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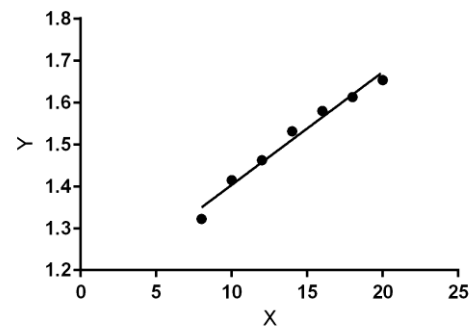
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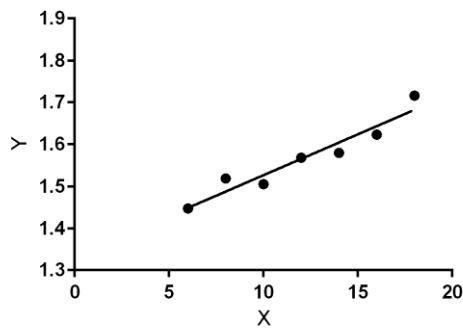
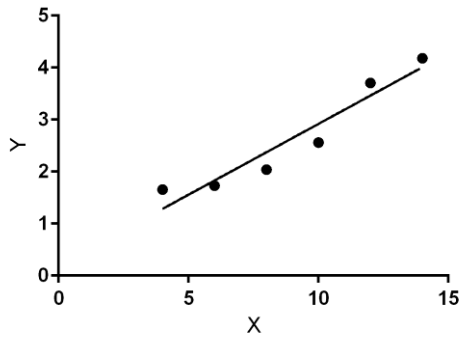Black cod 12°C R_2=0.9255
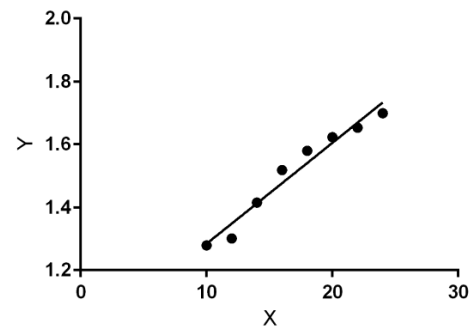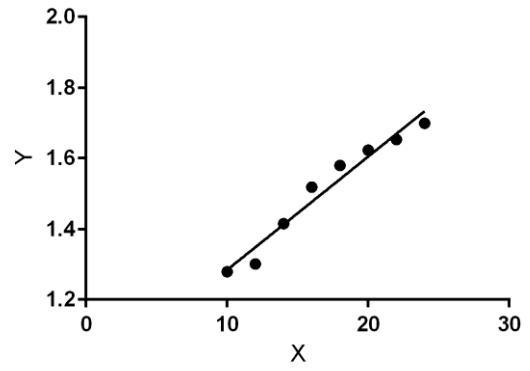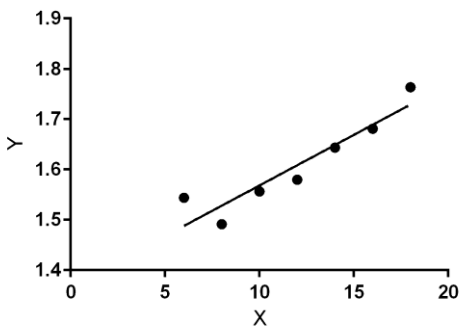

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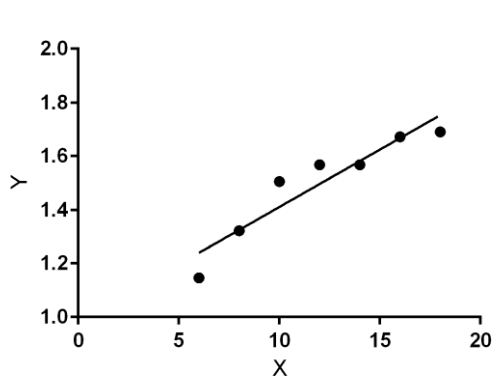
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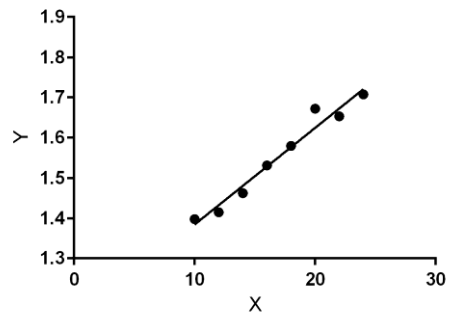

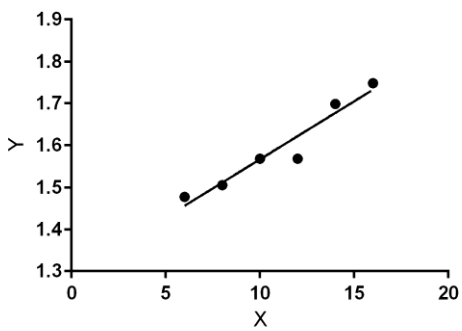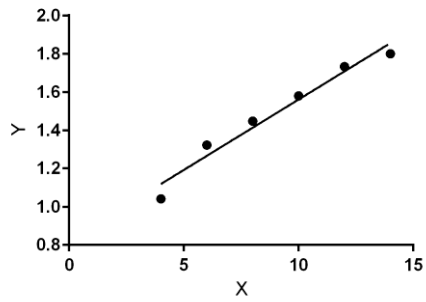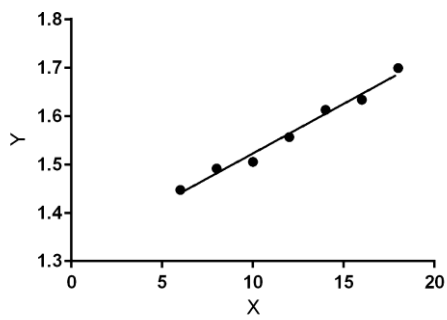Emerald cod 2°C R_2=0.9746

Heart rate

Black cod 8°C R_2 =0.9845

Blue cod 12°C R_2=0.8522

Black cod 12°C R_2=0.8703

Blue cod 18°C R_2=0.9638
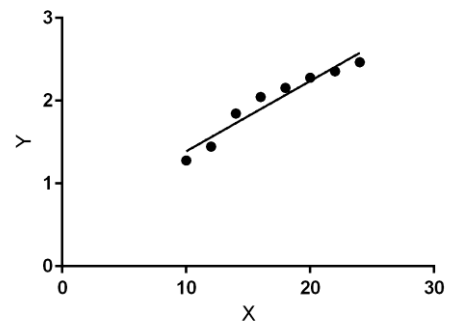

Blue cod 8°C R_2=0.9291

Oxygen consumption
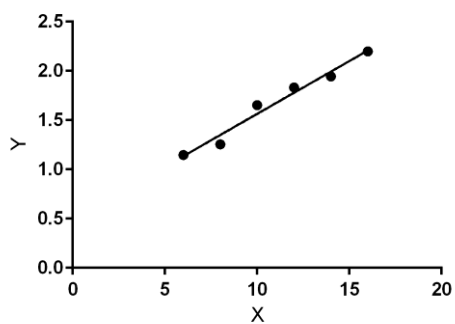

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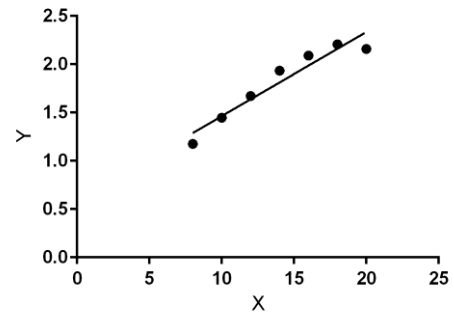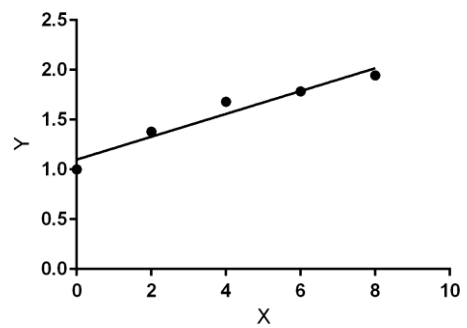

Blue cod 12°C R_2=0.9208

Black cod 12°C R_2=0.9821

Blue cod 18°C R_2=0.9349

Blue cod 8°C R_2=0.9556

Emerald cod 2°C R_2=0.9411