THE EFFECT OF TEMPERATURE CHANGE ON THE NEW ZEALAND MARINE FISH, *NOTOLABRUS CELIDOTUS*

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Abstract

Physiological responses of the labrid fish *Notolabrus celidotus* to temperature change, found thermal compensation to be more advanced in cold environmental temperatures compared to warm environmental temperatures. *N. celidotus* was acclimated for 28 days to 8, 14 and 24ºC and metabolism, ventilatory and circulatory function, condition factor, swimming ability, thermal tolerance and hypoxia tolerance investigated.

N. celidotus acclimated to 8ºC almost achieved full thermal compensation, which resulted in, resting and maximum oxygen consumption not being significantly different to 14ºC acclimated fish. In contrast, *N. celidotus* acclimated to 24ºC achieved only partial or no metabolic thermal compensation. This resulted in high resting oxygen consumption and a reduced aerobic scope for activity, which had detrimental affects on other physiological parameters investigated.

Thermal compensation was achieved for the resting ventilation rate of the 8ºC acclimated fish, which is most likely needed in order to meet the high oxygen demands incurred by metabolic thermal compensation. No thermal compensation was achieved for any other ventilation rate or for heart rate, at any acclimation temperature. Thermal compensation at the level of heart and ventilation rate of the 24ºC acclimated fish, would have been limited by the lack of metabolic thermal compensation. A low condition factor of the 24ºC acclimated fish would also have occurred due to the lack of metabolic thermal

compensation, which would have caused high-energy demands and the utilization of energy stores.

Thermal tolerance ranges shifted in the direction of temperature change for all acclimation groups, which indicates thermal compensation must have occurred to some degree at a variety of organizational levels. Swimming ability reflected metabolic thermal compensation, with the swimming ability of the 8ºC acclimated fish being similar to the 14ºC acclimated fish. In comparison, the 24ºC acclimated fish had a diminished swimming ability, which is likely to have occurred due to the reduced aerobic scope for activity of these fish and the low condition factor.

The findings suggest that the increasing temperatures associated with climate change will cause *N. celidotus* to migrate to cooler waters in order to survive. This response will have a large effect on New Zealand's marine ecosystem as *N. celidotus* is abundant in New Zealand waters and is an important part of the food chain. Additionally, the response of *N. celidotus* may be an indicator of the response of other New Zealand species to climate change, which could cause huge upset to New Zealand commercial fisheries.

1. General Introduction

1.1 Global Warming: A Brief Overview

"Temperature is amongst the most pervasive and important physical factors in the environment of an organism" (Reynolds and Casterlin 1980, p497).

Global climate change is projected to cause significant changes to many environmental conditions, including weather patterns, ocean levels and ocean composition, but the extreme sensitivity of animals to temperature fluctuations makes rising temperatures one of the greatest concerns. These rising temperatures are predicted to affect individual organisms, the size and structure of their populations, the species compositions of their communities, and the structure and functioning of ecosystems (Pörtner and Knust 2007). The basis of climate change is radiative forcing, which alters Earth's energy balance and triggers complex feedback systems (Fig. 1). Radiative forcing can arise by alterations in the intensity of incoming solar radiation, changes in the amount of solar radiation that is reflected by Earth and modification of longwave radiation emitted from Earth back into space (Le Treut 2007). Changes in radiative forcing are caused by natural variations in Earth's orbit and Earth's surface and atmospheric composition. However, since the industrial revolution anthropogenic input producing greenhouse gases has enhanced the intensity of radiative forcing so substantially it is causing damaging and potentially irreversible changes to Earth's environment (Stern 2007).

Milankovitch cycles correspond to movements in the Earth's orbit, which ultimately affect the amount of solar radiation reaching Earth and Earth's land masses (Muller and

MacDonald 1997). Parameters of the Milankovitch cycle include Earth's eccentricity, axional tilt, precession and orbital inclination. All of these follow individual cycles, which oscillate Earth's orbit towards and away from the sun, but have a combined effect that determines the total amount and location of solar radiation reaching Earth (Muller and MacDonald 1997). The region of Earth solar radiation strikes is important because land has a greater susceptibility to warming than the ocean; hence, the Northern Hemisphere (large land mass) undergoes greater warming than the Southern Hemisphere (large ocean mass) (IPCC 2007). The Milankovitch cycles work on large timescales with a current dominant cycle at approximately 100,000 years and are responsible for Earth's rhythmic cooling and warming phases (Muller and MacDonald 1997). Earth is currently in a warming phase, which is expected to last for a further 30,000 years (Jansen et al. 2007).

Solar radiation is either reflected or absorbed by Earth depending on the atmospheric and surface composition. Cloud cover and aerosols in the atmosphere and light-coloured surfaces such as ice, snow and deserts reflect solar radiation; the remaining radiation is absorbed by Earth's surface and atmosphere (Le Treut 2007). In order to maintain a relatively constant temperature Earth has to balance the amount of incoming energy absorbed from solar radiation with the amount of energy it emits, via longwave infrared radiation (Le Treut 2007). However, the phenomenon known as the "greenhouse gas effect" offsets this balance by preventing the longwave radiation from leaving Earth's atmosphere, as the infrared rays are not permeable to the "blanket" of gases (Le Treut

2007). In comparison, solar radiation is permeable to greenhouse gases, so a net increase in energy is experienced and the atmosphere warms (Stern 2007).

Changes in global temperature prior to the industrial revolution (1750) were predominantly regulated by changes in Earth's orbit and volcanic activity (Jansen et al 2007). Milankovitch cycles causing Earth to move closer to the sun resulted in warming periods, whereas cycles taking Earth's orbit away from the sun resulted in global cooling and associated glaciations (Jansen et al 2007). Volcanic activity had a large effect on global temperature, as this releases a large amount of aerosols, which has an initial cooling effect (Forster et al. 2007). However, aerosols are cleared from the atmosphere quickly (2-3 years) compared to carbon dioxide, which is also released by volcanic activity, and causes a secondary warming phase due to the greenhouse gas effect (Forster et al. 2007).

The industrial revolution (1750) brought with it a significant increase in greenhouse gases due to anthropogenic influences, which has ultimately shifted greenhouse gases to the dominant control in global temperature (Forster et al. 2007). The most damaging greenhouse gases produced by anthropogenic input are carbon dioxide (CO_2) and methane (CH_4), with carbon dioxide representing 77% of total anthropogenic greenhouse gas emissions in 2004 (IPCC 2007). Carbon dioxide is produced predominantly from the burning of fossil fuels and deforestation, and methane from agriculture, natural gas distribution and landfills (Forster et al. 2007). Carbon dioxide concentrations have increased by just over one third from pre-industrial levels of 280ppm to today's 380ppm

and the rate of increase continues to accelerate, which could lead to a concentration of 550ppm being reached by as early as 2035 (Stern 2007). Methane levels remained relatively stable at 700ppb until the 19th century, during which they accelerated to 1,745ppb in 1998 and to a further 1,774 in 2005 (Le Treut 2007). Atmospheric concentrations of carbon dioxide and methane are currently higher than any natural levels produced over the last 650,000 years (IPCC 2007).

This offset in Earth's energy balance has caused global mean surface temperatures to increase by 0.74°C ±0.18°C over 100 years (1906-2005) and a further increase of 0.2°C per decade is expected over the next twenty years (IPCC 2007). The rate of warming is also accelerating with the rate over the last 50 years (0.13°C ± 0.03°C per decade) being almost double that over the last 100 years (0.07°C ± 0.02°C per decade) (Trenberth et al. 2007). Ocean temperatures are increasing, although this is much slower than land temperature with a rate of 0.13°C per decade compared to 0.27°C per decade, since 1979 (Trenberth et al. 2007). The global ocean temperature has increased to depths of at least 3000m with the ocean taking up over 80% of the heat being added to the climate system (IPCC 2007). Over the period 1961 to 2003 the global ocean temperature increased by 0.10°C from the surface to a depth of 700m (Bindoff 2007). Ocean acidification has also been a problem since the industrial revolution, as the ocean absorbs a large proportion of atmospheric carbon and this causes it to become more acidic (IPCC 2007).

The climate's positive feedback system is already apparent, with the melting of large ice and snow masses, thawing of permafrost and inhibition of carbon absorption (IPCC

2007). The melting of snow and ice masses reveal darker Earth surfaces that allow for increased solar radiation absorption; hence, increase global warming. The thawing of permafrost releases methane into the atmosphere and rising temperatures and changes in rainfall patterns hinder the ability of Earth's natural processes to absorb carbon dioxide, which both enhance the effects of the greenhouse gases (Stern 2007). This positive feedback as well as the ever-increasing carbon dioxide and methane concentrations from anthropogenic production is increasing the rate of warming so substantially, it is expected the global temperature could increase a further 3-10°C by 2100 (Stern 2007).

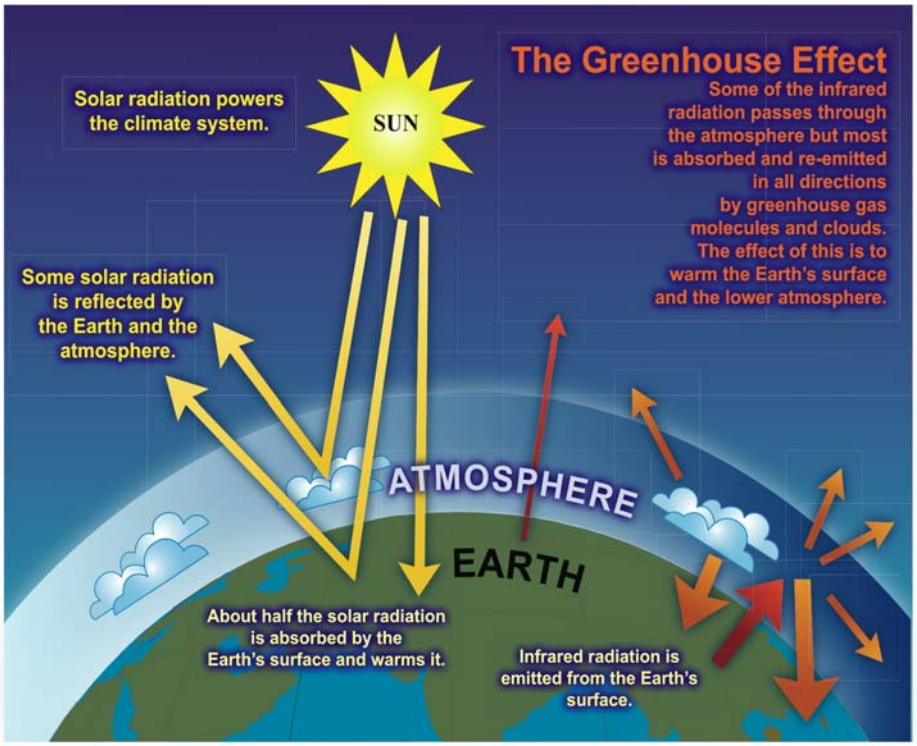

Fig. 1.1 Earth climate model (Le Treut et al. 2007).

1.2 The effect of global warming on terrestrial animals

Animals are very sensitive to temperature and use it as an important factor in habitat selection and as a stimulus for cyclic biological events, such as breeding and migration. Therefore, changes in global temperatures are predicted to have huge effects on ecosystems, with mass movements of species distribution, alterations in the timing of cyclical biological events and species extirpation and extinction (Rosenzweig et al. 2007). These changes have already been observed in a number of species with a notable interspecies variance in response times and size, which ultimately cause large disruptions to the highly sensitive ecosystem.

A study by Parmesan and Yohe (2003) looking at a large range of species found that distribution range limits have moved an average of 6.1±2.4 km per decade pole-ward in response to global warming, and more than 95% of the 1,700 species investigated have shifted in accord with climate change predictions within the last thirty years. Furthermore, a study of 677 species found 62% showed trends towards spring advancement in events such as breeding, nesting and the arrival of migrant birds, by a mean of 2.3 days per decade (Parmesan and Yohe 2003). A second study (Root et al. 2005) looking at phenological shifts in 130 species support these results. Root et al. (2005) found an average spring advancement of 3.2 days per decade but also noted variance in advancement depending on species and habitat latitude. European species advanced by 5.0 days per decade, Northern hemisphere species north of 45° latitude experience higher spring advancement (4.4 days per decade) compared to species south

of 45° (2.0 days per decade) and birds experienced the greatest phenological shifts with a spring advancement of 5.1 days per decade (Root et al. 2005). Of the species investigated 92% have undergone phenological shifts in the direction expected by global warming (Root et al. 2005). Additionally, egg laying dates have advanced in many birds, hibernation periods have been reduced in some mammals, some amphibians have experienced earlier calling, mating and maturation and several bird species no longer migrate out of Europe (Rosenzweig 2007).

The changes associated with animal responses to global warming are increasing the risk of species extinction, with 15-40% of species potentially facing extinction with a 2°C temperature increase (Stern 2007). Invasion of non-native species and disruptions of predator/prey relationships are a major factor for extinction (Rosenzweig 2007). For example, increasing temperatures in sub-Antarctic islands will allow for invasive species, such as the house mouse (*Mus musculus*) and springtails (*Collembola spp*) to become established (Rosenzweig 2007). With limited predators these invasive species will proliferate and diminish resources, which will put increased pressure on the native species and could lead to their extinction (Rosenzweig 2007). Increased temperatures are also associated with increase in disease, which can result in whole populations becoming extinct. For example, the extinction of approximately 75 species of frogs indigenous to the American tropics was most likely due to the outbreak of a pathogenic fungus, which is greatly enhanced by global warming (Rosenzweig 2007). Extinction may also result when distribution shifts are restricted due to geological barriers, or when suitable habitats are unavailable (Perry et al. 2005).

1.3 Effects of global warming on marine ecosystems

The effects of climate change on marine ecosystems are more apparent than terrestrial ecosystems, due to fewer constraints on animal dispersal in the ocean (Perry et al. 2005). Responses are seen in all trophic levels via individual species responses to the increasing temperature or via forced responses due to related changes lower in the food chain. For example, a temperature increase of 0.8-1°C above the summer maximum for extended periods (at least 4 weeks) results in coral bleaching (Rosenzweig 2007). Overall plankton biomass and seasonal length has changed with increasing temperatures (Rosenzweig 2007), and krill abundance in the Southern Ocean has declined (Atkinson et al. 2004). These changes greatly disrupt the ecosystem causing changes in ecosystem functioning and productivity, which lead to changes in species distributions and abundance.

The responses to climatic warming vary between different functional groups and trophic levels and cause a mismatch in timing between them in regard to seasonal cycles, which ultimately disrupts the mandatory species interactions needed for survival (Edwards and Richardson 2004). Other responses to warming include the occurrences of sub-tropical species in temperate waters, shifts from cold-adapted to warm-adapted communities, phenological changes, and an increase in harmful algal blooms (Rosenzweig 2007). A study by Perry et al. (2005) found nearly 2/3 of the 36 demersal fish species investigated dispersed to cooler waters via distribution shifts in either latitude or depth with a 1.05°C temperature increase. The study was conducted in the North Sea between 1977-2001 and found a mean distribution shift of 2.2km per year (Perry et al. 2005).

Changing marine ecosystems have huge implications on commercial fisheries. New Zealand fisheries harvest 600,000 tonnes of their 130 target species each year, with values of approximately $1.2-$1.5 billion per annum (Ministry of Fisheries 2008). From the 130 species targeted in New Zealand the main species are squid, hoki, ling, oreo dories, orange roughy and silver warehou (Ministry of Fisheries 2008). It is possible commercial fisheries will face declining numbers if the target species undergo distribution shifts, in response to increasing temperatures. However, little is known about the response New Zealand fish will have to global warming. Therefore, the purpose of the current work is to determine the effects of temperature change on the New Zealand marine fish *Notolabrus celidotus* (spotty). *N. celidotus* represents species in the centre of the food chain, hence, will provide a good insight into the effects global warming will have on the ecosystem, as well as on individual species responses.

1.4 Notolabrus celidotus (spotty)

N. celidotus is a native New Zealand wrasse from the labridae family and is located all around New Zealand, to a depth of approximately 30 metres, but has not dispersed to surrounding Islands, such as the Snares Islands, Chatham Islands, or the Three Kings Islands (Ayling and Cox 1982). It is a carnivore, feeding on benthic invertebrates, although the composition of the diet varies with age, as does the fish's distribution (Jones 1988). The common name "spotty" arises from the trademark black spot in the centre of each flank, which is more pronounced in the female (Fig. 1.2) (Ayling and Cox 1982).

Fig. 1.2 A female *N. celidotus,* with the trademark black spot.

N. celidotus, like most wrasses, is a protogynous hermaphrodite, with all individuals starting life as females and changing sex to become males later in life (Jones 1980). Sexual dimorphism occurs via the trademark black spot migrating upwards and dispersing into a group of irregular black spots immediately below the dorsal fin and body colour changing from the female's pale grey or yellow-brown to the male's grey or grey-blue, which occurs at lengths between 180-220mm (SL) (Fig. 1.2) (Clarke 1993). On some occasions the fish will change sex without undergoing sexual dimorphism and use its appearance to masquerade as a female. This allows it to remain undetected in the territory of another male, so it can join the spawning process of the territory male and mating female at the crucial fertilization point (Jones 1980). This is beneficial for the deceiving male as it has the greater testis size, so produces more sperm than the territorial male; hence, has a greater chance of fertilising the egg (Jones 1980). The males are very territorial with mating success positively correlated with the quality of the territory (Ayling and Cox 1982). Females have a preference for territories with patches of broken rock and kelp to provide shelter for young fish and mature females; as well as a large bare

patch of open rock, where the mating process can take place (Ayling and Cox 1982). The mating ritual consists of the male chasing the female into this open area and swimming rapidly around her in circles, with the yellow front portion of his dorsal fin raised as a courting signal (Ayling and Cox 1982). If the female is receptive she settles on the bottom of the open rock and the male approaches the female, ensuring close proximity as both fish then swim rapidly upwards for approximately 3m releasing eggs and sperm, and then rapidly swim back to the bottom (Ayling and Cox 1982). This spawning process occurs during the winter-spring months of July-November and the resulting pelagic eggs accumulate at or near the surface of the water column and hatch within approximately 120 hours, the larvae then live pelagically between August and December (Carbines 1993).

Between October and February the larvae (15-20mm) recruit to the fronds of the common kelp *Ecklonia radiata* and remain sheltered here for the first year of life, growing rapidly to 80-100mm (Carbines 1993). The diet of these juveniles originally consists of the amphipods and copepods from the frond surfaces, but as they grow their dependency on shelter from the kelp fronds is reduced and their diet consists more dominantly of amphipods and isopods from the rock strata (Carbines 1993). Female maturity is reached at about 120mm and these adult fish disperse to greater depths and feed predominantly on molluscs and crabs, with alternate feeding on other benthic and pelagic invertebrates such as hermit crabs, amphipods, shrimps, barnacles, various shellfish and worms, which they use their sharply pointed teeth to extract from rock surfaces (Ayling and Cox 1982). The maximum length reached by an adult male is 260mm, with the lifespan of *N. celidotus*

being approximately seven years (Ayling and Cox 1982). Although *N. celidotus* reaches depths of about 30m it is most abundant in shallower waters, with the greatest distribution in depths less than 8m (Jones 1984). They are opportunistic feeders and are attracted to areas in the environment that have undergone disturbance, which combined with their high preference for mussels make them a problem fish around mussel farms (Clarke 1993).

The time of spawning is regulated by water temperature for many fish, as young fish are generally less tolerant to extremes in temperature; hence, it is expected they will be brought into the environment at a temperature close to the centre of the adult's temperature range (Carbines 1993). *N. celidotus* spawns at a time when the water temperatures are relatively cool, which most likely allows embryo development to coincide with optimal incubation temperatures. High temperatures during fish embryonic development have been found to be detrimental due to reduced incubation periods leading to premature hatching and resulting increases in mortality (Petereit et al. 2008). The larval stage of *N. celidotus* coincides with warming water, which is associated with peaks in the abundance of phytoplankton and common zooplankton (Jones 1980). The waters are also relatively warm during the recruitment phase allowing for optimal growth in juveniles, as well as the adults who have now ceased breeding (Jones 1980).

N. celidotus is an important part of New Zealand's marine ecosystem as it is a very abundant species and is a food source for a number of finfish. Therefore, determining the effects of temperature change on *N. celidotus* will give an insight into the response of

other fish to global warming, due to direct effects on individual species from increasing temperatures or alterations in the food chain causing indirect distribution changes on species higher up.

1.5 Physiological responses to temperature change

Fish are ectotherms as they are unable to retain heat or generate enough heat to maintain a constant body temperature, so this is determined by their surroundings (Randall et al. 2002). This makes them very sensitive to the fluctuating temperatures they are exposed to by the environment, as metabolic rates are positively correlated with temperature. The Q_{10} effect is the factor by which reaction rates alter in response to a 10°C shift in temperature, fish generally have a Q_{10} of 2 or 3 in regard to metabolic rate (Saenger and Holmes 1992). Therefore, ambient temperature changes altering the metabolic rate cause associated changes in all aspects of fish physiology, such as circulation, respiration, digestion, growth, reproduction and swimming ability (Randall et al. 2002). Fish possess a thermal tolerance range that allows them to survive natural temperature fluctuations and within this is an optimum temperature at which they perform at their best (Rantin et al. 2007). Variations in either direction from the optimum temperature diminish performance, with fish functionality being jeopardised at the extremes. The thermal tolerance range differs with species, age and environmental stability (Saenger and Holmes 1992). Generally, adult fish have a greater thermal tolerance range than larvae and juveniles, which is why the seasonal timing of biological events, such as spawning, is so crucial for species survival (Saenger and Holmes 1992). Stenothermal animals inhabit

environments with a relatively constant temperature and include polar and tropical fish. As a result, they have small thermal tolerance ranges and generally do not possess the mechanisms to adapt to new temperatures (Kinne 1963). In comparison, eurytherms do experience variations in ambient temperature due to small fluctuations on a day-to-day scale and large temperature shifts with the seasons. To overcome this they have large thermal tolerance ranges and possess mechanisms to adapt to these changes by upward or downward tolerance shifts. These are generally temperate fish (Kinne 1963).

Temperature change has a huge range of complex, integrating effects on fish physiology. Molecular and cellular alterations in metabolism and neuronal pathways are responsible for changes observed at higher levels (Saenger and Holmes 1992). Decreasing ambient temperatures are associated with an overall reduction in metabolic rate and resulting decrease in oxygen demand; whilst increasing ambient temperatures are associated with an increasing metabolic rate creating a greater oxygen demand (Kinne 1963). Detrimentally, water oxygen saturation is inversely related to temperature, so the greater demand for oxygen experienced at higher temperatures coincides with less oxygen available for uptake (Rantin et al. 2007).

As the whole body of a fish is ectothermic, decreasing temperatures cause a similar decrease in the metabolic rate of the fish, and the cardiac and ventilatory systems; therefore, a relatively close match prevails between the metabolic need for oxygen and the supply of oxygen (Haverinen 2008). Decreasing temperatures reduce the velocity and force of muscle contractions and decrease the activity of the autonomic nervous system,

which limits locomotor, cardiac and ventilatory functions and diminishes the overall performance of the animal (Jones 1994, Haverinen 2008). Oxygen uptake becomes less efficient because the reduced ventilation rate lowers the amount of water pumped over the gills for oxygen and ion exchange (Rantin et al. 2007). The viscosity of blood is inversely related to temperature, so the higher viscosity in cooler waters and reduced heart rate decrease the efficiency of the circulatory system and associated oxygen transport (Bushnell et al. 1992). Therefore, less oxygen reaches areas of demand such as the muscles, which places a second limitation on muscle performance. The reduction in swimming ability has huge implications on the survival of the animal, as it limits the ability of the fish to produce speeds efficient enough to catch prey and escape from predators. Likewise, cellular membrane viscosity is inversely related to temperature, so cooler temperatures increase the viscosity and disrupts the critical processes that help to maintain homeostasis, such as membrane transport and maintenance of ion gradients (Randall et al. 2002). Other metabolic functions such as reproduction, digestion and growth are also reduced due a combination of the Q_{10} effect and limitations in metabolite availability from reduced food and oxygen uptake (Saenger and Holmes 1992).

Increasing temperatures invoke the opposite response with the Q_{10} effect creating an increase in reaction rates (Saenger and Holmes 1992). Accordingly, muscle contraction is rapid and the activity of the autonomic nervous system increases, which results in increased maximum swimming speeds, ventilation rates and heart rates (Jones 1994, Haverinen 2008). The greater resting metabolic rate creates a high demand for oxygen, which is achieved via the increased ventilation and heart rates and decreased blood

viscosity allowing for sufficient oxygen uptake and oxygen transport (Rantin et al. 2007). However, at critical maximum temperatures there is a rapid decline in ventilation and heart rates due to oxygen demand exceeding supply; the animal dies soon after. Additionally, maximum oxygen uptake does not increase or only increases slightly due to limitations in oxygen carrying capacity and exchange. In comparison, resting oxygen consumption increases significantly with an increase in temperature, which ultimately causes a decrease in the scope for activity (Fig 1.3) (Fry 1971). The scope for activity is the difference between resting and maximum oxygen consumption, which allows for extra activities such as swimming, reproduction and growth (Fry 1971). At high temperatures the energetic cost of simply living is so high there is little energy available for these other essential activities, which again threatens the survival of the animal.

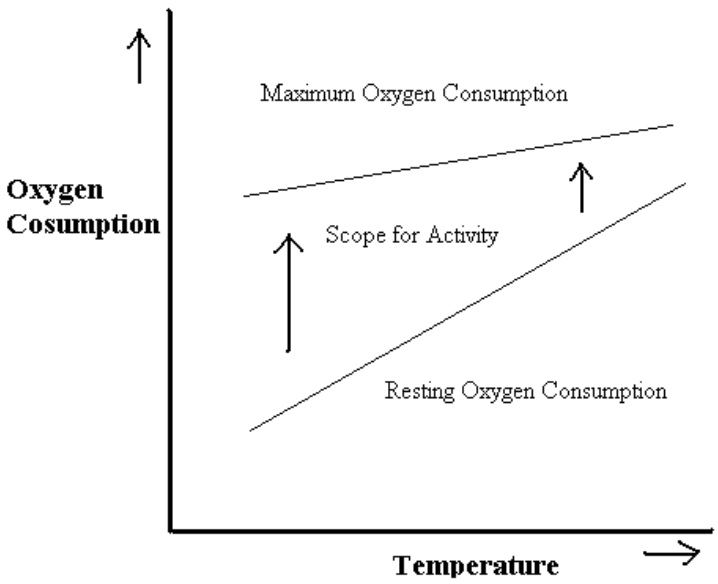

Fig. 1.3 Alterations in the "scope for activity" in response to temperature change.

Compensation for the above physiological changes is possible in some species and involves a shift in thermal tolerance ranges, in the direction of temperature change. The degree and rate of compensation depends on the species, environmental history and the rate of temperature change (Kinne 1963). Generally, species exposed to varying temperatures in their environment have a greater ability to adapt to the new temperatures, compared to species living in more constant environments. Furthermore, if the rate of temperature change is slow animals are able to compensate more readily. Compensation can result in the physiological responses following temperature change, returning to initial values (values before temperature change), returning partially to initial values or remaining at new values; termed full, partial and no compensation respectively (Precht et al. 1973). Additionally, over-compensation can occur causing physiological responses to rebound to levels beyond the initial values; or a rare inverse acclimation can occur, which causes the values to move in the opposite direction of the initial rates (Precht et al. 1973).

As the primary effects of temperature change are seen at the molecular level with reaction rates, it is conceivable that compensation will also be seen here. Temperature compensation can, in fact, be achieved at this molecular level via alterations in enzyme kinetics (Hochachka and Somero 1984). Increasing enzyme quantity and affinity allows for a greater chance a reaction will occur, which counter-effects the decreased reaction rates in colder temperatures and the reverse is true in warmer temperatures (Hochachka and Somero 1984). Temperature compensation is also apparent in cellular membranes, with alterations in lipid bilayer saturation regulating membrane viscosity. Increasing saturation in warmer temperatures increases membrane viscosity and decreasing

saturations in cooler temperatures decreases viscosity (Randall et al. 2002). Additionally, cholesterol increases membrane viscosity, so increasing cholesterol composition during warmer temperatures and decreasing it in cooler temperatures achieves further temperature compensation (Randall et al. 2002). Neuronal pathways also need to undergo thermal compensation, which is achieved by alterations to cell membrane ion pumps to maintain transmembrane electrical potentials (Römisch and Matheson 2003). Additionally, thermal compensation is needed to alter temperature-senstitive rates of neurotransmitter release, diffusion, binding and breakdown (Römisch and Matheson 2003). The red muscle fibres of fish, which are slow, aerobic fibres located superficially under the skin, undergo further thermal compensation via alterations in capillary and mitochondrial densities, myosin heavy and light chain isoforms, lipid concentrations and the relative volume of red muscle fibres (Day and Butler 2005). In cooler waters there is an increase in capillary and mitochondrial densities, which increase ATP production and blood flow to aerobic muscle (Day and Butler 2005). Increases in the aerobic capacity of mitochondria can also achieve thermal acclimation to cold temperatures, but this is secondary to the increase in mitochondrial volume (Johnston et al. 1994). Alterations in myosin heavy and light chain isoforms in cooler temperatures involve faster-contracting and higher tension fibre types, which work to offset the decrease in muscle contractibility (Hammill et al. 2004). Increases in lipid concentrations in red muscle fibres in cooler temperatures enhance oxygen transport, as well as act as an oxygen store (Day and Butler 2005). The increase in relative volume of red muscle fibres in cooler waters is via increased fibre diameters and allows for steady swimming to be supported by aerobic muscular activity up to higher velocities, which delays the recruitment of fast, anaerobic

white muscles, which fatigue rapidly (Hammill et al. 2004). Thermal compensation at the level of the temperature-dependent activity of the autonomic nervous system results in alterations in the heart and ventilation rates (Farrell and Jones 1992, Haverinen 2008).

N. celidotus is a eurytherm as it is a temperate fish inhabiting shallow water, so is exposed to significant seasonal and daily temperature fluctuations and is therefore expected to possess the mechanisms to adapt to temperature changes. Behavioural activities are a form of adaptation that do not require physiological changes and include the animal remaining in waters of their preferred temperatures, which requires migration. *N. celidotus* is a non-migratory fish, which supports the idea of physiological adaptation to temperature change. A second behavioural adaptation is hibernation/aestivation, which involves the animal effectively entering a state of torpor allowing it to tolerate the physiological changes associated with the extreme temperatures, without any thermal compensation. There have been reports of *N. celidotus* hibernating, which suggests it does not have the ability to undergo physiological changes for thermal compensation to low temperatures (Clarke 1993). There is a lack of research on the physiology of *N. celidotus*, hence, the degree of thermal compensation in this species is unknown.

1.6 Thesis aims

The aim of the present study is to investigate the physiological responses of *N. celidotus* to temperature change. The fish were acclimated to extreme temperatures of 8 and 24°C and a median temperature of 14°C, for a period of 28 days. This allowed the degree of thermal compensation of investigated parameters to be determined.

The physiological parameters investigated include: -

- Resting and maximum oxygen consumption (metabolic scope for activity)
- Heart and ventilation rate
- Swimming ability
- Critical thermal maximum and minimum
- Hypoxia tolerance

The outcome of the study will allow for an estimation of the response of *N. celidotus* to increasing ocean temperatures associated with global warming. The results can be used to predict the response of other New Zealand fish species, including species important for New Zealand commercial fisheries. As *N. celidotus* is a eurytherm it is expected to undergo some degree of thermal compensation, which will allow it to adapt to the temperature effects of global warming. If no thermal compensation is achieved it will most likely migrate to cooler waters in response to global warming. This will cause disruptions to the marine ecology and suggests other New Zealand fish will also follow

suit and migrate to cooler waters. Additionally, tropical fish may migrate to New Zealand waters from warmer climates, further disrupting New Zealand's marine ecosystem.

2. General Methods

2.1 Capture

Notolabrus celidotus was caught using collapsible traps that were set in coastal harbours in the Canterbury region of New Zealand's South Island. The traps were set at distances approximately 20 metres from the shoreline around Lyttelton Harbour (Te Whaka-raupo), Diamond Harbour and Akaroa Harbour. Crushed mussels were used primarily as bait and crabs and squid were used when available. The traps were left for 30-120 minutes before being checked or on some occasions were left over-night.

Once caught the fish were held in an aerated 40L barrel and transported to the aquarium in the Zoology Building at the University of Canterbury. The aquarium consists of a closed recirculating system where the water temperature is maintained at 14°C. The duration of the transport process was approximately 2 hours and there were no mortalities over the entire catch, which consisted of 60 fish.

Capture success varied throughout the year with a substantial decline in fish numbers encountered during the autumn and winter seasons: the fish that were caught during this time were predominately small (less than 130mm). Numbers caught increased during the warmer spring and summer seasons. Clarke (1993) also observed this seasonal change in abundance in the Marlborough Sounds region and observed fish in torpid states during the winter season, suggesting decreasing water temperatures can trigger periods of

hibernation in *N. celidotus*. The concept of winter hibernation in fish is supported by studies by Campbell et al. (2008) that found the Antartic fish *Notothenia coriiceps* enters a dormant state in winter, which is similar in many ways to hibernating vertebrates. The increase in abundance of *N. celidotus* during the warmer seasons is due to increased activity, arising due to the warmer water temperatures as well as from the initiation of spawning behaviour (Clarke 1993).

Maturation as females occurs at approximately 120mm (SL) (Jones 1980), hence, any fish less than 120mm(SL) were released immediately. Early experiments determined fish greater than 160mm had the potential to out swim the swim tunnel during the Ucrit test. Therefore, fish greater than 160mm were also released immediately.

2.2 Husbandry

The aquarium room is a closely monitored system. It is maintained at an air temperature of 10°C and a water temperature of 14 ± 0.5°C and is subjected to a 12-hour light and 12-hour dark photoperiod (12L: 12D). Salinity, ammonia levels and nitrite levels are monitored weekly. Salinity is maintained at 33ppt, ammonia levels below 1.2mg/L and nitrite levels below 0.2ppm.

On arrival all fish were placed into 60L tanks in the aquarium room, regardless of their designated acclimation temperature. They were held there for a minimum of 14 days

before being transferred to the acclimation tanks to allow for recovery from transportation. Experiments by Klesse (1996) allowed 1 week for recovery before beginning the acclimation process. A comparable factor to determine transportation recovery between Klesse (1996) and the current experiment was food acceptance. Fish in a stressed state experience a loss of appetite; hence, food acceptance indicates the fish have recovered from the stress of transportation (Klesse 1996). All spotties accepted food within the 14 days.

Fish were fed twice weekly on the New Zealand green-lipped mussel (*Perna canaliculus*) The mussel varied between fresh and thawed and were broken in half before being fed to the fish. Any uneaten food was removed from the tank at the end of the day.

N. celidotus often seeks cover under sessile invertebrates, rocks and microalgae in its natural environment (Carbines 1993). Therefore, empty mussel shells and rocks were placed in the tanks to allow cover for the fish while in captivity.

2.3 Acclimation

Fish were selected to be acclimated to 8, 14 or 24°C. These temperatures were selected after preliminary experiments found that 8 and 24°C were the minimum and maximum temperatures that *N. celidotus* could survive in, for the duration of the acclimation period. Surpassing these temperatures resulted in fish mortalities within the first 1-2 weeks of the

acclimation period; there were no mortalities at 8 and 24°C over the 28-day acclimation period. The intermediate temperature of 14°C was selected because it is the average temperature *N. celidotus* is exposed to in its natural environment. The experimental design required ten fish per acclimation temperature, per experimental set. All acclimation temperatures were tested with two sets of fish, hence, a total of 60 fish were used over the entire experimental process.

Fish acclimated to 14°C were kept in the aquarium for a further 28 days post transportation recovery period. The fish were transferred to new (60L) tanks in the aquarium at the beginning of the acclimation period. All husbandry described in section 2.2 was continued. Feeding was stopped 14 days prior to experiments to allow the fish to be in the correct post absorptive state for resting oxygen consumption experiments.

Fish acclimated to 8 and 24°C were transferred in 10L buckets to their respective temperature controlled rooms. They were left in the buckets, with aeration, for a number of hours to allow for a gradual change in the water temperature. When the water reached acclimation temperature the fish were transferred into 60L tanks. The system consisted of two 60L tanks in a closed recirculating system containing a 40L reservoir, and this required partial water changes of approximately 1/10 (16L) total system volume every two days. Salinity was maintained at 34ppm, ammonia levels less than 1.2mg/L and nitrite below 0.2ppm. The acclimation period was 28 days and feeding was ceased two weeks prior to the experiments. Fish acclimating to 8 and 24°C had diminished appetites. This is most likely due to the physiological stress of the extreme ambient temperature

change (Klesse 1996). Transportation stress could also be a factor, however, as 14°C fish were also transported to different tanks with no observed change in appetite this factor appears insignificant. A large individual difference was observed in the time frame of loss of appetite. Some fish recovered after only a few days, while other fish had diminished appetites for the entire feeding period.

2.4 Anaesthetic and ECG Wire Insertion

The first procedure the fish were subjected to was an operation to insert ECG wires. Fish were anaesthetised in 0.08 $g.L^{-1}$ of MS-222 (3-aminobenzoic acid ethyl ester) and ECG wires were inserted subcutaneously close to the heart using a syringe needle. The wires were sutured to the fish to secure them at two points. One point was between the pelvic and pectoral fins and the second point was immediately below the dorsal fin, approximately 1/3 from the anterior end. Care was taken to ensure the wires did not interfere with the movement of any fins.

Once the operation was complete the fish was weighed and the total length (TL) of the fish measured while it was still under anaesthetic. It was then transported to a closed-box respirometer (1.43L), which was left open to allow for water flow. It was left in the respirometer for a minimum of 24 hours to allow for recovery. A study by Hill and Forster (2005) found that 6-9 hours was a sufficient recovery period for *N. celidotus* subjected to handling stress and recovery from MS-222 was approximately 3 hours.

Therefore, 24 hours ensured the fish was no longer under the effects of the anaesthetic and had recovered from handling stress. The fish was placed into a closed-box respirometer to allow for resting oxygen consumption to be measured after the 24-hour recovery period. The respirometer was in a recirculating system holding approximately 44L of aerated seawater; ¾ of the closed box respirometer was submersed to prevent significant temperature change. The water temperature was maintained at the fish's acclimation temperature.

The fish underwent two days of experiments, which are described in relevant sections. The first day of experiments began by determining resting oxygen consumption, heart rate and ventilation rate. This was followed by hypoxia tolerance experiments and recovery from hypoxia. After the fish had recovered it was transferred to a swimming tunnel and swum to the point of exhaustion. When this point was reached maximum oxygen consumption, heart rate and ventilation rate were measured, as well as recovery from exercise. Once the fish had recovered the experimental system was set-up for the second day of experiments (see section 5.2.) and the fish was left in this system over-night. On the second day of experiments the critical thermal maximum was measured followed by the critical thermal minimum. On completion of these experiments the fish were anaesthetised again in MS-222 and the ECG wires removed. The fish was then acclimated to 14°C in the aquarium room for 28 days before being released in the Te Whaka-raupo region. The experimental system was then returned to the set-up used for day one experiments. The system was replaced with fresh seawater each day, as well as between the critical thermal maximum and critical thermal minimum experiments.

3. Metabolism, Respiration and Circulation

3.1 Introduction

Metabolism is the sum of all reactions occurring in an animal and is highly sensitive to ambient temperature change in ectotherms because of the Q_{10} effect, which affects all individual enzymatic reaction rates (Randall et al. 2002). The number of metabolic reactions in the whole animal is immense, so the combined effect of temperature change from all of these individual reactions can produce a large overall change in whole animal metabolism. Metabolism produces the energy source ATP (adenosine triphosphate) from food molecules and is the basis for all functions of the animal at all organizational levels, from cellular activities to whole animal behaviour. There are two components of metabolism, one of these is the extraction of chemical energy (ATP) from food molecules and the channeling of that energy into useful functions; such as muscle contraction, ciliary movements, the active transport of molecules by membranes and ultimately the activity of the animal (Randall et al. 2002). The second metabolic component involves chemical alterations and the rearrangement of food derived molecules into precursors of other kinds of biological molecules allowing for cellular repair, cellular regeneration, animal growth and reproduction (Randall et al. 2002).

Metabolism is greatly altered with acclimation to long-term temperature change, due to thermal compensation primarily acting at the molecular level to alter reaction rates (as described in chapter one), which allows for optimal functioning of the animal at different

temperatures. Therefore, in determining the effects of temperature change on ectotherms it is imperative to investigate metabolic rate, which is achieved indirectly by using oxygen consumption as a parameter. It is plausible to use oxygen consumption, because metabolic reactions utilize oxygen as a final electron acceptor in the electron transport chain so oxygen consumption reflects metabolic rate (Clarke and Fraser 2004). Anaerobic metabolism can occur when the oxygen demand is too high to meet the requirements of tissues, which results in an oxygen debt as the anaerobic end products must eventually be oxidized and removed, and this requires the consumption of oxygen when it becomes available (Randall et al. 2002). This anaerobic component of metabolism makes it important to measure oxygen consumption during recovery from periods of high-oxygen demand, in order to investigate all metabolic components.

Fish performance is dependent on the efficiency of its respiratory and circulatory system, as these regulate oxygen uptake and transport to meet metabolic demands. The transportation process of oxygen from the environment to tissues consists of four steps. Firstly, convection of water over the gill arches is created by ventilation of the gills, which then allows for diffusion of oxygen from water to blood (Jensen et al. 1993). Once the oxygen is in the blood stream it is transported to the tissues where it undergoes the final step, which is diffusion from the blood to the tissue cells (Jensen et al. 1993). Ambient temperature changes can affect all four stages, which result in alterations in the ability of the animal to meet oxygen demands. Detrimentally, the respiratory and circulatory system are driven by metabolism, as the energy produced by metabolic

reactions fuel respiratory and cardiac muscle contraction (Jones 1994). In times of high energy demands respiration and heart rates increase in order to increase oxygen availability to the tissues, but this response in turn creates an additional increase in oxygen demand (Jones 1994).

3.1.1 Respiratory System

Fish ventilation rates are regulated in part by the oxygen concentration of the water, which is determined by oxygen sensing receptors that are located on the first gill arch (Rantin et al. 2007). Blood oxygen and carbon dioxide levels and pH also regulate ventilatory control via detection by appropriate receptors (Heath 1973). As increasing temperatures are associated with decreased oxygen solubility an exponential increase in ventilation rate occurs (Rantin et al. 2007). A simple respiratory equation developed by Dejours (1981) to explain this is:

$$\mathbf{VO_2 = V.C_IO_2.EO_2}$$

Where VO_2 = oxygen uptake; V = ventilation of the gills; C_IO_2 = oxygen concentration of the inspired water and EO_2 = extraction of oxygen from the inspired water.

The extraction of oxygen from inspired water is high in teleosts due to their countercurrent gas exchange system allowing for efficient gas diffusion; the degree of

extraction changes little with temperature (Rantin et al. 2007). Therefore, ventilation rate must increase in proportion to the decrease in the oxygen concentration of the inspired water and associated increase in oxygen demand resulting from increased temperatures (Rantin et al. 2007). Additionally, higher temperatures are associated with increased autonomic nervous system activity and allows for faster respiratory muscle contraction and shorter periods between contractions, which are essential in order for ventilation to meet the oxygen demands (Haverinen 2008).

However, the increased ventilation rate at rest is energetically costly and reduces the respiratory scope for activity. In comparison, decreasing temperatures are associated with an increased oxygen solubility of the water, which stimulates a reduction in ventilation rate (Jones 1994). Lower temperatures may also reduce respiratory functionality by limiting respiratory muscle contraction and contraction frequency (Jones 1994). Both temperature responses limit the ability of the animal to perform essential life functions such as, growth, locomotion and reproduction making temperature compensation important for long-term survival (Kinne 1963).

3.1.2 Circulatory System

The circulatory system is responsible for transporting oxygen from the gills to oxygen-requiring tissue cells. Oxygen from the inspired water diffuses across the gill epithelia into the blood due to an oxygen gradient, which is achieved by the high oxygen partial

pressure of inspired water (PO_2) compared to the low venous oxygen partial pressure (Rantin et al. 2007). Oxygen tension of the blood is kept low as oxygen molecules are bound by haemoglobin molecules within erythrocytes and carried in this form to the tissues (Randall et al. 2002). The amount of oxygen bound per unit volume of blood depends on the number of erythrocytes, concentration of haemoglobin within the erythrocytes, prevailing oxygen partial pressures, and the oxygen binding affinity of the haemoglobin molecule (Nikinmaa and Salama 1998). The arterial oxygen partial pressure (PaO_2) decreases in tissue capillaries where carbon dioxide is produced, which alters the oxygen affinity of haemoglobin by causing a rightward shift on the oxygen dissociation curve, so more oxygen is released allowing it to diffuse into the tissue cells (Nikinmaa and Salama 1998).

Increases in temperature reduce the PO_2 of the ambient water, which in turn reduces the oxygen gradient across the gill epithelia and associated uptake of oxygen. Furthermore, temperature is inversely related to the oxygen affinity of haemoglobin so at high temperatures the oxygen dissociation curve is shifted to the right, although haemoglobin-oxygen saturation is still likely at the gill, if pH is normal (Nikinmaa and Salama 1998). This decreased oxygen affinity can be beneficial while oxygen uptake continues at the gills, because the decreased affinity makes oxygen more available at tissue capillaries (Nikinmaa and Salama 1998). Additionally, haemoglobin affinity is reduced by organic phosphates (mainly ATP and GTP), which bind to the deoxygenated form of the haemoglobin molecules inhibiting oxygen binding (Nikinmaa and Salama 1998). Erythrocytic organic phosphate content initially increases with temperature, although this

content is reduced when temperatures become threatening (Nikinmaa and Salama 1998). Decreasing pH is associated with increasing temperature, which also causes a rightward shift in the oxygen dissociation curve (Nikinmaa and Salama 1998).

In order to meet the animal's oxygen demands, the cardiac output may increase to overcome the reduced oxygen carrying capacity. This is achieved by increasing the heart rate, which then increases the blood flow to the gills and tissues and allows for increased oxygen loading and unloading (Rantin et al. 2007). The decreased $PaO_{2,}$ increased autonomic nervous system activity and release of catecholamines associated with high temperatures stimulates the sympathetic nervous system, which causes an increase in heart rate (Butler and Metcalfe 1983, Haverinen 2008). Additionally, the rate of cardiac pacemaker depolarization increases with temperature in ectotherms; hence, temperature affects heart rate directly (Butler and Metcalfe 1983, Haverinen 2008). It is also possible that the high temperatures allow for faster cardiac muscle contractions and contraction frequency (Farrell and Jones 1992). Additionally, erythrocytes can be liberated from storage organs, such as the spleen, to counter-act the decrease in haemoglobin affinity (Nikinmaa and Salama 1998). Seasonal adaptations are apparent in some fish to modify oxygen transport. For example, in the cooler seasons the demand on the circulatory system is reduced so erythropoiesis is switched off in salmon, there is an increase in methaemoglobin (a form of haemoglobin that cannot bind oxygen) levels in temperate fish, and ß – adrenergic responsiveness of trout red blood cells is decreased (Jensen et al. 1993). The reverse adaptations occur in the warmer seasons where there is a greater demand for oxygen (Jensen et al. 1993).

In a similar fashion to the respiratory system, temperature compensation within the circulatory system is important for long-term survival. High-energy costs at high temperatures and decreased cardiac ability at low temperatures limit the performance of essential activities such as prey capture, predator escape, reproduction, digestion and growth (Driedzic 1992). In regard to the whole animal, a secondary measure to determine the degree of physiological stress of a fish is the condition factor, which to a degree determines the body fat content of the fish in regard to its weight-length relationship (Elliot and Russert 1949, Dahlberg 1969). A high condition factor is associated with a high fat content and indicates low physiological stress; whereas, a low condition factor is associated with a low body fat content and indicates the fish is under high physiological stress, which could be hindering digestion, growth and possibly food uptake (Elliot and Russert 1949, Dahlberg 1969).

3.1.3 Current experiment

The current experiment investigates the aerobic scope for activity, heart and ventilation rate, and oxygen consumption during recovery from periods of high oxygen demands, and the condition factor of *N. celidotus* acclimated to 8, 14 or 24°C. The purpose of this is to determine whether *N. celidotus* is able to modify its metabolic, respiratory and cardiac systems when acclimated to temperatures at their upper and lower limits. The outcome of this will allow for predictions to be made about the fate of these fish in response to increased temperatures resulting from global warming. If no thermal compensation occurs it is likely the fish will be forced to migrate to cooler waters. If

thermal compensation does occur it suggests that *N. celidotus* will be able to adapt to the temperature changes it will be subjected to in its natural environment.

3.2 Materials and Methods

All oxygen consumption experiments were conducted in a cylindrical polyurethane closed-boxed respirometer (1.43L) with a perspex lid. Approximately ¾ of the respirometer was immersed in seawater, that was maintained at relevant acclimation temperatures, to prevent temperature change in the respirometer over the experimental period. The perspex lid had two circular holes that were securely fitted with bungs; one hole allowed for the ECG wires that were attached to the fish to be connected to electrodes outside the respirometer, and the second contained a 1mL syringe used to take water samples from the respirometer and a second 12mL syringe containing air saturated seawater that was used as a reservoir. An aquarium pump drove water flow through the respirometer and this flow could be stopped during experiments using taps that were connected to the respirometer inflow and outflow tubes. No mixing apparatus was used as it could introduce a stress factor to the animal. Additionally, studies by Wells (1987) found opercular and pectoral fin movements sufficient to circulate water in the respirometer.

The oxygen tension of the water was determined by passing the 1mL sample through a Strathkelvin oxygen microcell that contained an oxygen electrode. The oxygen electrode

was connected to a Strathkelvin oxygen meter, which displayed the partial pressure of the sample (PO_2) in millimeters of mercury (mmHg), the reading was taken 90 seconds post sample injection to allow the meter to stabilise. Sodium hypochlorite was injected into the Strathkelvin oxygen microcell at 3-4 weekly intervals to destroy any microorganism build up that may affect the reading. The oxygen meter was calibrated prior to all experiments using air-saturated water at the experimental temperature. A control experiment measuring the PO_2 in the closed respirometer with the absence of a fish, found an insignificant change in PO_2 over a period of one hour (± 0.4 mmHg).

The heart rate and ventilation rate of the fish was determined using ECG wires. The ECG wires that were inserted into the fish prior to experiments (see section 2.4) were connected to a BIOAmp and PowerLab A/D system. The readings were displayed on a connecting laptop using PowerLab software.

3.2.1 Resting Oxygen Consumption, heart rate and ventilation rate.

The fish was placed into the respirometer 24 hours prior to resting oxygen consumption (VO_{2rest}) experiments to allow the fish to be in a resting state. Additionally, the feeding of the fish was ceased two weeks prior to the experiment to ensure the fish was in the required post-absorptive state for VO_{2rest} experiments. All air bubbles were removed from the respirometer on placement of the fish. If any air bubbles entered the system over the 24-hour recovery period they were removed before the experiment commenced. After the

recovery period the water flow through the respirometer was turned off and a PO_2 sample taken. Immediately after the sample was taken the ventilation rate was recorded by counting the number of opercular beats per min, two counts were made and the mean ventilation rate calculated. Samples were taken every 10 minutes over a 30-minute period; the experiment was terminated if the PO_2 dropped below 90mmHg. Care was taken to ensure the fish was not disturbed in any way during the experiment. 100mmHg is the more common threshold before termination of oxygen consumption experiments (Lowe and Davison 2006, Robinson and Davison 2007). However, hypoxia tolerance experiments (refer chapter 6) found *N. celidotus* can tolerate very hypoxic conditions in comparison to other species. Therefore, 90mmHg is adequate for the threshold in these fish.

Heart rate and ventilation rate from ECG recordings could not be measured along with $VO_{2rest,}$ as preliminary experiments found a high probability of the fish losing their ECG wires if they were connected to recording equipment for the 24-hour recovery period. Therefore, on completion of the VO_{2rest} experiment the respirometer was re-opened and the ECG wires were connected to the recording system. The respirometer was then re-sealed and all air-bubbles removed. This procedure caused some disturbance to the fish so heart rate and ventilation rate (from ECG recordings) may be slightly elevated from resting levels, thus a 30-minute recovery period followed to minimize this stress effect. Heart rate and ventilation rate was then recorded over a 30-minute period; PO_2 was recorded simultaneously at 10-minute intervals to ensure levels did not drop below 90mmHg. Analysis of this oxygen consumption data found, in most cases, it was not

significantly elevated from resting oxygen consumption experiments. This suggests that heart and ventilation rate during this time are an effective measure of resting rates.

3.2.2 Maximum oxygen consumption, heart rate and ventilation rate, and recovery

In order to achieve maximum oxygen consumption (VO_{2max}), heart rate and ventilation rate the fish was swum to exhaustion in a Blazka-style swimming tunnel (see chapter 4) and then **immediately** transferred back to the closed-box respirometer. The respirometer was sealed and air-bubbles removed, ECG wires were threaded through the perspex lid at this time but not connected to the recording equipment in order to reduce time loss between the fish reaching exhaustion and the first PO_2 sample. The ECG wires were connected to the electrodes after the first water sample was taken and heart rate and ventilation rate recorded at the same time as PO_2 samples for the remaining experimental duration. PO_2 samples were taken at 5-minute intervals for the first 30-40 minutes and then at 10-minute intervals thereafter. VO_{2max} was taken as the largest value post exercise, as was maximum heart and ventilation rate. Opercular beats per minute were recorded with every PO_2 recording. In a number of fish the heart and ventilation rates (from ECG recordings) were unable to be measured during this experiment because the ECG wires became displaced while swimming to exhaustion. When PO_2 levels dropped below 90mmHg the respirometer was flushed with fresh seawater for 5 minutes before being resealed. If any air bubbles entered the respirometer during flushing they were removed. The experimental period was 90-120 minutes.

Care has to be taken when using the term VO_{2max} in experiments with a delay between exhaustion from exercise being reached and oxygen consumption being measured. This is because it is likely VO_2 has declined from maximum levels over the transition phase. However, in *N. celidotus* it was found that VO_2 increased over the first 5-10 minutes post-exercise, which indicates this species has a lag in exhaustion being reached and VO_{2max} (Fig. 3.4). Therefore, it is likely the maximum oxygen consumption measured in the current experiment represents the true VO_{2max}.

3.2.3 Data Analysis

Oxygen consumption (mg O_2 kg^{-1} h^{-1}) was determined using the equation:-

$$\mathbf{VO_2 = \frac{\Delta PO_2 . C . V . 31.999}{t.m}}$$

Where ΔPO_2 = change in PO_2 over the measurement period (mmHg), C = oxygen capacity of water at a given temperature ($\mu mol.L^{-1}.mmHg^{-1}$), V = volume of water in respirometer (L), 31.999 = molecular weight of oxygen ($kg.kmol^{-1}$), t = time interval of ΔPO_2, and m = mass of fish (g).

ΔPO_2 was calculated at 10-minute intervals with the exception of the first 30-40 minutes post swimming exhaustion, where ΔPO_2 was calculated at 5-minute intervals. VO_{2max} was taken as the largest VO_2 value post swimming, hence, VO_{2max} occurred at varying time

intervals in individual fish. Confidence that this is the true VO_{2max} is warranted, as oxygen consumption increased with time over the first 5-10 minutes, indicating that VO_{2max} did not occur in the period between exhaustion being reached and the first VO_2 measurements. GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA) was used for statistical analysis and graphing. A repeated measure two-way analysis of variance (ANOVA) with Bonferroni's Multiple Comparison post-hoc test and a repeated measure one-way ANOVA with Dunnett's Multiple Comparison post-hoc test was used to compare maximum and resting oxygen consumptions within groups and between acclimation temperatures, statistical significance was taken as $P<0.05$. Factorial aerobic scope was analysed using a Kruskal-Wallis non parametric one way analysis of variance (ANOVA) with Dunn's Multiple Comparison post-hoc test. The standard one-way analysis of variance (ANOVA) test could not be used as the Bartlett's test found the variance between groups differed. Equal variance is a prerequisite for ANOVA. Values used are acclimation group means and associated standard errors.

Heart rate and ventilation rate were calculated at parallel time intervals to oxygen consumption. At each time interval the mean heart and ventilation rate were calculated from two consecutive samples. Heart rate was determined from the ECG recordings, whereas ventilation rate was recorded from opercular beats.min^{-1} as this was able to be recorded in a larger number of fish compared to the ECG recordings. The same statistical analysis applied to oxygen consumption was conducted on heart rate and ventilation rate, with an additional linear regression analysis. The values used are also acclimation group means and associated standard errors.

Oxygen consumption during recovery was calculated as the mean VO_2 within acclimation groups at set time intervals. A two-way ANOVA with a Bonferroni post-hoc test was used to compare the oxygen consumption of the different acclimation groups in response to time. Heart rate and ventilation rate were also analysed in this manner. All values used are means and associated standard errors. Sample sizes for oxygen consumption during recovery were 14, 17 and 17 for the 8, 14 and 24°C acclimated fish respectively. Sample sizes of heart rate during recovery were 7, 10 and 9 for the 8, 14 and 24°C acclimated fish respectively and ventilation rate sample sizes during recovery were 13, 17 and 17 for the 8, 14 and 24°C acclimated fish respectively.

The weight and length of the fish was measured prior to oxygen consumption experiments (see chapter 2). The condition factor of the fish was determined using the equation: -

$$\mathbf{K = \frac{100000.W}{L^3}}$$

Where K = condition factor, W = weight in grams and L = length in mm and 100000 is a scaling constant used to bring K to a more meaningful number.

A one-way ANOVA with Bonferroni's Multiple Comparison post-hoc test was used to analyse the difference of mean values and associated standard errors between acclimation groups.

3.3 Results

3.3.1 Oxygen consumption

The mean resting oxygen consumption (VO_{2rest}) differed little between fish acclimated to 8 and 14°C ($P>0.05$) (68.45 ± 6.64 mg O_2 $kg^{-1}h^{-1}$ and 74.25 ± 4.22 mgO_2 $kg^{-1}h^{-1}$ respectively) (Fig 3.1). In comparison, the mean VO_{2rest} of the 24°C acclimated fish (156.70 ± 10.24 mgO_2 $kg^{-1}h^{-1}$) was significantly higher than the 14°C acclimated control group ($P<0.01$) (Fig 3.1). Exercise to exhaustion resulted in maximum oxygen consumption (VO_{2max}) causing a substantial increase from a resting state. The trend was similar to that of VO_{2rest} with the 8 and 14°C acclimated fish having very similar values (270.6 ± 16.50 mg O_2 $kg^{-1}h^{-1}$ and 271.5 ± 17.37 mg O_2 $kg^{-1}h^{-1}$ respectively). These values were greater than VO_{2rest} by a factor of 4.02 ± 0.70 for 8°C acclimated fish and 3.92 ± 0.30 for 14°C acclimated fish; hence, the aerobic scope is large enough to allow for activities such as locomotion and reproduction. In comparison, the 24°C acclimated fish appeared to have a comparatively greater VO_{2max} (330.60 ± 20.65 mg O_2 $kg^{-1}h^{-1}$) although this was not significantly different to the other acclimation groups ($P>0.05$). The factorial aerobic scope of the 24°C acclimated fish was 1.89 ± 0.22, which is significantly smaller than the other groups ($P<0.05$) (Fig 3.1).

3.3.2 Heart rate and Ventilation rate

Heart rate was positively correlated with acclimation temperature (R^2=0.9517 for resting rates and R^2=1 for maximum rates) and increased significantly from resting levels during times of VO_{2max} ($P<0.0001$) (Fig 3.2). Heart rate increased by only a factor of approximately 1.5-2 after exhaustive exercise, which is low in comparison to the difference in oxygen consumption during these periods. This difference between parameters is common in other species (Heath 1973). The difference between resting heart rates of the 8, 14 and 24°C acclimated groups (48.42 ± 5.22 min^{-1}, 64.94 ± 3.40 min^{-1} and 146.10 ± 8.52 min^{-1} respectively) were extremely significant ($P<0.0001$). The Q_{10} between the 8 and 14°C acclimated groups was 1.63 and was 2.25 between the 14 and 24°C acclimated groups. The difference in maximum heart rate between the acclimation groups was also extremely significant ($P < 0.0001$) (90.50 ± 6.29 min^{-1}, 130.29 ± 5.40 min^{-1} and 197.80 ± 8.88 min^{-1} respectively) with a Q_{10} of 1.84 between the 8 and 14°C acclimated groups and 1.52 between the 14 and 24°C acclimated groups.

Ventilation rate and acclimation temperature were not as strongly correlated compared to heart rate (Fig. 3.3). This was because the resting (87.09 ± 4.08 min^{-1}) and maximum (96.92 ± 2.92 min^{-1}) rates of the 8°C acclimated fish were not significantly different ($P > 0.05$). Additionally, the resting ventilation rate of the 8°C acclimated fish was not significantly different to that of the 14°C acclimated fish. In comparison, resting and maximum ventilation rates were significantly different in the 14 and 24°C acclimated fish

($P<0.001$) and all maximum rates were positively correlated with acclimation temperature ($R^2=0.9934$).

3.3.3 Recovery from exercise

Oxygen consumption after swimming to exhaustion initially increased with time in all acclimation groups over the first 5-10 minutes (N = 14, 17 and 17 for the 8, 14 and 24°C acclimated fish respectively) (Fig. 3.4). A sharp peak occurred at the 5-minute interval in the 14 and 24°C acclimated fish, indicating that most individuals achieved VO_{2max} at this time. In comparison, the 8°C acclimated fish had a more uniform spread of data over the first 5-10 minutes, which indicates individuals reached VO_{2max} at varying times over this period. Once VO_{2max} was reached there was an exponential decline in VO_2 towards resting levels over the following 90-110 minutes. All three groups had a similar rate of decline in VO_2 over the recovery period; although the 8°C acclimated fish appeared to have a slight delay in recovery. However, the 24°C acclimated fish had a significantly higher VO_2 in comparison to the other groups ($P < 0.0001$) and the VO_2 of the 8°C acclimated fish was significantly lower ($P < 0.0001$). All groups reached VO_2 close to that of resting levels by 90 minutes. An observed increase in VO_2 occurred after each flushing of the respirometer with oxygen-saturated water.

Heart rate remained at high levels in all three groups for the duration of measurement and did not significantly decline with time (N = 7, 10 and 9 for the 8, 14 and 24°C acclimated fish respectively) ($P = 0.5963$) (Fig. 3.5). At the end of the recovery period the heart rates

of the 8 and 14°C acclimated fish were still at approximately 80 and 70% of maximum levels, whereas rates of the 24°C acclimated fish had declined below 50% of maximal levels. Heart rates of the 24°C acclimated fish were significantly higher than the other two groups ($P<0.0001$) and rates of the 8 °C acclimated fish were significantly lower ($P<0.0001$). As with VO_2, flushing of the respirometer caused an initial increase in heart rate, which was also observed with ventilation rate (Fig.3.6).

Ventilation rate showed a small initial increase over the first 10 minutes and then gradually declined towards resting levels (N = 13, 17 and 17 for the 8, 14 and 24°C acclimated fish respectively) (Fig. 3.6). In the 8°C acclimated fish the ventilation rate was not significantly different to that of resting levels at the end of the recovery period. The ventilation rate of the 24°C acclimated fish dropped to approximately 20% of maximum levels and 14°C acclimated fish only dropped to approximately 60% of maximum levels, by the end of the recovery period. The same trend observed in VO_2 and heart rate was also apparent with ventilation rate with the 24°C acclimated fish having a significantly higher ventilation rate than the other two groups ($P<0.0001$) and 8 °C acclimated fish having a significantly lower ventilation rate ($P<0.0001$).

3.3.4 Condition Factor

Condition factors varied between the different acclimation temperatures (Fig. 3.7). The condition factor of the 24°C acclimated fish was significantly lower than the other two groups ($P < 0.01$). The 14°C acclimated fish appeared to have the greatest condition factor, although this was not significantly different to the 8°C acclimated fish ($P > 0.05$).

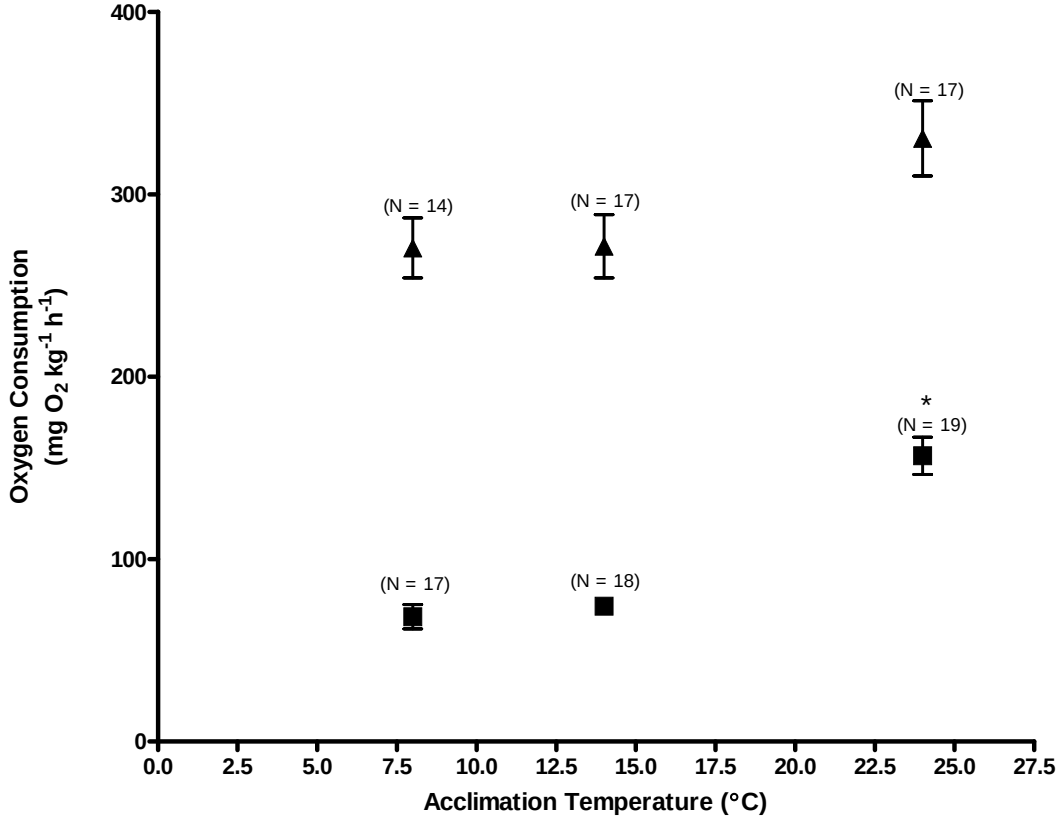

Fig. 3.1 Resting and maximum oxygen consumption (VO_{2rest} and VO_{2max}) of fish acclimated to 8, 14 and 24°C. Scope for activity is the difference between VO_{2rest} (squares, ■) and VO_{2max} (triangles, ▲) and was significantly reduced in the 24°C acclimated fish. Values are the mean ± standard error for each acclimation group. VO_{2max} was achieved by exercise to exhaustion in a swim tunnel. * indicates value is significantly different from other acclimation groups. The resting oxygen consumption of the 14°C acclimated fish has a standard error of 4.22 mg O_2 kg^{-1} h^{-1} but this is too small to appear on the graph.

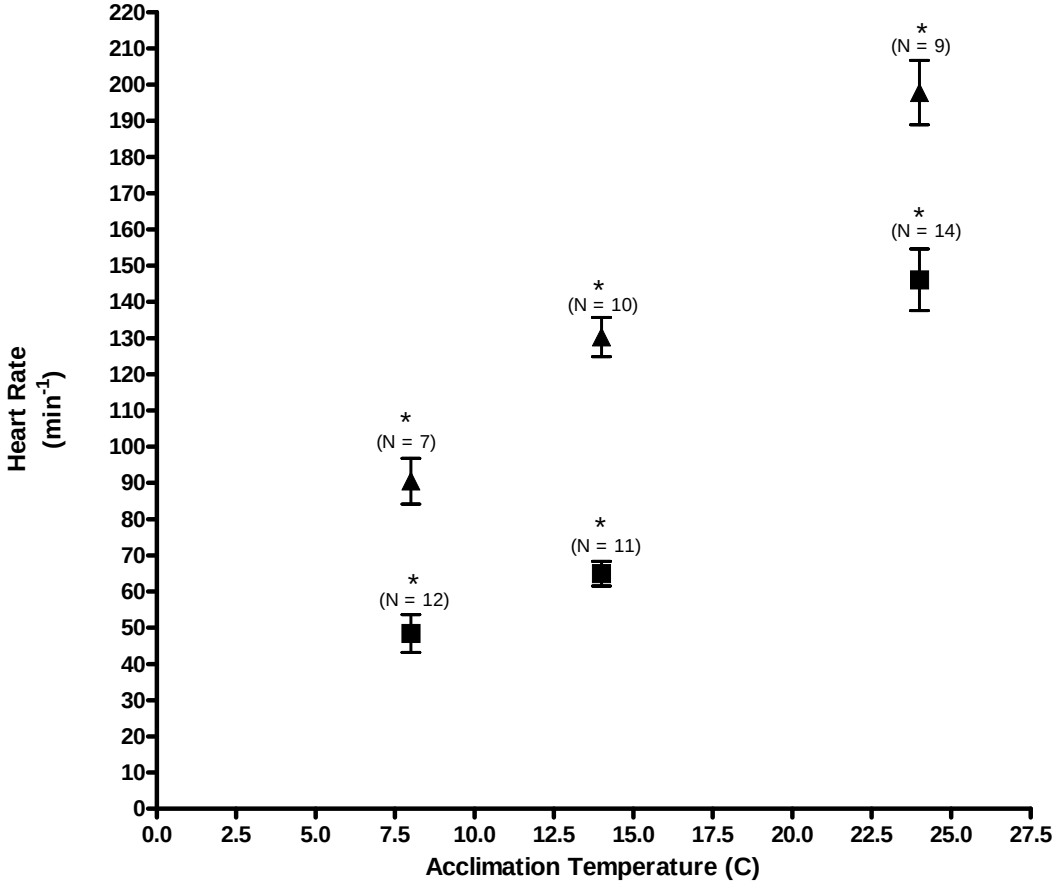

Fig. 3.2 Resting and maximum heart rates of fish acclimated to 8, 14 and 24°C. Heart rate increased significantly with acclimation temperature and maximum heart rate (triangles, ▲) was significantly greater than resting heart rates (squares, ■). * indicates value is significantly different from other acclimation groups.

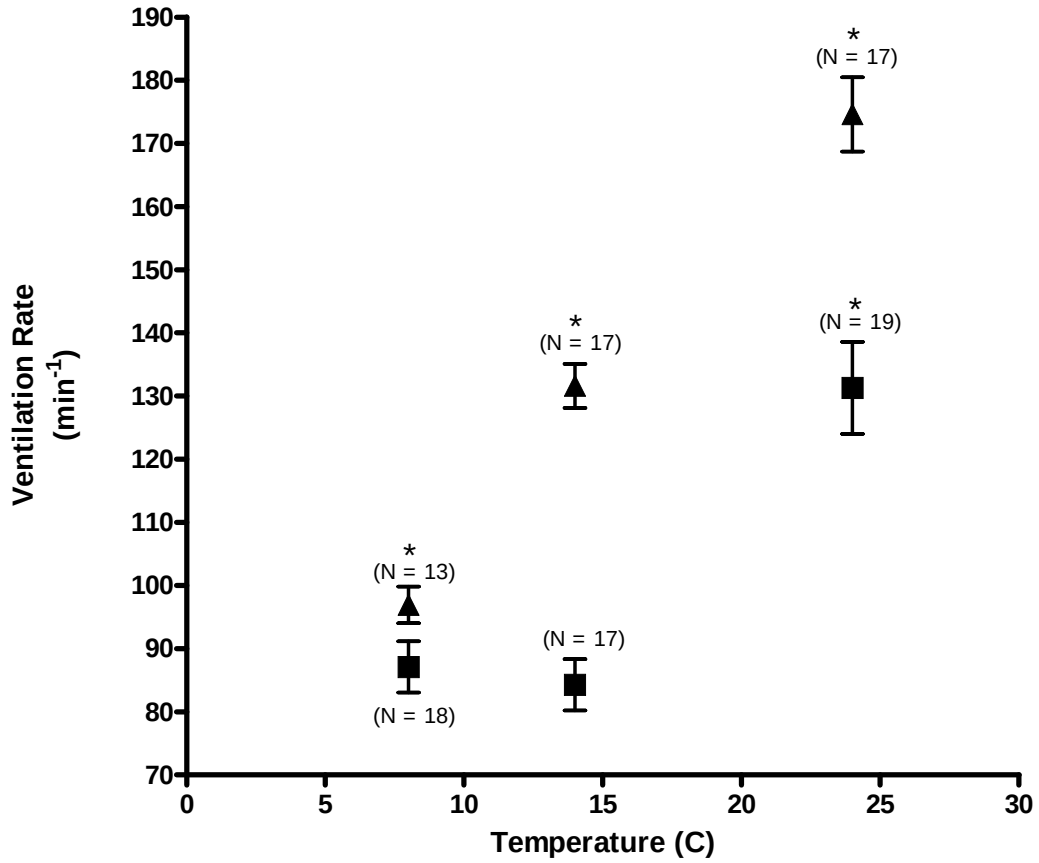

Fig. 3.3 Resting (squares, ■) and maximum (triangles, ▲) ventilation rates of fish acclimated to 8, 14 and 24°C. Maximum rates are significantly different between groups, as are the resting rates of the 24°C acclimated fish compared to the 8 and 14°C acclimated fish. All maximum rates are significantly higher than resting rates. * indicates value is significantly different from other acclimation groups.

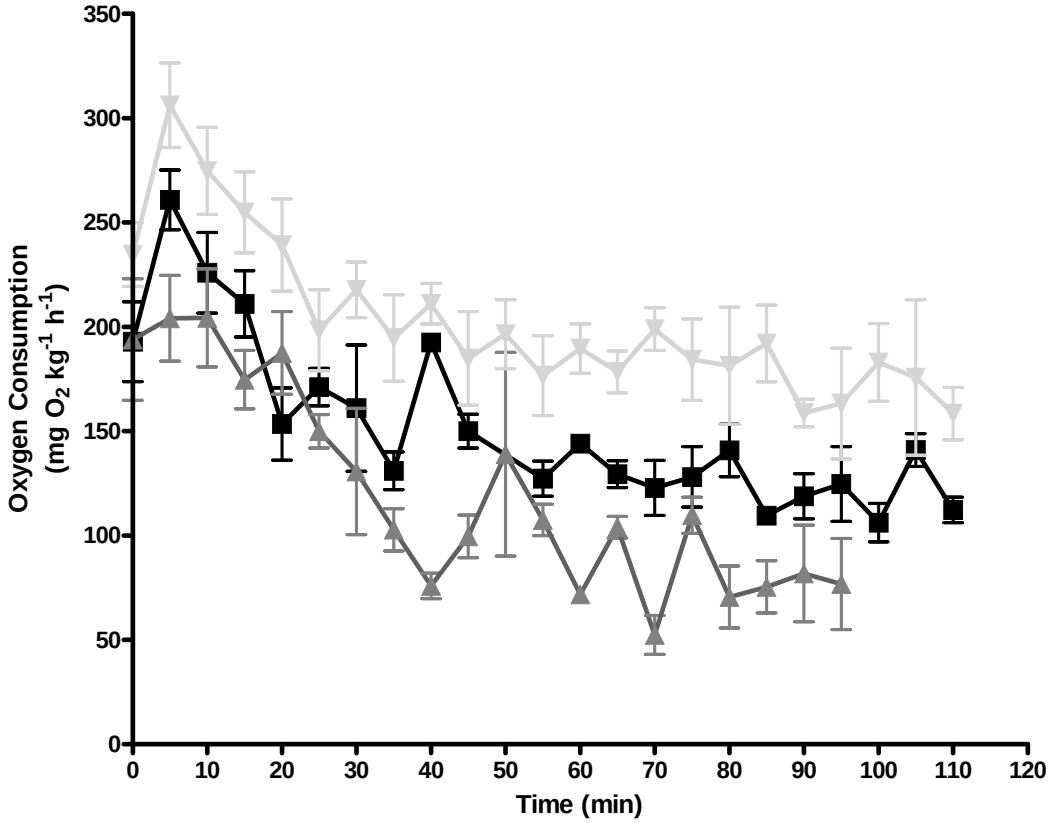

Fig. 3.4 Oxygen consumption of 8, 14 and 24°C acclimated fish immediately after exhaustive exercise and the associated recovery period from VO_{2max}. Oxygen consumption during this recovery period was significantly different between the three acclimation groups (8°C acclimated fish, ▲; 14°C acclimated fish, ■; 24°C acclimated fish, ▼).

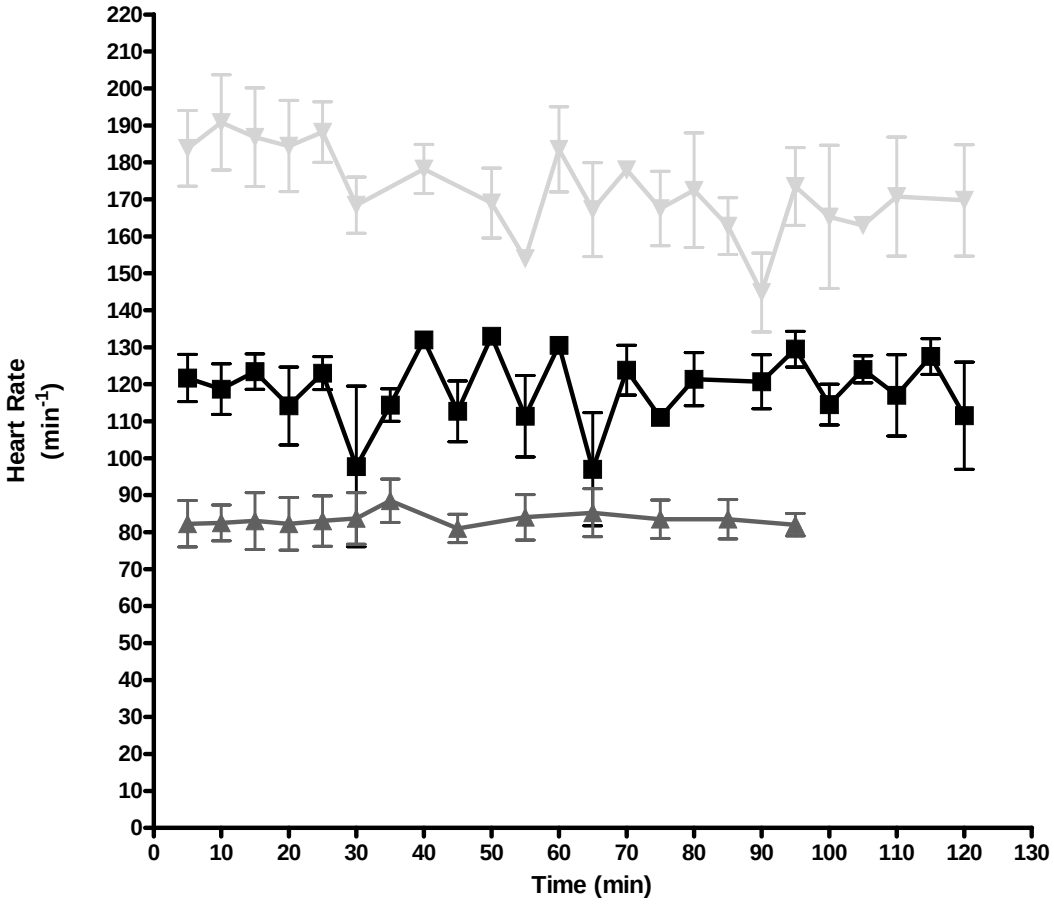

Fig. 3.5 Heart rate following exercise to exhaustion in fish acclimated to 8 (▲), 14 (■) and 24°C (▼). Data includes maximal heart rates and a recovery period of 95-120 minutes. Heart rate differed significantly between acclimation groups.

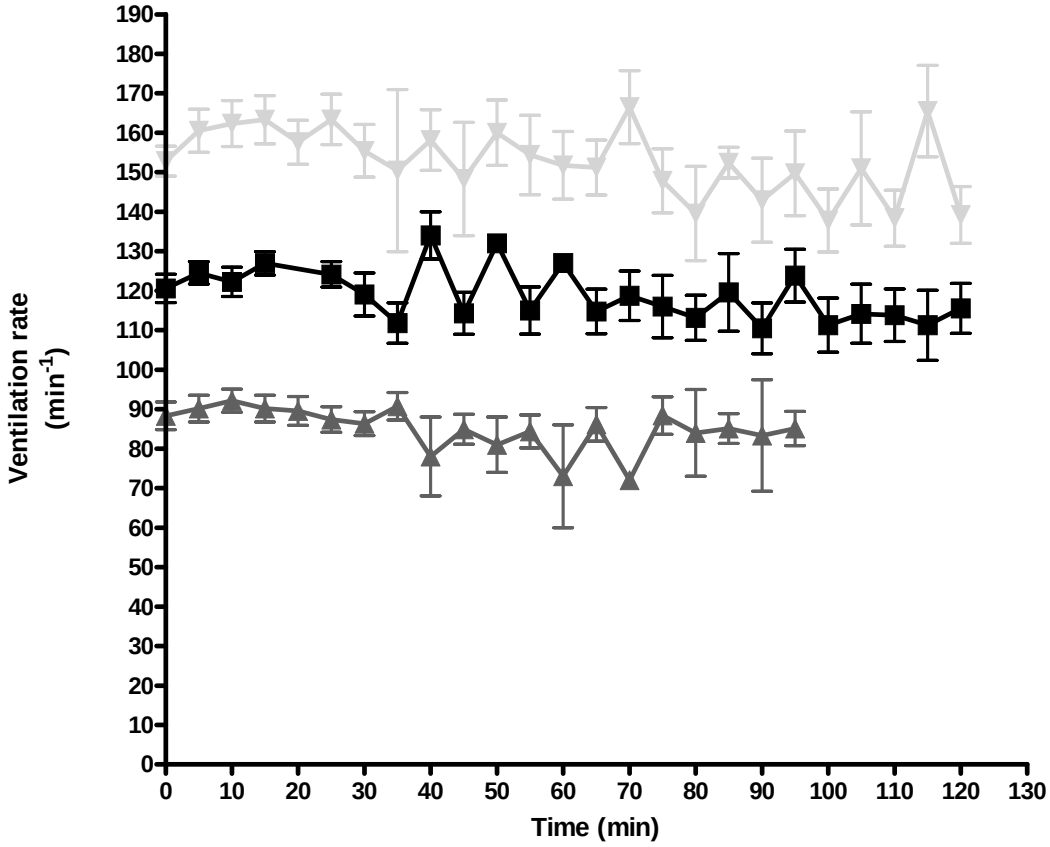

Fig. 3.6 Ventilation rate of fish acclimated to different temperatures after exercise to exhaustion. Rates differed significantly between acclimation groups (8°C acclimated fish, ▲; 14°C acclimated fish, ■; 24°C acclimated fish, ▼).

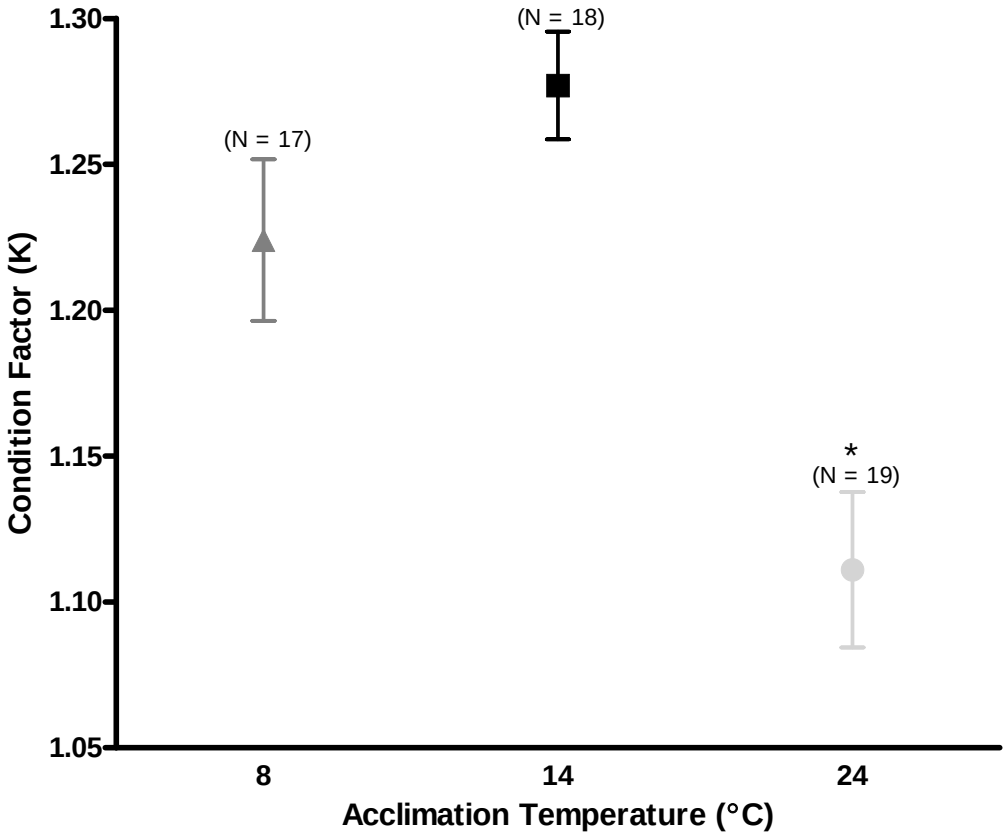

Fig. 3.7 Condition factors of fish acclimated to 8, 14 or 24°C for 28 days. Fish acclimated to 24°C (●) had the lowest body fat content, 14°C (■) acclimated fish had a comparably larger body fat content and 8°C (▲) fish were not significantly different to this group. * indicates value is significantly different from other acclimation groups.

3.4 Discussion

Thermal compensation is required to overcome the high physiological challenges associated with changes in environmental temperature; otherwise animal survival is threatened with long-term exposure (Kinne 1963). The current experiment found thermal compensation does occur at some levels in *N. celidotus* when acclimated to extreme temperatures for 28 days. Thermal compensation was achieved to a much greater degree in cold acclimated fish compared to warm acclimated fish. Maximum and resting oxygen consumption of individual acclimation groups indicated a very high degree of thermal compensation in the 8°C acclimated fish, with little to no compensation in the 24°C acclimated fish. The factorial aerobic scope of the 24°C acclimated fish was significantly smaller than the other two acclimation groups. In comparison, heart rate, ventilation rate and oxygen consumption during recovery from exercise to exhaustion showed substantial shifts with acclimation temperature and little thermal compensation. The condition factors of the different acclimation groups support the idea that temperature is related to physiological stress, which affects the overall condition of the animal.

3.4.1 Maximum and resting oxygen consumption

Maximum oxygen consumption did not differ significantly between the acclimation groups, which is often found with warm acclimation but suggests thermal compensation occurred in the 8°C acclimated fish (Robinson and Davison 2007). Maximum oxygen

consumption is often limited in warm acclimated fish by oxygen carrying capacity and oxygen uptake, so lack of changes in this parameter between the acclimation groups does not necessarily indicate the fish has undergone thermal compensation. In the current study there was a substantial increase in maximum heart and ventilation rate in the 24°C acclimated fish. This allows for a greater volume of oxygenated water to be pumped over the gills per unit time and a greater volume of oxygenated blood reaching tissues (Rantin et al. 2007). However, this response did not achieve an increase in maximum oxygen consumption, which suggests that oxygen consumption in *N. celidotus* is limited by circulatory factors other than heart rate. In some species long-term exposure to high temperatures stimulates erythropoiesis and the release of erythrocytes from the spleen in order to increase the amount of blood haemoglobin available for oxygen uptake (Jensen et al. 1993, Nikinmaa and Salama 1998). A slight rise in maximum oxygen consumption indicates this may happen to a degree in *N. celidotus* but this effect may be counteracted by the rightward shift in the oxygen dissociation curve resulting from high temperatures (Rantin et al. 2007).

In comparison, the maximum oxygen consumption of the 8°C acclimated fish was similar to the 14°C acclimated fish. In cooler waters maximum oxygen consumption is decreased due to reduced metabolism and reduced contraction velocities and frequencies of the skeletal, cardiac and respiratory muscles (Rantin et al. 2007). This diminishes the performance of the fish and important activities such as predator escape and prey capture are compromised. Thermal compensation is needed to overcome this by increasing metabolism and the ability for efficient muscle contractions, which allows the fish to

perform at its best (Kinne 1963). Resting oxygen consumption of the 8°C acclimated fish was also similar to the 14°C acclimated fish due to thermal compensation. This high resting metabolism is beneficial to the fish as it causes an overall increase in activity allowing for better locomotion, growth, digestion and reproduction, hence, increases fish survival. These findings are consistent with the concept of metabolic cold adaptation, in which metabolic rates are elevated from levels expected at the experimental temperature (Holeton 1974, Clarke 1991). Although this concept has received a great deal of criticism it is commonly applied to polar fish, and indicates full thermal compensation has been achieved which is crucial for survival in cold habitats (Holeton 1974, Clarke 1991).

The resting oxygen consumption of the 24°C acclimated fish was significantly higher than the other two groups, suggesting a high oxygen demand was present and thermal compensation had not occurred. The factorial aerobic scope for activity of these fish was 1.89 ± 0.22, which was significantly lower than the factorial aerobic scopes of the 8 and 14°C acclimated fish (4.02 ± 0.70 and 3.92 ± 0.30 respectively). The scope for activity found for *N. celidotus* in its natural environmental temperature (14°C) is low in comparison to other polar, tropical and temperate fish, which have a factorial aerobic scope of approximately 6-7 (Duthie 1982, Lee et al. 2003, Lowe and Davison 2006). The reduced aerobic scope suggests no thermal compensation occurred in the 24°C acclimated fish (Fry 1971). However, it is possible partial compensation of resting oxygen consumption occurred, which would have resulted in the aerobic scope gradually increasing over the acclimation period. An additional experiment investigating the resting oxygen consumption of fish acclimating to 8 and 24°C at day 10, 20 and 28 of the

acclimation period supports this (Appendix 1). However, small sample sizes (N=3) in this experiment prevent accurate statistical analysis so conclusions cannot be made confidently. This experiment found the resting oxygen consumption of both acclimation groups moved in the direction predicted by thermal compensation during the acclimation period. Therefore, it is possible partial thermal compensation did occur to a certain degree in fish acclimated to 24°C but this needs to be further investigated.

3.4.2 Heart rate, ventilation rate and recovery from exercise to exhaustion

Heart rate and ventilation rate both showed substantial temperature responses as is expected with acute temperature changes, especially in heart rate whose single most important determinant is temperature (Lowe et al. 2005). Resting and maximal levels both increased with temperature, with the exception of the resting ventilation rate of the 8°C acclimated fish. This resting ventilation rate of the 8°C acclimated fish was the same as the maximum ventilation rate of this group and the resting ventilation rate of the 14°C acclimated fish, which suggests full thermal acclimation of this parameter. However, experimental observations found ventilation to be very shallow during this time, which would result in a decreased respiratory stroke volume. This consequently reduces the amount of water that perfuses the gills so less oxygen is available for diffusion across epithelial cells (Rantin et al. 2007). At maximum ventilation rates ventilation was much deeper, creating a larger respiratory stroke volume so greater oxygen perfusion, which is needed to increase in oxygen consumption to maximal levels. Increases in respiratory

stroke volume are a common response to increasing oxygen demands, while ventilation frequency remains unchanged (Smith and Jones 1981).

The positive correlation of heart and ventilation rate with temperature suggests cardiac and respiratory muscles are highly sensitive to temperature change in *N. celidotus* and do not undergo thermal compensation. Studies on Antarctic fish by Robinson and Davison (2007) found a different response with full thermal compensation of the ventilation rate. This is surprising as Antarctic fish are stenotherms so thermal flexibility is expected to be less than in eurytherms, such as *N. celidotus.* A further study by Aho and Vornanen (2001) found adaptations to heart rate via partial thermal compensation when rainbow trout was acclimated to different temperatures. The positive correlation of both heart and ventilation rate with temperature in *N. celidotus* suggests cardio-ventilatory coupling. Cardio-ventilatory synchrony is thought to exist at certain times in fishes and to be sensitive to many environmental conditions (Hughes 1972, Butler and Metcalfe 1983). It has been observed in some species under temperature stress and during MS 222 anaesthesia and hypoxia (Randall and Smith 1967, Heath and Hughes 1971). In the current experiment the concept of cardio-ventilatory coupling is supported with a 1:1 ratio between heart and ventilation rate during resting and maximum metabolic rates. This was observed in all acclimation groups with the exception of resting metabolism in the 8°C acclimated fish, in which a 1:2 ratio existed.

Recovery from exercise to exhaustion also shows a strong acclimation temperature response. Oxygen consumption, heart rate and ventilation rate are all significantly higher

in the 24°C acclimated fish and significantly lower in the 8°C fish. However, acclimation groups show similar rates of declines over the recovery period suggesting that the functionality of these systems is not jeopardized with temperature.

3.4.3 Condition factor

The fish acclimated to 24°C underwent little-to-no thermal compensation, hence, had high energy costs to fuel increased metabolism and associated increases in heart and ventilation rates for the entire acclimation period. Additionally, these fish had very diminished appetites before feeding was stopped, and had high metabolic oxygen demands and activity levels which may have resulted in blood flow to the intestines being redistributed, so the absorption of any food that was consumed may have been limited (Thorarensen et al. 1993). Detrimentally, if the energy intake drops below the amount required for maintenance the fish starts to consume its own energy stores. The comparably low condition factor of these fish support the idea of high physiological stress of fish exposed to high temperatures, which results in high utilization of energy stores and over time a low body fat content. The lower energy costs of the 8 and 14°C acclimated fish would result in them having larger energy stores than the 24°C acclimated fish and hence a larger condition factor.

3.4.4 Summary

The aerobic scope was not significantly different between the 8 and 14°C acclimated fish, indicating thermal compensation. However, the aerobic scope of the 24°C acclimated fish was significantly reduced, which suggests no thermal compensation, or only partial compensation was achieved in this group. The resting and maximum oxygen consumption of the 8°C acclimated fish was similar to the 14°C acclimated fish, which supports metabolic cold adaptation and full thermal compensation in cold acclimated fish. In contrast, fish acclimated to 24°C had a significantly larger resting oxygen consumption, which was the cause of the reduced aerobic scope for activity. Heart rate and ventilation rate showed high temperature sensitivity and no thermal compensation. Recovery from exercise to exhaustion in terms of oxygen consumption, heart rate and ventilation rate showed very similar trends, however, all parameters were significantly higher in 24°C acclimated fish and significantly lower in 8°C acclimated fish. The high condition factor in the 8 and 14°C acclimated fish support the idea that these temperatures incur low physiological stress for *N. celidotus* and the significantly lower condition factor of the 24°C acclimated fish support the idea that these fish are at a very high level of physiological stress.

4. Swimming Ability

4.1 Introduction

Locomotion is achieved in fish by myotomal muscle contractions on alternating sides of the body, which generates waves of flexion that travel the length of the body, from nose to caudal fin (Lindsey 1978). Additionally, undulations or oscillations of the pectoral fins can also achieve locomotion (Lindsey 1978). These motions exert a force against the surrounding water column, which propels the fish in the opposite direction (Willmer et al. 2000). The mode of locomotion differs with species depending on morphology and the swimming activity level (Lindsey 1978). *N. celidotus* is regarded as a labriform swimmer as locomotion is dominantly at slow speeds via oscillations of the pectoral fins (Tuckey and Davison 2004). This labriform swimming is common in reef dwelling fish as it allows for increased maneuverability; the benefits of this override the associated trade-off in speed in these habitats (Blake 1983). As swimming speeds increase to intermediate levels intermittent use of carangiform swimming is employed, which involves myotomal muscle contractions at the posterior end of the body (Tuckey and Davison 2004). At high swimming speeds carangiform swimming is continuous, which leads to rapid fatigue as it involves the use of anaerobic muscle (Tuckey and Davison 2004).

Muscular tissue forms a large part of the body mass of fish, with approximately 40-60% of the total body mass being locomotor musculature (Bone 1978). This is required to

generate the sufficient amount of power needed for rapid swimming (Bone 1978). These muscle fibres are separated into red, white and pink fibres in fish, each with different properties and functions. Red muscle fibres are utilized during slow swimming speeds and are non-fatiguing (Bone 1978). They are very well oxygenated due to a small diameter, high capillary density and abundance of myoglobin and have a high mitochondrial density, which allows for efficient aerobic function during activation and is the reason for these muscles being non-fatiguing (Bone 1978). Mitochondrial density is inversely related to the number of muscle fibres, hence, red muscle has a comparatively low amount of muscle fibres (Bone 1978). This decreases the ability for strong muscle contractions, so sufficient power for fast swimming speeds cannot be produced. White muscle fibres are utilized for fast swimming speeds but fatigue rapidly (Bone 1978). These fibres are poorly vascularized, do not posses myoglobin and have a low mitochondrial density, which means activity of these muscles is fueled by anaerobic activity and can only function until glycogen resources are exhausted, with a resulting production of lactate (Johnston 1981). The low mitochondrial density gives rise to a high content of muscle fibrils, which allows for sufficient power for fast swimming speeds. There is also propagation of action potentials in white muscle fibres, which allows for action potentials from a single stimulus to be conducted long distances causing a greater number of muscle fibril contractions contributing to fast swimming speeds: there is no propagation in red muscle fibres (Bone 1978). Pink muscle fibres are an intermediate between the two and are utilized at intermediate speeds (Bone 1978).

In fish, the muscle fibres are anatomically separated within the myotome, with red muscle fibres usually located subcutaneously, white muscle fibres deeper within the body and pink muscle fibres located between the two (Johnston 1981). However, studies by Davison (1988) have found that the specialized labriform locomotion of *N. celidotus* has resulted in a modification of the location of these muscle fibres. As the pectoral fins are the sole producer of slow swimming speeds, which is the dominant mode of locomotion in these fish, there is a high content of red muscle fibres powering the pectoral fins (Davison 1988). Pectoral fins continue to produce locomotion during high swimming speeds, which is produced by the additional presence of white muscle fibres. Carangiform swimming is restricted to fast, burst swimming, which has resulted in the myotome losing all red muscle fibres and consisting only of white muscle fibres and leads to rapid fatigue when this mode of swimming is employed (Davison 1988).

The intensity of swimming differs in fish depending on speed requirements and is classified into three classes, which are burst swimming, sustained swimming and prolonged swimming (Beamish 1978). Burst swimming is considered as high speed swimming that lasts less than 20 seconds and ends in fatigue, hence utilizes white muscle fibres (Beamish 1978). This consists of an initial acceleration phase of unsteady swimming followed by a steady phase termed sprint, before exhaustion is reached (Beamish 1978). Behaviours involving predator avoidance, prey capture and negotiation of rapid currents employ this mode of swimming (Beamish 1978). Sustained swimming is considered as slow speeds that can be maintained for extended periods (more than 200 minutes) and do not end in fatigue, hence utilize red muscle fibres (Beamish 1978). This

mode of swimming is employed for behaviours such as migration, schooling, foraging and maintaining position in the water column against buoyancy and current pressures (Beamish 1978). Prolonged swimming is an intermediate mode of swimming and lasts between 20 seconds and 200 minutes and usually ends in fatigue, it requires the use of both red and white muscle fibres (Beamish 1978). Prolonged swimming is used in the laboratory to determine the swimming ability of fish. It is measured as the critical swimming speed (Ucrit), which involves swimming the fish in a flume with incremental increases in water velocity until the fish reaches exhaustion (Beamish 1978).

Temperature has a large effect on the critical swimming speed of fish due to direct thermodynamic effects on swimming muscles and indirect effects regarding the delivery of oxygen to muscles. Optimal swimming ability is achieved within temperatures the fish are exposed to in their natural environment; ability is reduced with temperature shifts in either direction from this optimal level. Decreases in temperature reduce the swimming ability due to insufficient red and white muscle contraction rates (Day and Butler 2005). The velocity and frequency of muscle contractions are determined by reaction rates, so decreases in rates ultimately result in reduced muscle contraction velocity and frequency. Thermal compensation in cooler temperatures involves increases in capillary and mitochondrial density in red muscle fibres, which increases energy production and blood flow (Day and Butler 2005). Alterations in myosin heavy and light chains and increases in the amount of red muscle fibres produce faster contracting and higher tension fibre types (Ball and Johnston 1996). Additionally, muscle enzyme quantity and affinity increase, which increases the chance of a reaction occurring (Hochachka and Somero

1984). All of these modifications increase the force and velocity of muscle contraction in cold temperatures, which allow for increased swimming speeds and greater swimming ability.

In comparison, decreases in swimming ability with increased temperatures are associated with limitations in oxygen delivery to the muscles. This is due to decreased water oxygen tensions, high oxygen demands of the whole body and limitations on oxygen transport and uptake (see chapter 1). Therefore, the efficiency of the respiratory and circulatory system at high temperatures and aerobic scope for activity ultimately determine the swimming ability of the fish, in regard to meeting swimming muscle oxygen demands (see chapter 3). Therefore, high temperatures most likely affect aerobic red muscle fibres, which are active at all swimming speeds. Thermal compensation is required at the whole animal level to decrease resting metabolism, which then increases the scope for activity so sufficient oxygen is available to fuel locomotor muscle activity (Randall et al. 2002). This is achieved by decreasing the enzyme affinity and quantity in the whole body, including swimming muscles (Hochachka and Somero 1984). Additionally, alterations to the circulatory system to increase the oxygen carrying capacity can improve swimming ability (see chapter 3).

4.1.1 Current Experiment

The current experiment investigated the prolonged swimming ability (Ucrit) of *N. celidotus* acclimated to 8, 14 or 24°C. The purpose of this was to determine whether or

not thermal compensation at the level of the locomotory musculature is achieved. Swimming ability is an essential activity needed for such behaviours as predator escape, prey capture and reproduction. Therefore, for *N. celidotus* to be able to survive in the warming temperatures associated with global warming it must have the ability to undergo thermal compensation. If thermal compensation is not achieved then *N. celidotus* will have to migrate to cooler waters to ensure survival.

4.2 Materials and Methods

These experiments were conducted in an 80L Blazka-style swimming tunnel on the same day as oxygen consumption experiments. The swimming tunnel is made of clear perspex with plastic mesh at each end (Fig. 4.1). A revolving impeller generated water flow and the velocity of the impeller could be altered during the experiment to alter the speed of water flow. Preliminary studies found that the fish would not swim in the tunnel unless the front end was covered in dark plastic. *N. celidotus* often seeks cover in its natural environment, so the dark plastic most likely encouraged the fish to swim, as it could be used as cover (Carbines 1993). Experiments by Davison (1988) encountered the same problem and used a cloth over the front end of the swim tunnel to achieve swimming.

During experiments on the 8 and 14°C acclimated fish the swim tunnel was stationed in the aquarium room, which has an air temperature of 10°C. This was done to prevent significant water temperature change during the experiment. The temperature was

maintained sufficiently at 14°C but to maintain 8°C cold seawater had to be added throughout the experiment. Seawater was cooled in the University's Antarctic room prior to the experiment, to first fill the swim tunnel at the beginning of the experiment and then for additional use throughout the experiment. During experiments on the 24°C acclimated fish the swim tunnel was stationed in a laboratory, this allowed for a warmer air temperature to help maintain a constant 24°C throughout the experiment. Seawater was heated prior to the experiment by the use of aquarium heaters to achieve the required 24°C. Temperature was maintained throughout the experiment, with the exception of some experiments where the fish swam for extended periods of time. In these experiments the temperature started to increase and cold water had to be added to the tunnel to maintain 24°C. The temperature did not vary by more than 0.8°C over all experiments.

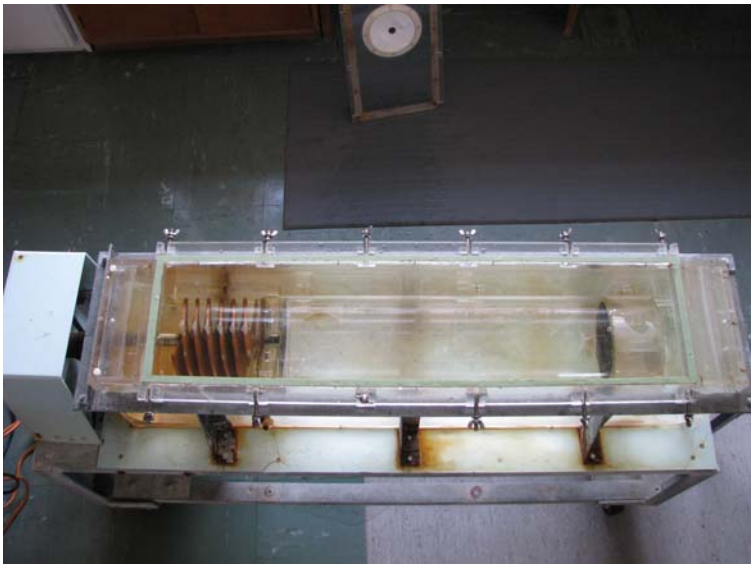

Fig. 4.1 Blazka-style swimming tunnel used for Ucrit experiments.

ECG wires were still attached to the fish during Ucrit experiments to allow for heart rate measurements of VO_{2max} (see sections 2.4 and 3.2). Therefore, to prevent the wires from being suctioned into the impeller they were secured with suture thread at lengths no longer than that of the fish. This may reduce the Ucrit of the fish in these experiments due to the production of a drag force. However, the diameter of the wire is only 0.0055 inches so the drag force produced by this is likely to be minimal and as all fish were subjected to the same treatment it does not alter the outcome of the experiment.

An individual fish was initially placed in the swim tunnel with a water flow of 0.5 body lengths per second (bl s^{-1}) for 15 minutes, which was essentially allocated as an acclimation period. After 15 minutes the experiment officially began and the water velocity was increased to 1.5 bl s^{-1}. From this point on the water velocity was increased by 0.5 bl s^{-1} every 15 minutes until the fish reached exhaustion. Exhaustion is defined as the point in which a fish can no longer maintain position in the chamber against the current and becomes impinged on the rear mesh of the swim tunnel (Kolok 1999). To ensure exhaustion was reached the fish was manually placed back into the water current after the first two instances of becoming impinged on the mesh. This method of manual replacement was also employed by Gregory and Wood (1998) for Ucrit measurements of rainbow trout. Time of exhaustion was recorded at the point the fish was impinged on the mesh for the third time, swimming velocity at this time was also recorded.

4.2.1 Data Analysis

Ucrit was determined using the equation (Brett 1964): -

$$\mathbf{Ucrit = Uf + ([Tf/Ti]Ui)}$$

Where, Uf = the highest speed maintained for the entire speed increment (15 minutes), Tf = the time taken to reach exhaustion in the final speed interval, Ti = the time interval length (15 minutes) and Ui = the speed increment (0.5 bl s^{-1}).

GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA) was used for statistical analysis and graphing. A Kruskal-Wallis non parametric one way analysis of variance (ANOVA) with Dunn's Multiple Comparison post-hoc test was used to compare the Ucrit between the acclimation groups. The standard one-way analysis of variance (ANOVA) test could not be used as the Bartlett's test found the variance between groups differed. Equal variance is a prerequisite for ANOVA. Statistical significance was taken as $P < 0.05$ and values recorded are mean and associated standard error.

4.3 Results

Initially fish swam using purely labriform locomotion and easily maintained position under the dark plastic, at the front end of the swimming tunnel. As speeds increased,

pectoral fin movements become more rapid and the fish regularly lost position from under the plastic cover, and had to further increase pectoral fin movements to produce forward movement. It was not until high speeds were reached that the fish began using carangiform swimming and at first this was used only intermittently to produce forward motion. This worked to propel the fish forward when it had lost position from under the plastic cover. For a short duration before the fish reached exhaustion carangiform swimming was employed continuously. Labriform swimming was used for the entire duration of the experiment.

The Ucrit of 24°C acclimated fish (2.38 ± 0.20) was significantly lower than the other two acclimation groups (Fig. 4.2). The Ucrit of the 8 and 14°C acclimated fish (2.70 ± 0.07 and 3.26 ± 0.08 respectively) were not significantly different with a Q_{10} of 1.37 (Fig. 4.2). Two individual fish out-swam the swimming tunnel, hence, never reached the point of exhaustion. Both of these fish were 14°C acclimated fish. On one occasion a fish reached exhaustion during the acclimation period, this was a 24° acclimated fish. Results of condition factor measurements are re-introduced here to show the comparison with Ucrit results (Fig 4.3). Both show that the 14°C acclimated fish have the best performance, followed closely by the 8°C acclimated fish, and the 24°C acclimated fish perform significantly worse than the other two acclimation groups (Fig. 4.2 and Fig 4.3). This indicates temperature has immediate effects on swimming ability as well as indirect, long-term effects that alter the amount of energy stores available to fuel locomotion.

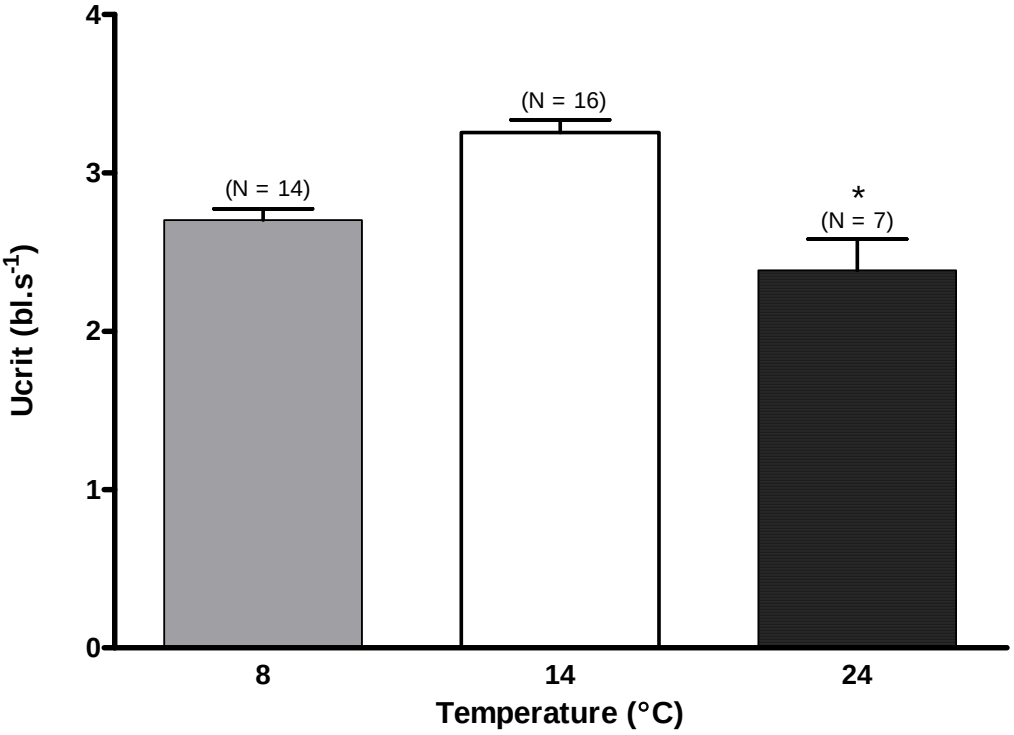

Fig. 4.2 The Ucrit of fish acclimated to 8, 14 and 24°C. The fish were swum to exhaustion in a swimming tunnel as the water current velocity increased by 0.5 bl s^{-1} every 15 minutes. * indicates value is significantly different from other acclimation groups.

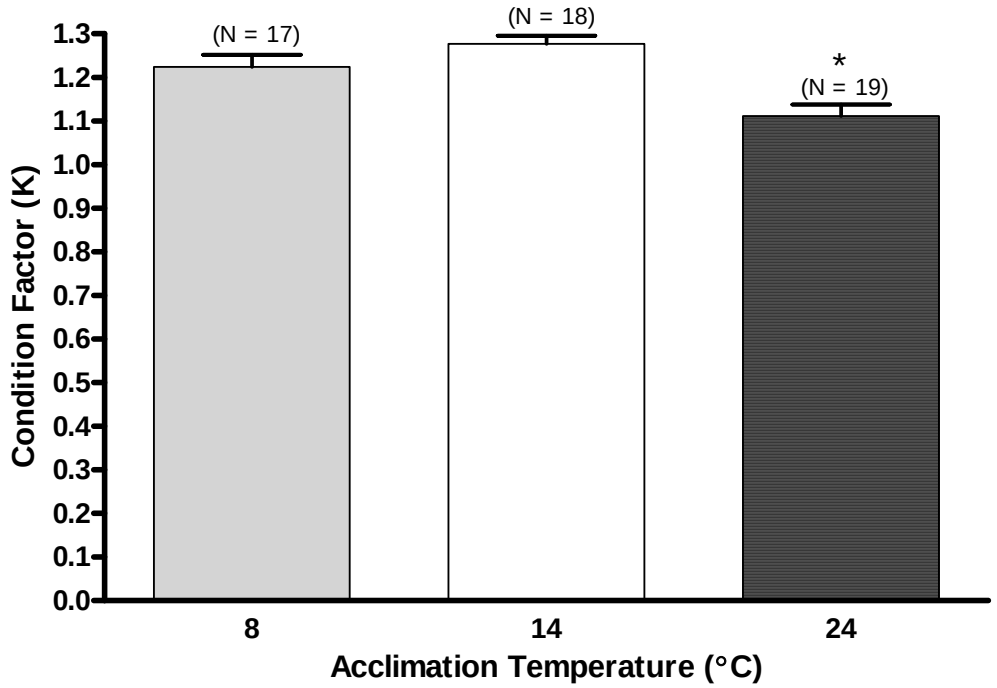

Fig. 4.3 Condition factors of fish acclimated to 8, 14 or 24°C for 28 days. * indicates value is significantly different from other acclimation groups.

4.4 Discussion

The swimming ability of a fish has a large impact on overall performance, as it is an essential component of many activities. Feeding, reproduction, foraging and the guarding of territory are all required for survival and are dependent on the efficiency of burst, prolonged and sustained swimming (Beamish 1978). Therefore, thermal compensation is imperative with changing temperatures, to overcome the associated physiological stresses and maintain optimal swimming ability. The current experiment found that after a 28 day acclimation period, thermal compensation had occurred in the 8°C acclimated fish as the Ucrit of this group was not significantly different to the 14°C acclimated fish (Fig. 4.2). However, there was no thermal compensation in the 24°C acclimated fish, as this group had the lowest Ucrit (Fig. 4.2). Higher temperatures are associated with high physiological stress, which can cause not only an immediate effect on aerobic scope but also an accumulative response on fish condition (Beamish 1978). Additionally, high and low temperatures incur different physiological constraints on swimming ability, which consequently require different mechanisms to achieve thermal compensation. High temperatures require alterations to the metabolism of the animal and to circulatory and respiratory systems in order to improve swimming abilities. In comparison cold temperatures predominantly require modifications to muscles, which is a much less complex process.

In cold temperatures the swimming ability is limited by the muscle contraction velocity and frequency (Day and Butler 2005). Generally, oxygen demands of the muscles are met

as the overall demand on the body is low and oxygen tension of the water is high (Rantin et al. 2007). Oxygen delivery only becomes a limiting factor at the critical thermal minimum, at which the functionality of respiratory and cardiac function is diminished preventing sufficient oxygen uptake and transport (Randall et al.2002). This leads to a loss of homeostasis and a cold induced coma, which is closely followed by death (Randall et al. 2002). However, these temperatures were not reached in the current experiment. Therefore, in resting states the oxygen demands are easily met due to low metabolic rates, which results in low physiological stress in fish subjected to cold temperatures (Kinne 1963).

In comparison, the swimming ability of fish at high temperatures is limited by the animal's ability to met oxygen demands. This has the greatest effect on swimming produced by the aerobic red muscle fibres, which means even slow swimming speeds are limited. The resting metabolic rate of the fish is increased at high temperatures, creating a large oxygen and metabolite demand, which reduces the scope for activity of the fish and creates high physiological stress even during resting states. Detrimentally, high metabolic oxygen demands of organs, essential to maintain immediate survival at high temperatures, causes blood flow to be redistributed from organs such as the intestine, which reduces nutrient absorption and transport (Thorarensen et al. 1993). High temperature is also a suppressor of appetite, which reduces the food uptake and associated supply of metabolites (Beamish 1978, Klesse 1996). This low energy uptake requires energy stores to be utilized to a large extent at resting states and further more during activity, which causes a large reduction in fish condition over time (Fig. 4.3).

Therefore, at high temperatures the swimming ability of fish is limited by the aerobic scope for activity and the decreased condition of the animal.

The Ucrit of the 14°C acclimated fish (3.26 ± 0.08) from the current experiment is consistent with the Ucrit found in experiments by Davison (1988) and Tuckey and Davison (2004) (3.64 ± 0.08 and 2.71 ± 0.07 respectively). Additionally, the Ucrit found by Tuckey and Davison (2004) is almost identical to that of the 8°C acclimated fish from the current experiment. This supports the idea that the 8°C acclimated fish have achieved thermal compensation to increase their swimming ability. However, the swimming ability of the 24°C acclimated fish was significantly less in comparison to all studies, which indicates thermal compensation was not achieved.

Thermal compensation in the 8°C acclimated fish involves alterations predominantly at the level of the muscles. Alterations include increases in mitochondrial density and enzyme quantity and affinity to increase energy production and reaction rates (Day and Butler 2005). Additionally, the myosin heavy and light chains are altered to allow for faster muscle contraction and frequencies (Ball and Johnston 1996). In comparison, thermal compensation in the 24°C acclimated fish involves alterations at the level of the whole animal. This is needed to reduce the resting metabolic rate of the animal to allow for a greater scope for activity, and increase oxygen uptake and transport by modifications to the circulatory and respiratory systems (Kinne 1963, Driedzic 1992, Randall et al. 2002). Therefore, it is likely modifications at the muscular level are much easier to achieve compared to complex modifications of the whole animal. This would

allow for a large degree of thermal compensation at low temperatures compared to high temperatures and could be an additional factor in the difference between swimming abilities of the 8 and 24°C acclimated fish.

4.4.1 Summary

The critical swimming speed of the 8°C acclimated fish was significantly higher than the 24°C acclimated fish. This is because low temperatures incur lower physiological stress so have less of an effect on swimming ability and thermal compensation is less complex than at high temperatures. Low temperatures simply affect the contraction efficiency of locomotor muscles, whereas high temperatures decrease the availability of oxygen for the locomotor muscles and this requires whole body modifications to overcome.

5. Thermal Tolerance

5.1 Introduction

Eurytherms are exposed to considerable temperature fluctuations in their environment, so require the ability to tolerate a large range of temperatures for survival (Randall et al. 2002). The ability to shift this tolerance range between summer and winter regimes is also a necessity, to ensure optimal performance and ultimately survival throughout the seasons (Pörtner 2001). Thermal tolerance ranges differ between species depending on the range of environmental temperature fluctuations to which they are exposed (Brett 1956). The width of this range is limited by the ability of the animal to meet oxygen demands (Pörtner 2001). Oxygen limitations at the extremes of the tolerance range cause a transition to anaerobic metabolism followed closely by functional failure of the whole animal (Pörtner 2001). A major outcome of this is the disorganization of locomotor activity and loss of the animal's ability to escape from conditions that will promptly lead to its death (Scott 1987). Therefore, as first discovered by Fry (1947), environmental temperature can act as a lethal factor when its effect is to destroy the integrity of an organism. Optimal performance of the animal is associated with the ability to reach maximal PaO_2, which occurs over a narrow range of "optimum" temperatures that are well within the thermal tolerance range (Pörtner 2001). In the laboratory the thermal tolerance range is determined using the critical thermal methodology (CTM). The critical thermal maximum (CTMax) and critical thermal minimum (CTMin) are determined by exposing an animal to a constant rate of water temperature increase or decrease

(respectively) until a non-lethal endpoint is reached (Mora and Ospina 2002, Schulte 2007). This endpoint is defined as the loss of equilibrium, which is observed as the animal losing the ability to stay upright in the water column (Scott 1987, Mora and Ospina 2002). This indicates the loss of homeostasis of the animal due to oxygen limitations destroying animal functionality.

Optimum temperatures are associated with maximum aerobic energy available for the functioning of all physiological factors, including respiration, circulation, metabolism, locomotion, growth, reproduction and digestion (Pörtner 2001). As temperatures exceed the narrow optimal temperature range and approach the thermal tolerance extremes there is a sequential impairment of some of these physiological factors (Pörtner 2001). This is due to limitations on oxygen supply meaning the oxygen demands cannot be met for all physiological activities. Growth and reproduction are among the first activities to become oxygen limited (Brett 1956). Metabolic maintenance, the most essential component of immediate survival gets priority over oxygen supply so is the last physiological factor to deteriorate before loss of equilibrium (Pörtner 2001). As activities, such as reproduction and growth are necessary for long-term survival, the onset of oxygen limitations for these activities essentially represent a long-term thermal tolerance range, any tolerance past this point is time limited (Brett 1956). Therefore, the thermal tolerance range determined by a constant temperature change over a matter of hours, is much greater than the long-term thermal tolerance range. However, the critical thermal methodology is an ecologically relevant measure, as fish may be exposed to acute temperature fluctuations of these magnitudes in their natural environment (Currie et al. 1998).

The onset of oxygen limitations with decreasing temperatures are associated with a reduction in heart and ventilation rates (see section 3.1. and 3.4.). This results in oxygen uptake and transport being insufficient in its ability to meet demands, hence, there is a decrease in aerobic scope (Pörtner 2001). In comparison, oxygen limitations due to increases in temperature arise from a related increase in oxygen demand. This demand cannot be met when it exceeds the maximum oxygen consumption of the animal, which again results in a reduced aerobic scope (Pörtner 2001). Changes in the distribution of fish populations due to global warming have been found to be caused by limitations of cardiac function (Pörtner and Knust 2007). Other physiological effects of high temperatures include loss of protein function, neuromuscular failure, and the heat shock response (Römisch and Matheson 2003). However, these occur immediately prior to death, it is oxygen limitations that are primarily responsible for functionality failure and the onset of sequential physiological deteriorations leading to death (Pörtner 2001).

Exceeding both CTMax and CTMin results in arterial hypoxia so the animal is forced to employ anaerobic metabolism. This occurs firstly in tissues with a high oxygen demand such as the liver, cardiac muscles and respiratory muscles (Pörtner 2001). Anaerobic metabolism of the heart and respiratory muscles result in the transition towards death as it causes further reductions in oxygen delivery, which is closely followed by failure of animal functionality (Lannig et al. 2004). Marine fish deaths from natural environmental temperature fluctuations are predominantly due to cold exposure, with only a few reported cases of death due to exposure to high temperatures (Brett 1956). Severe cold fronts occurring at approximately 10 yearly intervals across south-eastern U.S.A. have

resulted in hundreds of millions of fish mortalities (Gunter and Hildebrandt 1951, Reber and Bennett 2007). The rate of cooling has a large impact on survival with slower rates allowing for a better match with thermal compensation (Reber and Bennett 2007). The chance of survival during extreme cold events is enhanced during seasons of low seasonal temperature, as a large amount of thermal compensation needed to survive the cold snap has already been achieved (Reber and Bennett 2007). With cold temperatures being the biggest threat to survival in nature it is understandable *N. celidotus* has not dispersed to Islands surrounding New Zealand with cooler waters such as the Snares Islands and Chatham Islands (Ayling and Cox 1982, Reber and Bennett 2007).

Thermal compensation causes a shift in the thermal tolerance range, which is in the direction of the temperature change. The mechanisms for this involve alterations of the aerobic scope. Thermal compensation in cold temperatures is achieved by a rise in mitochondrial density or mitochondrial aerobic scope (Pörtner 2001). This increases the amount of energy available to fuel activities such as respiration and cardiac function, which are the physiological factors that deteriorate and result in oxygen limitations in cold temperatures (Pörtner 2001). Increasing respiratory and cardiac function allows for sufficient oxygen uptake and delivery, which increases the aerobic scope and reduces the chance of arterial hypoxia (Pörtner 2001). Thermal compensation to high temperatures involves opposite mechanisms via decreases in mitochondrial density or mitochondrial aerobic scope. This reduces the resting metabolic oxygen demand, which serves to increase the aerobic scope (Pörtner 2001). In addition to an increase in mitochondrial ATP production with temperature, there is also an increase in proton leakage across the

inner mitochondrial membrane (Brookes et al. 1998). Compensation for this involves a large energy cost, which contributes approximately 20-30% of the resting metabolic rate (Pörtner 2001). Therefore, a reduction in mitochondrial density with thermal compensation at high temperatures, also acts to reduce energy costs associated with proton leakage (Pörtner 2001). These mitochondrial adaptations are the major effectors for shifts in thermal tolerance ranges. However, adaptations at different organizational levels also act to increase the aerobic scope (Pörtner 2001). These adaptations are described in chapter three and include compensations at the level of enzymes, muscle contractibility and lipid saturation.

5.1.1 Current Experiment

In the current experiment *N. celidotus* was acclimated for 28 days to 8, 14 or 24°C. The thermal tolerance range was then determined to see if a shift in the tolerance range resulted. If thermal compensation was achieved a resulting tolerance shift would occur in the direction of the temperature change. If this was the outcome then it would indicate that *N. celidotus* is capable of acclimatizing to changing temperatures associated with climate change. The temperatures the fish were acclimated to are the maximum and minimum temperatures *N. celidotus* can survive at, for the duration of the acclimation period. These temperatures are almost certainly outside the optimal temperature range, so activities such as reproduction and growth would be jeopardized without compensation. Therefore, if no shift in the thermal tolerance range occurred, long-term survival of this

species with naturally increasing environmental temperatures would be threatened and migration to cooler waters would be crucial.

5.2 Materials and Methods

Experiments on the thermal tolerance range of *N. celidotus* were conducted in the same closed-box respirometer used for the oxygen consumption experiments (section 3.2). This was used because the clear perspex lid allowed for effective monitoring of the fish throughout the experiment, as well as recordings of ventilation rate via counting opercular movements (Fig 5.1). The water flow through the respirometer remained on for the duration of the experiment and circulated through a closed system. The system consisted of aerated seawater in a 10L bucket that was sitting in a water-bath filled with tap water. The water flowed from the bucket through the respirometer by the use of an aquarium pump. Temperature was controlled using the opposing effects of a cooling refrigeration unit and a heating temperature control unit that were powering the water-bath. This control of the water-bath temperature sequentially controlled the seawater temperature in the bucket, with a slight lag in time. Temperature was monitored using the temperature control unit in the water-bath and a thermometer for both the tap water and seawater. Temperature of the seawater in the bucket and the respirometer were not significantly different (± 0.2°C).

Thermal tolerance experiments were commenced the day following experiments on oxygen consumption. The fish was placed into the thermal tolerance setup at the conclusion of the oxygen consumption experiments and left over-night to acclimate. The temperature in the respirometer was initially set to the relevant acclimation temperature. The ECG wires that were used to measure heart rate in prior experiments were still attached. This is because the removal of these wires would cause stress to the animal and may affect thermal tolerance results. Heart rate could not be recorded during the experiment as the water flow through the respirometer created too much electrical interference and distorted the recordings.

CTMax experiments were always conducted first, which involved a gradual increase in seawater temperature. The rate of warming for the 8 and 14°C acclimated fish was initially 1°C per 10 minutes, until a set temperature was reached (20°C and 25°C respectively). After this point the rate was reduced to 1°C per 30 minutes. The rate of warming of the 24°C acclimated fish was 1°C per 30 minutes from the onset of the experiment. This rate of warming is larger than other studies on marine fish that employed a rate of 1°C h^{-1} (Mora and Ospina 2002, Lannig et al 2003). When the fish lost equilibrium the temperature was recorded and the fish was immediately transferred to a reservoir of seawater, which was maintained at the relevant acclimation temperature. The fish was allowed 90 minutes to recover before it was returned to the respirometer. During the recovery time the water in the bucket and respirometer was replaced with fresh seawater and the temperature of the system was returned to the relevant acclimation temperature.

CTMin experiments were then conducted, which involved a gradual decrease in water temperature. The rate of cooling for the 14 and 24°C acclimated fish was initially 1°C per 10 minutes until a set temperature was reached (8°C and 16°C respectively). After this point the rate was reduced to 1°C per 30 minutes. The rate of cooling of the 8°C acclimated fish was 1°C per 30 minutes for the entire experimental duration. The experiment ceased when the fish lost equilibrium and the temperature was recorded as the CTMin at this point. The fish was then immediately transferred to seawater at the relevant acclimation temperature. This was the last experiment conducted on the fish so from here it underwent procedures (section 2.4.) to remove ECG wires and re-acclimate the fish to environmental temperatures.

Respiration was monitored throughout the experiment by counting opercular movements. This was used as an indicator of the animal's level of physiological stress. When respiration rates dropped below resting levels or approached maximum rates (see section 3.3.2, Fig. 3.3) it was estimated that the fish had exceeded its optimal temperature range. The rate of temperature change had always been reduced to 1°C per 30 minutes by this time.

Fig. 5.1 The system setup to measure the thermal tolerance range of fish acclimated to 8, 14 and 24°C.

5.2.1 Data Analysis

GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA) was used for statistical analysis and graphing. A two-way analysis of variance (ANOVA) with Bonferroni's Multiple Comparison post-hoc test was used to compare the CTMax and CTMin between the acclimation groups as well as within the acclimation groups. Statistical significance was taken as $P < 0.05$ and values recorded are the mean and associated standard error.

5.3 Results

During CTMax experiments there was a general increase in activity level with temperature. At temperatures approximately 1-2°C below the CTMax violent bursts of activity lasting less than 5 seconds were frequently observed. This is possibly a last attempt from the fish to escape such unfavourable temperatures. Respiration rates were extremely high during this time reaching the maximal rates determined for the fish at their acclimation temperatures. During CTMin experiments the fish typically reduced their activity level in response to the decreasing temperatures. On some occasions the fish performed short bursts of activity prior to losing equilibrium. These bursts were not as violent as during CTMax experiments but most were most likely employed for the same escape behaviour. However, on other occasions no bursts of activity were observed and the fish remained extremely still. To ensure the fish could still actively maintain equilibrium and was not “frozen” into position the respirometer was manually tilted to one side for a brief moment to upset the balance of the fish. Fish were able to recover from this disruption to their balance right up to the point CTMin was reached. During this experiment the respiration rate of the fish dropped well below the resting rates (see section 3.3.2, Fig. 3.3) recorded at their acclimation temperatures.

A shift in the thermal tolerance range as predicted by the acclimation temperature occurred (Fig. 5.2). Both the CTMax and CTMin of the 8°C acclimated fish were significantly lower than the respective CTMax and CTMin of the other two acclimation groups ($P < 0.001$) (Fig. 5.2). Likewise, the CTMax and CTMin of the 24°C acclimated

fish were significantly higher than the respective CTMax and CTMin of the other two acclimation groups ($P < 0.001$) (Fig. 5.2). In all groups the CTMax was significantly larger than the CTMin ($P < 0.0001$). The size of the thermal tolerance ranges were not significantly different between the groups.

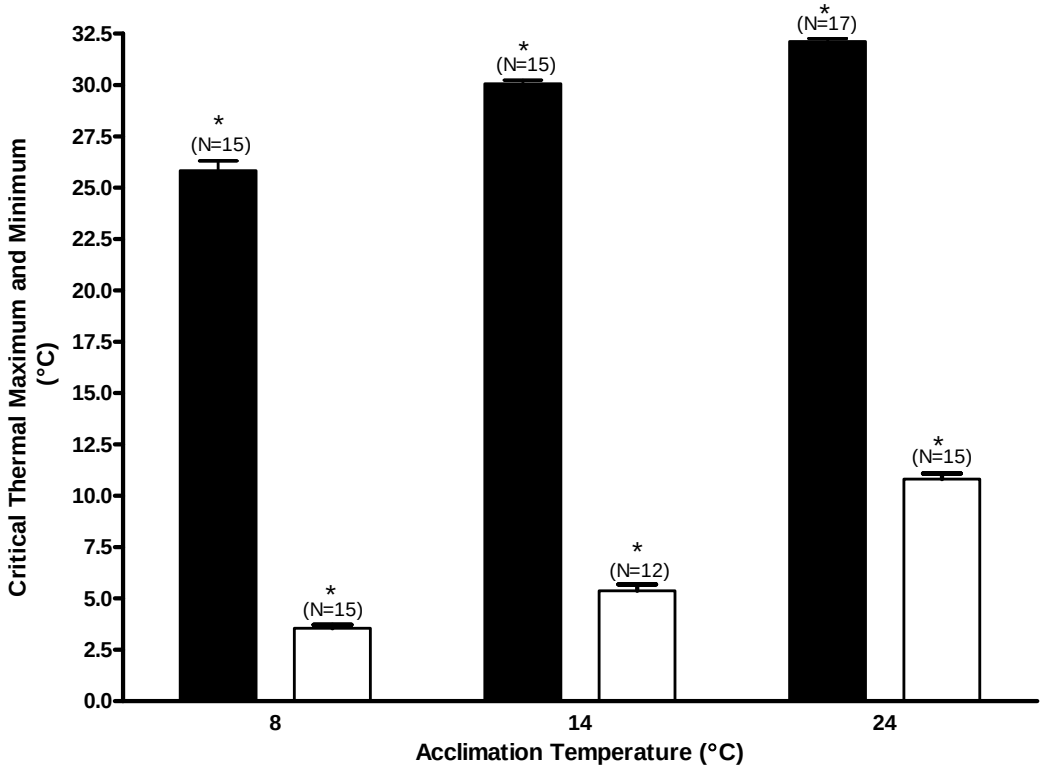

Fig. 5.2 The thermal tolerance range of fish acclimated to 8, 14 or 24°C. The critical thermal maximum (black column) and minimum (white column) are shown for each acclimation group. * indicates a significant difference of values between acclimation groups.

5.4 Discussion

N. celidotus is a shallow water marine fish so is exposed to relatively large seasonal temperature fluctuations. In order to survive these fluctuations *N. celidotus* is expected to possess mechanisms that cause the thermal tolerance range to shift in relation to seasonal temperature regimes (Pörtner 2001). This is a necessity to ensure optimal performance throughout the year. The results of the current study support the idea that *N. celidotus* is able to shift its thermal tolerance range in response to long-term temperature changes. Cold acclimation caused a 4°C drop in CTMax and a 2°C drop in CTMin, compared to the thermal tolerance range at the environmental temperature of 14°C; whereas, warm acclimation resulted in a 2°C rise in CTMax and 5°C rise in CTMin, compared to that of the 14°C acclimated fish. This is comparable to the results of a study on cold and warm acclimation of the three freshwater fish; channel catfish (*Ictalurus punctatus*), largemouth bass (*Micropterus salmoides*) and rainbow trout (*Oncorhynchus mykiss*) (Currie et al. 1998). In these species cold acclimation via a 5°C temperature decrease resulted in CTMax dropping by 2.3, 1.3 and 1.1°C respectively and CTMin decreasing by 3.8, 4.1 and 0.2°C, for catfish, bass and rainbow trout respectively. Warm acclimation via a 10°C increase in temperature resulted in CTMax rising by 3.9, 3.1 and 1.8°C and CTMin rising by 7.1, 7.5 and 2°C, for catfish, bass and rainbow trout respectively (Currie et al. 1998). This suggests that warm acclimation in *N. celidotus* is not as advanced as the freshwater species, which is most likely due to freshwater species having to adapt to greater natural temperature variations (Pörtner 2001). The CTMin of the cold acclimated catfish and bass was also more pronounced than *N. celidotus*. This shift in the thermal tolerance range in

response to long-term exposure to acclimation temperatures, suggests that *N. celidotus* will be able to acclimatize to the imminent increases in environmental temperature.

Both the current study and the study by Currie et al (1998) separated temperature changes into either cold or warm acclimation. In comparison, a study by Feldmeth et al (1973) looked at simultaneous cold and warm acclimation via exposure to cyclic temperatures changes, ranging from 15-35°C. This study found that pupfish (*Cyprinodon nevadensis amargosae*) are able to simultaneously compensate for both warm and cold temperatures by expanding both maximum and minimum temperature tolerance values. This supports the idea that the drop in CTMax with cold acclimation and rise in CTMin with warm acclimation observed in the current study, is not a necessity but instead a tactic to allow for more specialized animal functionality, within the smallest temperature range possible (Pörtner 2001).

The use of critical thermal methodology (CTM) to determine the thermal tolerance range, measures the animal's ability to meet metabolic oxygen demands and sustain immediate life. The functioning of physiological factors such as locomotion, growth and reproduction become limited within a much narrower temperature range (Pörtner 2001). This range is not measured by CTM but results in the survival of the animal becoming time-limited (Brett 1956). It can only be assumed that this narrower temperature range shifts in correlation with the thermal tolerance range. This would allow for optimal performance of all physiological activities at the acclimation temperature. However, limitations on growth and reproduction in the cooler seasons and observations of winter

dormancy suggest this may not be the case (Carbines 1993, Clarke 1993). It is possible that metabolic maintenance is the dominant physiological factor compensated for with long-term temperature variations to ensure immediate survival. Compensation for other physiological activities may be secondary, reduced or even lacking. If the animal is unable to compensate for these other physiological activities then eventual death of the animal is inevitable (Pörtner 2001). Chapter four investigates the level of thermal compensation on locomotion and found this to occur in the cold acclimated fish but not the warm acclimated fish. Further work needs to be done on the responses of growth and reproduction to temperature acclimation in order to determine the ability for long-term survival with global warming.

5.4.1 Summary

As expected *N. celidotus* does shift its thermal tolerance range in the direction predicted by the acclimation temperature. This indicates that this species has the mechanisms to acclimatize to increasing temperatures associated with global warming. Thermal compensation of the thermal tolerance range involves increasing the aerobic scope of the animal, to reduce oxygen limitations. However, this experiment only determines the ability of metabolic maintenance and immediate survival. The ability for long-term survival of this species in regard to other physiological factors such as growth and reproduction cannot be predicted.

6. Hypoxia Tolerance

6.1 Introduction

Coastal waters are exposed to a high level of nutrient loading and organic pollution from anthropogenic sources, making them susceptible to hypoxic conditions (Breitburg 2002). High nutrient loading promotes eutrophication, which increases the flux of organic carbon to the bottom of the water column and promotes bacterial growth and reproduction (Diaz & Rosenberg 1995). This sequentially results in an increase in oxygen demands via bacterial and other metazoan respiratory processes and ultimately creates environmental hypoxia (Diaz & Rosenberg 1995). Hypoxic water is generally a seasonal phenomenon coinciding with high surface temperatures and strong density stratification; it tends to remain at the bottom of the water column but can occur at intermediate depths along coastlines (Diaz and Rosenberg 1995, Breitburg 2002). Strong winds and tidal effects causing the mixing of the different stratification levels alleviate this by allowing for oxygenation of the bottom water layers (Breitburg 2002). Hypoxia caused by eutrophication and organic pollution is one of the major water pollution problems in the world and is an important factor in limiting species distribution (Rainer and Fitzhardinge 1981, Schiedek et al. 2007). Global warming is expected to enhance this problem, as the increasing temperatures will accelerate the effects of eutrophication and cause the problem to spread (Diaz and Rosenberg 1995).

High water temperatures enhance the effects of hypoxia in a variety of ways and cause major disruptions to exposed habitats. The intensity of eutrophication and water stratification increases with temperature, which causes a greater carbon flux to the bottom of the water column and limits the mixing of the different water stratification levels, which consequently enhances the severity of hypoxia (Breitburg 2002). Additionally, the solubility of oxygen in water is decreased with an increase in temperature, which simulates conditions similar to the low oxygen saturation of hypoxia, and ultimately reduces the amount of oxygen available for uptake (Breitburg 2002). This causes a "temperature-oxygen squeeze" as the bottom hypoxia and high surface temperature enclose on each other, making the area unsuitable to sustain life (Breitburg 2002). The spread of hypoxia throughout the water column can also be achieved after die-offs of extensive algal blooms (Breitburg 2002).

Many species employ a survival strategy when exposed to hypoxia, which involves rising in the water column to areas of higher oxygen content (Breitburg 2002). If hypoxic conditions are present throughout the water column this strategy is ineffective and species survival becomes threatened (Breitburg 2002). High temperatures also increase the oxygen demands of organisms by increasing the metabolic rate; and decrease the oxygen carrying capacity of the blood by decreasing the oxygen affinity of haemoglobin (Breitburg 2002, Valverde et al. 2006).

Fish avoid hypoxic water whenever possible by migrating to areas of higher oxygen saturation, as this is less costly than occurring the physiological stresses associated with

environmental hypoxia (Jensen et al. 1993, Breitburg 2002). Environmental hypoxia induces physiological responses similar to those experienced at high environmental temperatures, as both result in a reduced blood oxygen tension. Branchial and extra-branchial oxygen receptors detect low oxygen tension during times of environmental hypoxia and stimulate an immediate increase in ventilation rate (Jensen et al. 1993, Maxime et al 2000, Breitburg 2002, Valverde et al. 2006). This increases the amount of water that passes over the gills per unit of time and compensates for the reduced oxygen tension (Jensen et al. 1993). Oxygen diffusion across the gills is also improved during hypoxia via lamellar recruitment, which is stimulated by the circulating catecholamines released during hypoxia (Borch et al. 1993, Jensen et al. 1993, van den Thillart et al. 2002). High environmental temperatures stimulate an increase in heart rate in fish, which increases the blood flow to the gills and tissues and allows for increased oxygen loading and unloading (Rantin et al. 2007). However, hypoxic conditions have a very species-specific response in terms of heart rate and can cause bradycardia, tachycardia or no apparent change in heart rate (Fritsche 1990, Borch et al. 1993). The reason for species employing bradycardia during environmental hypoxia is not clear, but is thought to be a mechanism to increase the efficiency of gas exchange via ventilation/perfusion synchrony, or reduce metabolic costs by decreasing oxygen demands (Fritsche 1990). Many species increase the cardiac stroke volume to oppose the bradycardia and maintain a constant cardiac output during hypoxia (Berschick et al. 1987). The teleost heart is controlled by a double antagonistic cholinergic and adrenergic innervation and by circulating catecholamines (Borch et al. 1993, Fritsche and Nilsson 1993). Studies on species that employ bradycardia during environmental hypoxia have found that the

branchial and extra-branchial oxygen receptors stimulate both cholinergic and adrenergic innervation of the heart, but the cholinergic inhibitory effects dominate causing the bradycardia (Fritsche 1990, Fritsche and Nilsson 1993). Hypoxia also stimulates the release of erythrocytes from the spleen and stimulates erythropoiesis, which both increase haematocrit content and further increase the oxygen-carrying capacity of the blood (Jensen et al. 1993, Shimps et al 2005) Other mechanisms have been discovered during hypoxia that increase the blood carrying capacity,such as a reduction of erythrocytic ATP and GTP levels, which reduces the number of allosteric interactions of these phosphates with haemoglobin and increases the haemoglobin oxygen affinity (Randall 1982, Jensen et al 1993).

Hypoxia has a large impact on blood pH, which causes shifts in the oxygen dissociation curve and can cause either increased or decreased oxygen affinity of the haemoglobin (Jensen et al. 1993). A high oxygen affinity of the haemoglobin increases the oxygen carrying capacity of the blood and increases the amount of oxygen reaching the tissues (Jensen et al. 1993). The direction of the oxygen dissociation curve shift is dependent on the severity of hypoxia, as well as other environmental conditions. Increased ventilation during environmental hypoxia causes respiratory alkalosis by the rapid removal of carbon dioxide from the blood. The resulting increase in pH causes a leftward shift in the oxygen dissociation curve and an increase in the oxygen affinity of the haemoglobin (Jensen et al. 1993). Additionally, catecholamines released into the blood stream during hypoxia bind to β-adrenergic receptors on the red blood cells (Jensen et al. 1993). This causes an increase in cyclic adenosine monophosphate (cAMP) inside the cells, which is associated

with the activation of the Na^+/H^+ exchanger. As a result there is an efflux of H^+ from the cell and an increase in pH; hence, the oxygen affinity of the haemoglobin is further increased (Jensen et al. 1993). However, over prolonged exposure to elevated catecholamine levels the receptors may become desensitized and the response diminished (Jensen et al. 1993). Furthermore, when approaching critical blood oxygen tension levels anaerobic metabolism is employed, which increases blood lactatic acid levels causing a decrease in pH, a rightward shift in the oxygen dissociation curve and consequent reduction in haemoglobin oxygen affinity (Maxime et al. 2000). On exposure to a combined hypoxia-hypercapnia the high carbon dioxide levels oppose the effects of respiratory alkalosis and a respiratory acidosis results (Borch et al. 1993). This causes a decrease in pH and associated reduction in the oxygen affinity of the haemoglobin and oxygen transport (Jensen et al. 1993). Therefore, hypoxia-hypercapnia conditions are expected to produce a lower hypoxia tolerance, compared to pure hypoxia (normal carbon dioxide levels) (Jensen et al. 1993).

Reproduction, growth and locomotion are all essential activities for the survival of fish. However, as the oxygen tension of the ambient water decreases the animal is unable to meet oxygen demands to fuel all activities, so there is a sequential halt to some of them (Rantin and Johansen 1984, Breitburg 2002). During severe hypoxia only functions essential for immediate survival are fueled and oxygen delivery to fuel other activities is ceased. Therefore, long-term survival is jeopardized when the fish is exposed to hypoxia. Acclimation to hypoxia is limited but some mechanisms can be employed to decrease oxygen demands of the animal and increase oxygen uptake and transport. Decreasing the

metabolic rate of the animal, erythropoiesis and increasing the blood oxygen affinity are all strategies for this (Breitburg 2002). In the current study *N. celidotus* was not acclimated to hypoxia but was acclimated to different temperatures, which have different oxygen tensions.

At critical hypoxia tensions the animal is unable to uptake enough oxygen from the environment to meet its oxygen demands, so anaerobic metabolism is employed to compensate for the loss of aerobic energy production (Maxime et al. 2000). The ability to utilise anaerobic metabolism for energy production is limited and the fish rapidly loses homeostasis after the onset, due to a build up of lactate (Maxime et al. 2000). Anaerobic metabolism incurs an oxygen debt that needs to be repaid when the animal re-enters oxygenated water (Maxime et al. 2000). The ability of the fish to repay this debt rapidly is an essential part of hypoxia tolerance as the functionality of the fish is diminished during this time. Therefore, it is important to also measure recovery from critical hypoxia tensions when determining hypoxia tolerance.

6.1.1 Current experiment

Notolabrus celidotus inhabits shallow coastal waters so has the potential to be exposed to environmental hypoxia. Therefore, it is likely that they will have a relatively high tolerance to hypoxia. *N. celidotus* was acclimated for 28 days to 8, 14 and 24°C. The hypoxia tolerance of the fish was then measured to determine the degree of thermal

compensation of the different acclimation groups. A high hypoxia tolerance in fish acclimated to 24°C would indicate a high degree of thermal compensation in these fish. If the hypoxia tolerance is low and differs significantly among the acclimation groups it indicates *N. celidotus* does not have the mechanisms to undergo thermal compensation.

6.2 Materials and Methods

Hypoxia tolerance experiments were conducted immediately after resting oxygen consumption experiments. They were essentially a continuation of the experiment determining resting heart rate (section 3.2.1), hence, were conducted in the same closed-box respirometer described in section 3.2 and oxygen tension was determined using the same Strathkelvin oxygen microcell containing an oxygen electrode. The oxygen electrode was connected to a Strathkelvin oxygen meter, which was calibrated prior to the experiment using air-saturated water at the experimental temperature. The reading from the meter displayed the oxygen partial pressure (PO_2) of the sample in millimeters of mercury (mmHg). Control experiments on the closed-box respirometer with no fish found PO_2 did not change significantly (<0.4 mmHg) over a period of 60 minutes.

The fish was already in the closed-box respirometer prior to this experiment and the respirometer sealed with all air bubbles removed. Therefore, water samples were taken from the respirometer every 10 minutes to determine the PO_2. Ventilation rate was recorded immediately after the samples were taken by counting opercular beats per

minute, two counts were made and the mean ventilation rate calculated. Heart rate was also determined at this time using ECG wires that had been previously inserted into the fish (refer section 2.4). The ECG wires were connected to a system containing a BIOAmp and PowerLab A/D system and the readings were displayed on a connecting laptop. Hypoxia tolerance was recorded as the last PO_2 recorded before an end point was reached. The end point was when the fish lost equilibrium and/or the PO_2 differed insignificantly over three consecutive samples, which indicated that the fish had reached a PO_2 which was to low too for oxygen extraction from the water (termed the hypoxia tolerance threshold or critical hypoxia level).

When the end point was reached the respirometer was opened, allowing it to be flushed with oxygenated seawater. When the PO_2 of the seawater in the respirometer returned to levels greater than 130mmHg the respirometer was resealed and recovery from hypoxia recorded. It took approximately 3-5 minutes for 130mmHg to be reached. If any air bubbles entered the respirometer during this time they were removed. Samples were again taken at 10-minute intervals during recovery, with ventilation rate and heart rate being recorded at simultaneous 10-minute intervals. The respirometer was flushed with oxygenated seawater when the PO_2 fell below 90 mmHg, air bubbles were removed if they entered the respirometer during flushing. Recovery was recorded for 120 minutes in fish acclimated to 14 and 24°C and 90 minutes in fish acclimated to 8°C.

6.2.1 Data Analysis

Oxygen consumption (mg O_2 kg^{-1} $h)^{-1}$ was determined using the equation: -

$$\mathbf{VO_2 = \frac{\Delta PO_2 . C . V . 31.999}{t.m}}$$

Where ΔPO_2 = change in PO_2 over the measurement period (mmHg), C = oxygen capacity of water at a given temperature ($\mu mol.L^{-1}.mmHg^{-1}$), V = volume of water in respirometer (L), 31.999 = molecular weight of oxygen ($kg.kmol^{-1}$), t = time interval of ΔPO_2, and m = mass of fish (g).

GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA) was used for statistical analysis and graphing. A Kruskal-Wallis non parametric one-way analysis of variance (ANOVA) with Dunn's Multiple Comparison post-hoc test was used to compare the critical hypoxia tensions between the acclimation groups. The standard one-way analysis of variance (ANOVA) test could not be used as the Bartlett's test found the variance between groups differed. Equal variance is a prerequisite for ANOVA. Oxygen consumption, heart rate and ventilation rate during the gradual reduction in PO_2 were all calculated as the mean value, for each acclimation group, at set oxygen tensions. A repeated measure two-way analysis of variance (ANOVA) with Bonferroni's Multiple Comparison post-hoc test was used to compare oxygen consumption, heart rate and ventilation rate at different oxygen tensions between acclimation groups. The repeated measure two-way analysis of variance (ANOVA) with Bonferroni's Multiple Comparison post-hoc test was also used to compare oxygen consumption, heart rate and ventilation

rate of the different acclimation groups in response to time during the recovery period. Oxygen consumption, heart rate and ventilation rate during recovery were calculated as the mean value, within the acclimation group, at set time intervals. A two-tailed Mann Whitney non-parametric t-test was used to determine whether oxygen consumption, heart rate and ventilation rate were significantly different to the resting values (refer chapter 3) at the end of the recovery period. Statistical significance was taken as $P<0.05$. Values used are acclimation group means and associated standard errors. Sample sizes of the different acclimation groups are the same for all hypoxia experiments, including recovery. Samples sizes are 17, 18 and 19 for 8, 14 and 24°C acclimation groups, respectively.

6.3 Results

At the beginning of the experiment the 8 and 14°C acclimated fish were generally inactive, breathing was shallow and the fish was dark in colour. In contrast, the 24°C acclimated fish were more active from the initiation of the experiment, breathing was much deeper and the fish was relatively pale in comparison to fish in other acclimation groups. As the oxygen tension dropped activity increased, breathing became deeper and the colour of the fish began to fade in all acclimation groups. The point at which these changes took place differed between individuals but generally took place around 70-50 mmHg. As the fish approached its critical oxygen tension there were rapid bursts of activity and very heavy breathing. The fish became very pale and in some instances lost

equilibrium. If the PO_2 was unchanged over three consecutive samples the fish was relieved from the hypoxic conditions, before loss of equilibrium. However, in all of these cases the fish was obviously stressed with heavy gasping and severe active bursts. The duration of the experiment differed significantly between the acclimation groups with the 8°C acclimated group with the longest duration (7.32 ± 0.32 hours), followed by the 14°C acclimated group (3.75 ± 0.26 hours), with the 24°C acclimated group reaching their hypoxia tolerance levels rapidly in comparison (1.82 ± 0.14 hours).

Fish acclimated to 24°C were significantly less tolerant to hypoxia (33.07 ± 1.84 mmHg) than the other acclimation groups ($P < 0.001$) (Fig. 6.1). The hypoxia tolerance of the 8 and 14°C acclimated groups (17.28 ± 0.8937 and 16.01 ± 0.7397 mmHg respectively) were not significantly different. Oxygen consumption differed significantly with oxygen tension ($P < 0.0001$) and was significantly higher in the 24°C acclimated group compared to the other acclimation groups, at oxygen tensions greater than 50mmHg ($P < 0.001$) (Fig. 6.2). At a PO_2 of approximately 50mmHg acclimation temperature became an insignificant variable with oxygen consumption becoming similar across all groups and rapidly decreasing until critical hypoxia levels were reached.

Heart rate differed significantly with PO_2 ($P < 0.0001$) and was significantly higher in fish acclimated to 24°C, until low oxygen tensions were reached (Fig. 6.3) There were three stages the heart rate went through in response to water oxygen saturation. At high PO_2 the heart rate progressively increased with a reduction in PO_2, at intermediate PO_2 the heart rate stopped rising and reached a peak, and finally there was a progressive

reduction in heart rate at low PO_2, which fell below resting levels. The decline in heart rate of the 24°C acclimated fish was substantial and at approximately 50mmHg was not significantly different to the other acclimation groups.

Ventilation rate changed significantly with PO_2 ($P < 0.0001$) and acclimation temperature (Fig. 6.4). All acclimation groups followed a similar sigmoidal trend in ventilation rate with PO_2. There was an initial decline at high PO_2 before the ventilation rate began to increase. This was followed by a second decline in ventilation rate, at approximately 50mmHg in the 14°C acclimation group and 70mmHg in the 8 and 24°C acclimation groups. Ventilation rates of the 8 and 14°C acclimation groups were not significantly different at high PO_2, while 24°C acclimated fish had a significantly higher rate ($P < 0.001$). However, between 40-60mmHg the ventilation rate of the 8°C acclimated group became significantly lower than the other two groups ($P < 0.001$), while the 14 and 24°C acclimation groups were no longer significantly different.

Oxygen consumption changed significantly with time during recovery from critical hypoxia levels ($P = 0.0004$) (Fig. 6.5). All acclimation groups showed a similar recovery trend with a sharp initial decline in VO_2 for the first 60 minutes. At the end of the recovery period the VO_2 of all acclimation groups had returned to values similar to resting levels ($P = 1$). An observed increase in VO_2 occurred after each flushing of the respirometer with oxygenated seawater. This same response was seen with increases in heart and ventilation rate on flushing of the respirometer (Fig. 6.6 and 6.7). Heart rate did not change significantly with time ($P = 0.6544$) but did differ significantly with

acclimation temperature, 24°C acclimated fish had significantly higher heart rates and 8°C acclimated fish had significantly lower heart rates ($P < 0.0001$) (Fig. 6.6). Ventilation rate did differ with time in all three acclimation groups ($P = 0.0005$) (Fig. 6.7). There was an initial rise in ventilation rate of the 14°C acclimated group before it started to decrease. Ventilation rate of all groups gradually declined and reached values similar to resting levels over the recovery period ($P = 1$). However, after approximately 80 minutes there was an observed increase in ventilation rate of the 8°C acclimated fish.

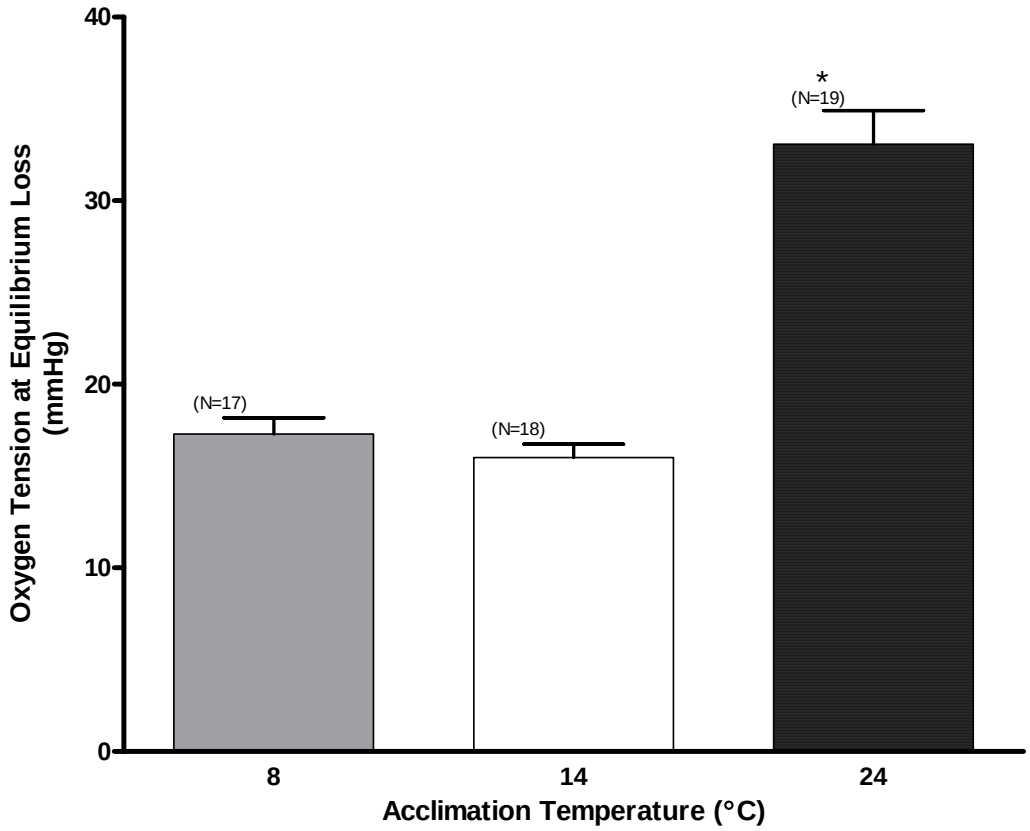

Fig. 6.1 Hypoxia tolerance of fish acclimated to 8, 14 or 24°C, after a gradual reduction in PO_2 over a number of hours. * indicates value significantly different to other acclimation groups.

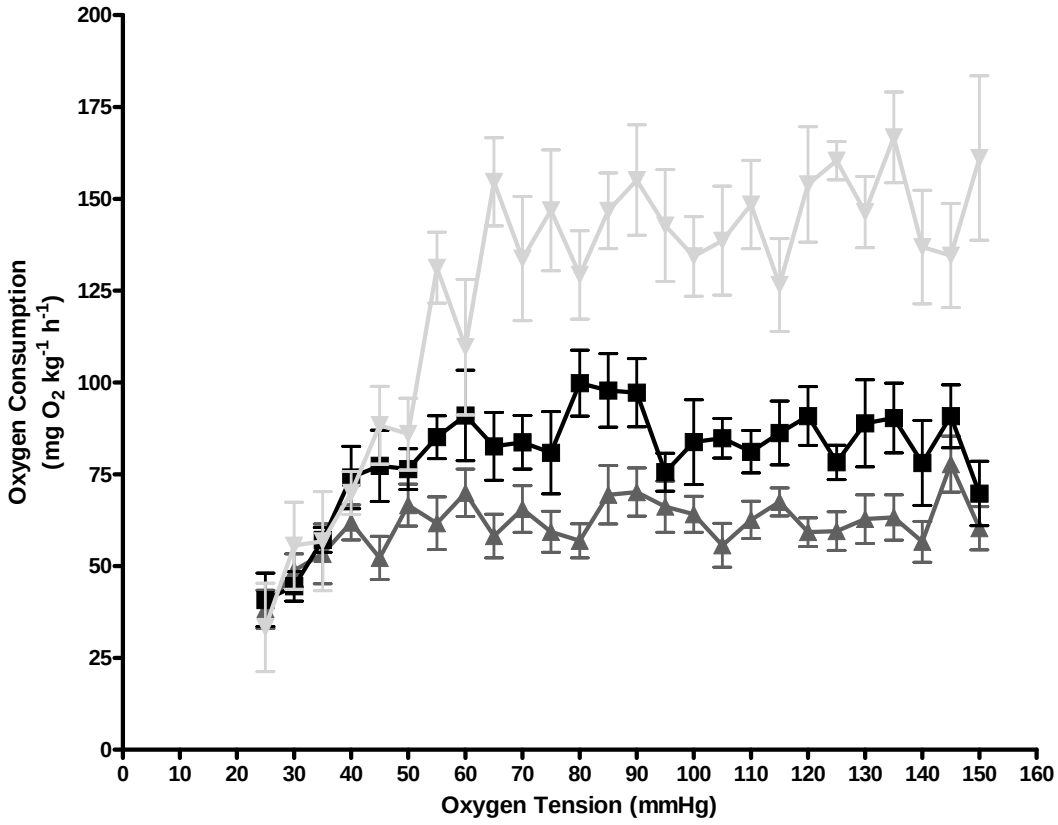

Fig. 6.2 Oxygen consumption of fish acclimated to 8,14 or 24°C at different oxygen tensions (8°C acclimated fish, N=17, ▲; 14°C acclimated fish, N=18, ■; 24°C acclimated fish, N=19, ▼).

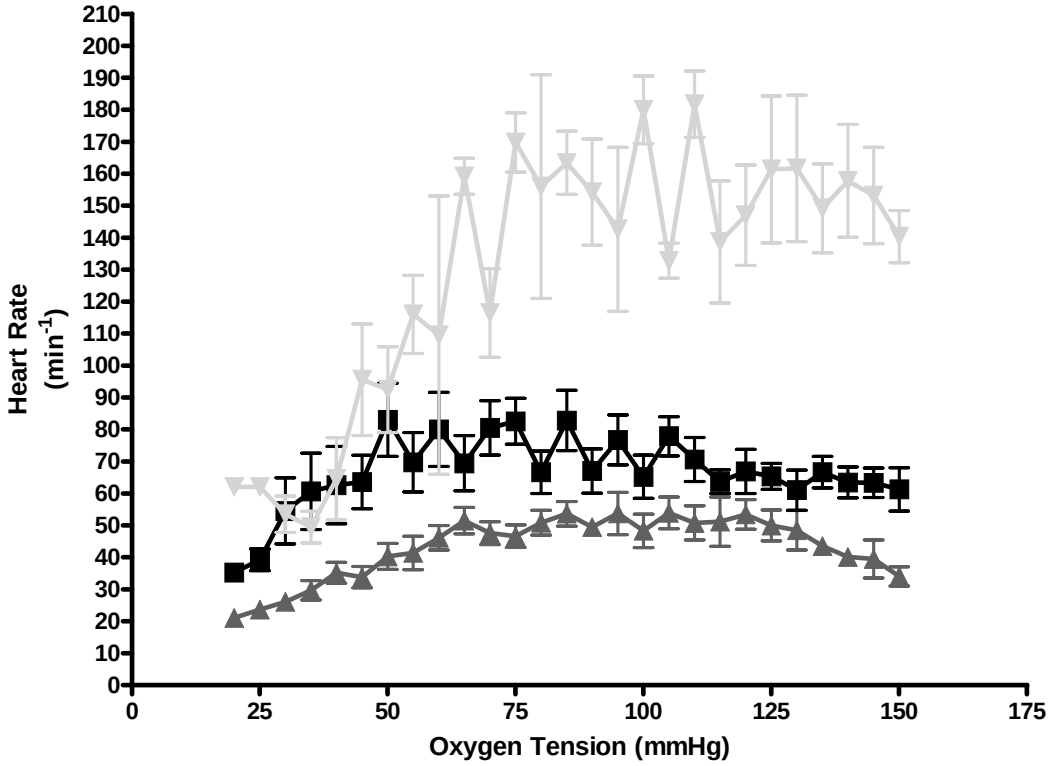

Fig. 6.3 Heart rate of fish exposed to a gradual change in oxygen tension. Fish had been acclimated for 28 days to 8 (N=17, ▲), 14 (N=18, ■) or 24°C (N=19, ▼).

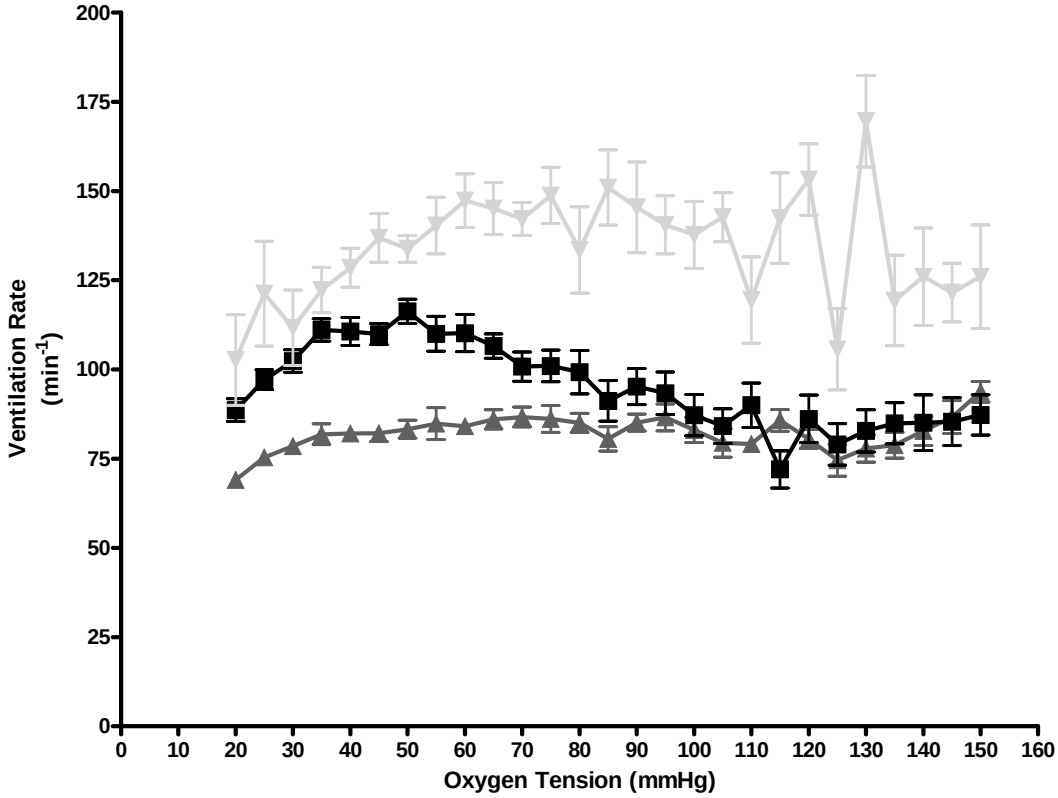

Fig. 6.4 Ventilation rate of *N. celidotus* exposed to a gradual reduction in $PO_{2,}$ until the critical oxygen tension was reached. *N. celidotus* was acclimated to 8,14 or 24°C at different oxygen tensions (8°C acclimated fish, N=17, ▲; 14°C acclimated fish, N=18, ■; 24°C acclimated fish, N=19, ▼).

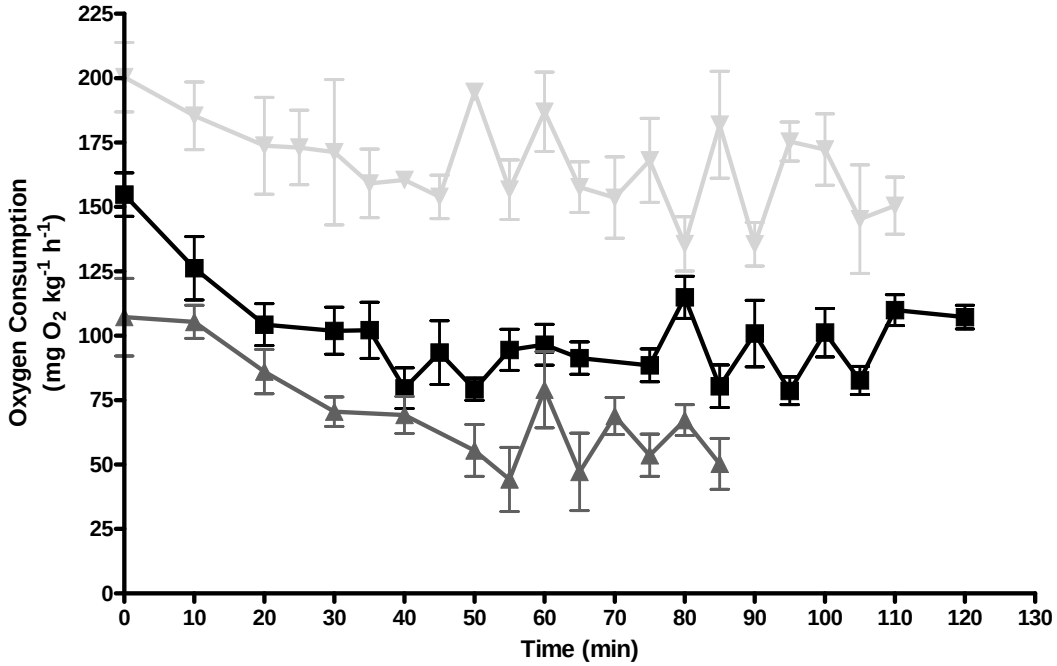

Fig. 6.5 Oxygen consumption of *N. celidotus* during recovery from exposure to its critical oxygen tension. Fish had been acclimated for 28 days to 8 (N=17, ▲), 14 (N=18, ■) or 24°C (N=19, ▼). Rapid increases in VO_2 are associated with the flushing of the respirometer with oxygenated seawater.

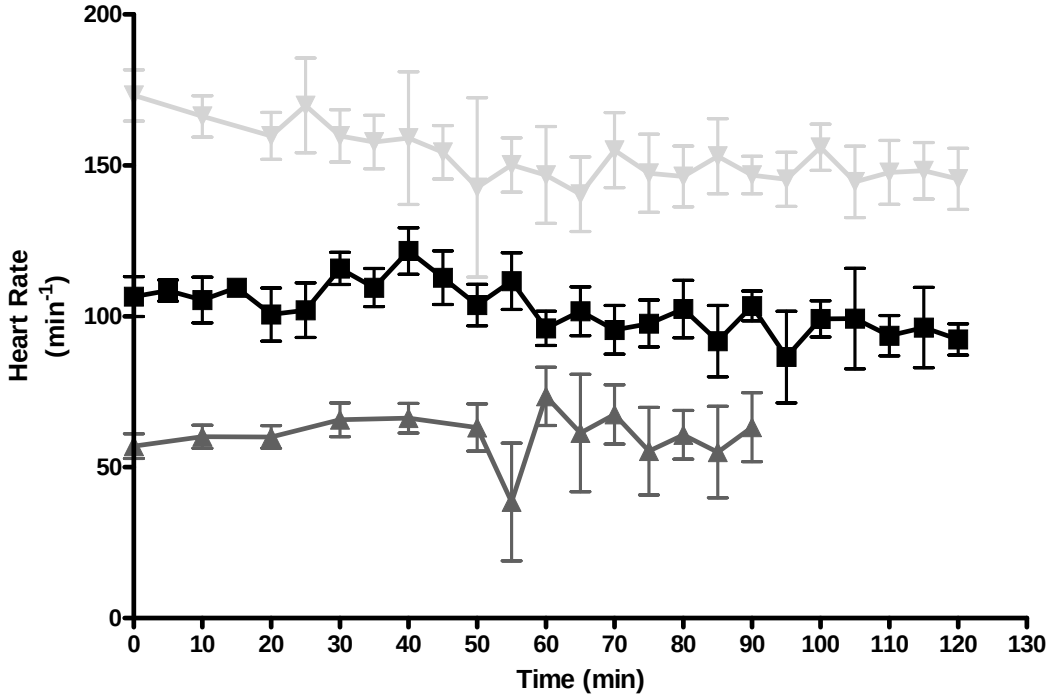

Fig. 6.6 Heart rate of *N. celidotus* immediately after exposure to critical PO_2 and during the associated recovery period. Fish had been acclimated for 28 days to 8 (N=17, ▲), 14 (N=18, ■) or 24°C (N=19, ▼).

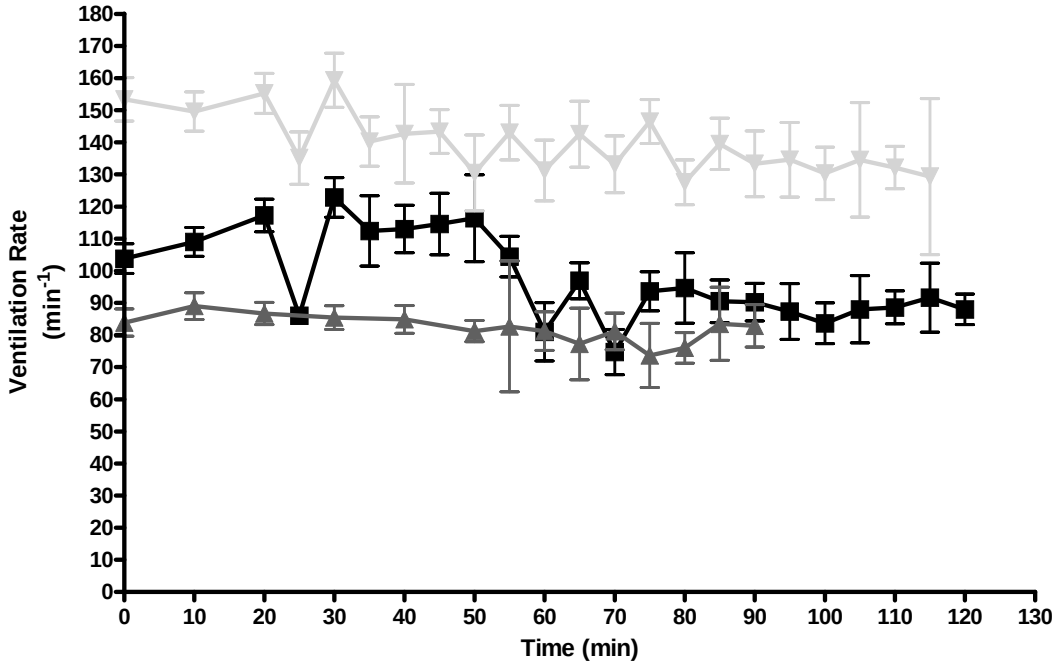

Fig. 6.7 Ventilation rate of *N. celidotus* immediately after exposure to critical PO_2 and during the associated recovery period. Fish had been acclimated for 28 days to 8 (N=17, ▲), 14 (N=18, ■) or 24°C (N=19, ▼).

6.4 Discussion

The current experiment found that *Notolabrus celidotus* has a very high hypoxia tolerance, even when subjected to a co-stressor of high environmental temperature. Oxygen consumption, heart rate and ventilation rate all differed significantly with changes in water oxygen saturation. Recovery rates of oxygen consumption, heart rate and ventilation rate were similar between the acclimation groups. The high hypoxia tolerance of the 24°C acclimated fish and the similarities between the 8 and 14°C acclimated fish suggest that there is some degree of thermal acclimation during the 28 day acclimation period.

Fish acclimated and tested at an ambient water temperature of 8 and 14°C had greater hypoxia tolerances (17.28 ± 0.8937 and 16.01 ± 0.7397 mmHg, respectively) than the 24°C acclimated group (33.07 ± 1.84 mmHg), but all groups have high tolerance in comparison to other species (Fig. 6.1). Studies on *Leiostomus xanthurus* (spot) and *Brevoortia tyrannus* (Atlantic menhaden) also found hypoxia tolerance to be inversely related to temperature (Shimps et al. 2005). Severe hypoxia is often referred to as 30mmHg, the hypoxia tolerance threshold of the 8 and 14°C acclimated groups are well below this and the 24°C acclimated group is not significantly higher than 30mmHg, which supports the idea *N. celidotus* has a high hypoxia tolerance regardless of temperature (Berschick et al. 1987, Maxime et al. 2000, Scott et al. 2008). Bigeye tuna (*Thunnus obesus*) is a hypoxia tolerant species and frequently inhabits water with oxygen tension of approximately 23.3mmHg (Lowe et al. 2000). In contrast, yellowfin and

skipjack tunas (*Thunnus albacares* and *Katsuwonus pelamis*) are much less tolerant to hypoxia and their depth distribution is limited by an oxygen tension of approximately 81.6mmHg (Lowe et al. 2000). Furthermore, other studies on teleosts with poor hypoxia tolerance have found the hypoxia tolerance threshold to be very high in comparison to *N. celidotus*; such as 116mmHg for bluegill, 93mmHg for trout and 91 mmHg for flounder (Rantin and Johansen 1984) Most hypoxia tolerant fish have a critical hypoxia tension between 20-40 mmHg (Rantin and Johansen 1984).

When subjected to 24°C *N. celidotus* incurs high-energy costs, as seen by a significantly higher oxygen consumption in the 24°C acclimated group (Fig. 6.2) and this group rapidly reaching its hypoxia tolerance threshold. The combined effect of high-energy costs and a reduced oxygen carrying capacity of the blood at high temperatures is expected to greatly reduce the hypoxia tolerance of the animal (Breitburg 2002). However, although this group is less tolerant in comparison to the other acclimation groups, its hypoxia tolerance threshold is still within the severe hypoxia range. Furthermore, lowered temperatures are expected to increase hypoxia tolerance (Diaz and Rosenberg 1995) but this was not observed in the current experiment. This either indicates acclimation to different temperatures in *N. celidotus* results in some degree of thermal acclimation, or the oxygen tension at the tolerance threshold is so low at 14ºC the fish does not have the ability to go any lower. Further research needs to be done to determine which mechanisms *N. celidotus* employs to achieve this. Studies on the high-energy teleost, bigeye tuna, have found it to have a significantly higher blood-oxygen affinity compared to other species of tuna and this high blood-oxygen affinity is

characteristic of many hypoxia-tolerant fish (Lowe et al. 2000, Scott et al. 2008). Therefore, it is expected that the hypoxia tolerant *N. celidotus* will also have a high blood-oxygen affinity (Lowe et al. 2000). A second major strategy to allow for high hypoxia-tolerance is metabolic depression; this reduces the oxygen demands of the animal predominantly through the down-regulation of protein synthesis and ion pumping, which are two of the most energy demanding cellular processes (Jensen et al. 1993, Scott et al. 2008). Alternatively, metabolic depression can be achieved by remaining inactive but this can only be employed as a short-term strategy (Jensen et al. 1993). Other possible mechanisms to allow for high hypoxia tolerance include lamellar recruitment to increase the gill diffusion conductance, and erythropoiesis and the release of red blood cells from the spleen, which both increase the haematocrit content of the blood and the oxygen carrying capacity (Jensen et al. 1993, Breitburg 2002).

While cardio-respiratory coupling has been suggested as a fixed relationship in some species, these two systems are not synchronized during gradual hypoxia in *N. celidotus* (Butler and Metcalfe 1983). Heart rate shows an initial rise in response to a reduction in PO_2, which gradually reaches a peak before starting to decrease and fall below resting levels (Fig. 6.3). In contrast, the ventilation rate has an initial decline and does not increase and peak until much lower PO_2 is reached (Fig. 6.4). Once it has reached a peak ventilation rate then begins to decline with oxygen tension (Fig. 6.4). Heart rate begins to decline at a relatively high PO_2, which suggests that *N. celidotus* employs the characteristic bradycardia response to hypoxia. The increase in ventilation rate at low oxygen tensions and the observed increase in the depth of ventilation (stroke volume)

supports the idea that a decrease in oxygen availability is compensated for by an increase in the water convection current across the gills (Jensen et al. 1993).

Oxygen consumption remains relatively constant in the 8 and 14°C acclimated fish until approximately 60mmHg, suggesting that *N. celidotus* is able to regulate uptake during this time (Fig. 6.2) (Berschick et al. 1987). The rapid decline in oxygen consumption, heart rate and ventilation rate as the fish approaches its hypoxia tolerance threshold indicates the animal no longer being able to uptake enough oxygen from the medium to meet oxygen demands, so the animal begins to lose cardiac and respiratory homeostasis and whole animal functions deteriorate. The decrease in oxygen consumption, heart rate and ventilation rate begins at much higher oxygen tensions in the 24°C acclimated fish compared to the other two groups. This further supports the idea that these fish have higher oxygen demands and thereby lose the ability to meet oxygen requirements at higher oxygen tensions.

N. celidotus recovered quickly from hypoxia with oxygen consumption and ventilation rate returning to values close to resting levels over 90-120 minutes (Fig. 6.5 and 6.7). However, heart rate did not change significantly over the recovery period and remained elevated from resting levels (Fig. 6.6). Oxygen consumption was initially high but rapidly fell over the first 40-60 minutes. The ability for *N. celidotus* to recovery quickly from hypoxia indicates it can efficiently repay the oxygen debt it incurs during hypoxia. This further supports the idea that it is a hypoxia-tolerant species.

6.4.1 Summary

In conclusion, *N. celidotus* is hypoxia tolerant even at high temperatures. The fish was able to function in severe hypoxia and recovery from critical hypoxia was rapid at all experimental temperatures. This suggests that *N. celidotus* undergoes some degree of thermal compensation to increase hypoxia tolerance at high temperatures.

7. General Discussion

The current work investigated different physiological responses of *Notolabrus celidotus* to temperature change, to determine the ability of this species to adapt to the increasing temperatures they will face with climate change. Climate change is projected to cause atmospheric temperatures to increase by 3-10ºC by 2100, which is likely to cause mass species extinction (Stern 2007). Marine ecosystems are affected by climate change, as there are few constraints on species dispersal in the ocean. This has the potential to cause large shifts in the distribution of some species, which would cause disruptions to mandatory species interactions and invasions from non-indigenous species (Edwards and Richardson 2004, Rosenzweig 2007). *N. celidotus* is an abundant New Zealand species and is in the middle of the food chain, so the behaviour of this fish will have a large consequence on the food web and ecosystem, which may be an indirect cause for distribution shifts of other New Zealand species. It may also be used as an indicator species, as to the response of other New Zealand species to increasing temperatures.

After acclimation for 28 days to 8, 14 and 24ºC *N. celidotus* achieved varying degrees of thermal compensation, at different organizational levels. In all physiological parameters investigated thermal compensation was more advanced in the 8ºC acclimated fish, compared to the 24ºC acclimated fish. This resulted in high physiological stress on the 24ºC acclimated fish for the entire acclimation and experimental period. Acclimation to temperature change is a very complex procedure, requiring the integration of thermal compensation at all organization levels, to achieve full thermal compensation of the

whole animal. Metabolism is a fundamental component of whole animal acclimation as it involves modifications at the cellular and molecular level, which has direct effects on other physiological parameters (Hochachka and Somero 1984, Randall et al. 2002, Haverinen 2008).

7.1 Metabolism

N. celidotus underwent metabolic thermal compensation to a larger degree at low temperatures compared to high temperatures (Fig. 3.1). There was almost full metabolic compensation at 8ºC and partial or no metabolic compensation at 24ºC, which indicates that alterations to cellular and molecular functions are more easily achieved in cold temperatures in this species. A high resting metabolic rate caused by high temperatures is expected to decrease the aerobic scope for activity of the animal (Fry 1971). This is because the maximum metabolic rate typically does not undergo a parallel temperature related increase, due to limitations on the oxygen carrying capacity of the blood (Fry 1971). This lack of change in maximum metabolic rate at different temperatures was true for *N. celidotus*, with the maximum rates not significantly different between the acclimation groups (Fig. 3.1). The elevated resting metabolic rate, with lack of a similar increase in maximum metabolic rate, caused a significant reduction in the scope for activity of the 24ºC acclimated fish. The effects of this were observed in other physiological parameters investigated and restricted thermal compensation of heart and ventilation rate, swimming ability, hypoxia tolerance and condition factor.

7.2 Respiratory and Circulatory Systems

Metabolic oxygen demands are meet by the respiratory and circulatory systems, so thermal compensation at the level of metabolism is essentially a form of thermal compensation in these systems (Jones 1994). However, these systems also incur their own temperature related responses, so further thermal compensation is required. Heart and ventilation rate are controlled by the autonomic nervous system, activity of which increases at high temperatures and decreases at low temperatures (Butler and Metcalfe 1983, Haverinen 2008). Therefore, thermal compensation to alter the rates of neurotransmitter release, diffusion and binding, and receptor responsiveness need to be made to return heart and ventilation rate to normal (Römisch and Matheson 2003). The term “normal” in this text refers to levels encountered at natural temperatures, in this case 14ºC. Heart and ventilation rate showed no thermal compensation in *N. celidotus*, with the exception of the resting ventilation rate of the 8ºC acclimated fish achieving full thermal compensation (Fig. 3.2 and 3.3). This suggests that the autonomic nervous system does not readily acclimate and activity remains low in cold temperatures and high in warm temperatures. Full thermal compensation of resting metabolic rate in the 8ºC acclimated fish as seen in section 7.1, results in a high oxygen demand (Fig. 3.1). Oxygen uptake and/or delivery need to increase to meet this oxygen demand. Full thermal compensation of resting ventilation rate indicates an increase in oxygen uptake achieves this in the 8ºC acclimated fish (Fig. 3.3). The lack of thermal compensation of resting

oxygen consumption in the 24ºC acclimated fish is also reflected by the lack of thermal compensation to the heart and ventilation rate. A high heart and ventilation rate is needed for the animal to meet the high metabolic oxygen demands.

7.3 Condition Factor

Condition factors are a measure of the degree of thermal compensation at the level of the whole animal (Elliot and Russert 1949, Dahlberg 1969). This measures the amount of physiological stress on the animal and reflects the amount of energy available for activities such as locomotion, reproduction, digestion and growth (Elliot and Russert 1949, Dahlberg 1969). The condition factor of *N. celidotus* was significantly lower in the 24ºC acclimated group, which supports the idea that these fish were under a great deal of physiological stress throughout the acclimation period (Fig. 4.2). The high metabolic rate and the high ventilation and heart rates would incur large energy demands. These fish also had a reduced appetite (see section 2.3), so would have been forced to utilise their own energy stores to meet energy demands. In comparison, the 8ºC acclimated group had significantly less physiological stress throughout acclimation, which was reflected by a high condition factor. At the initiation of the acclimation period these fish would have had a very low energy demand due to low metabolic rates. Energy demands would have increased with metabolic compensation, but would always be substantially less than the 24ºC acclimated group.

7.4 Swimming Ability

Locomotion is utilised for many higher-level functions of a fish including predator/prey interactions, migration, schooling and foraging; hence, a diminished swimming ability results in an overall reduction of fish performance (Beamish 1978). Swimming ability is largely dependent on the aerobic scope for activity of the animal, which is determined by the metabolic rate and the efficiency of the respiratory and circulatory systems in oxygen uptake and transport (Randall et al. 2002). Additionally, skeletal muscles are temperature-sensitive so need to undergo thermal compensation to achieve an adequate swimming ability. Full thermal compensation of the 8ºC acclimated fish was achieved (Fig. 4.2), this requires modifications to muscle fibres and the increase in metabolic rate seen in section 7.1 (Fig. 3.1). These changes allow for rapid muscle contractions needed to reach high swimming speeds. In comparison, the Ucrit of the 24ºC acclimated fish was significantly lower than the other groups (Fig. 4.2). At high temperatures a reduced scope for activity is expected to be the cause of reduced swimming ability, due to a high resting metabolic rate reducing the amount of oxygen available to fuel locomotion (Randall et al. 2002). Therefore, the lack of metabolic thermal acclimation in the 24ºC acclimated fish causing a reduced scope for activity, is responsible for the reduced swimming ability in *N. celidotus*. Additionally, it is likely the low condition factor of these fish, caused by high physiological stress for an extended period of time at high temperatures, results in a lack of energy stores available to fuel high speed locomotion.

7.5 Thermal Tolerance

Thermal tolerance ranges are also determined by the aerobic scope for activity, with optimum temperatures associated with minimal resting metabolic costs and the highest respiratory and cardiac functionality (Pörtner 2001). Therefore, shifts in thermal tolerance ranges are caused by thermal compensation at the level of metabolism and respiratory and circulatory systems. Thermal tolerance ranges of all acclimation groups shifted in the direction of temperature change (Fig. 5.2). This strongly indicates *N. celidotus* has the mechanisms to adapt to the increasing temperatures expected with climate change. Thermal tolerance shifts require compensation at all organizational levels, which indicates the resting metabolic rate of the 24ºC acclimated fish undergoes partial thermal compensation (see section 7.1 and 3.4.1). It also indicates the respiratory and circulatory systems have achieved some degree of thermal compensation.

However, the critical thermal methodology used in the current experiment does not take into account long-term survival, which is determined by activities such as growth and reproduction (Pörtner 2001). It can only be assumed that these activities also undergo a shift in thermal tolerance similar to metabolic compensation, but if this is not the case long-term survival would be limited.

7.6 Hypoxia Tolerance

Hypoxia tolerance is determined by the functionality of the respiratory and circulatory systems (Jensen et al. 1993). At high temperatures thermal compensation to increase oxygen uptake and delivery are required. However, as hypercapnia was a by-product in the hypoxia experiment, thermal compensation to increase hypoxia tolerance may reflect modifications of the oxygen carry capacity of the blood, in order to overcome decreases in pH that decrease the oxygen affinity of haemoglobin (Jensen et al. 1993). The hypoxia tolerance of the 24ºC acclimated fish was significantly lower than the other acclimation groups (Fig. 6.1). However, it is high in comparison to other species, which in combination with the co-stressor of high temperature, indicates a high degree of thermal compensation. Therefore, as mentioned above, this suggests that the oxygen carrying capacity of the blood is substantially enhanced. The significantly higher oxygen consumption, heart rate and ventilation rate of these fish during the hypoxia experiment show the increased physiological demands on these fish, which further enhances the idea that the circulatory system must undergo a large degree of thermal compensation.

7.7 Concluding Remarks

Based on the lack of full thermal compensation and high degree of physiological stress observed on the 24ºC acclimated fish, I would conclude that *N. celidotus* would be most likely to migrate to cooler waters in response to the increasing temperatures associated

with climate change. This is a response that has already been seen in some fish in the Northern Hemisphere (Perry et al. 2005). The effects of high metabolic demands are apparent with the reduced scope for activity, high respiratory and cardiac rates and decreased swimming ability. The diminished swimming ability has adverse effects on predator escape, prey capture, foraging, territory guarding and reproduction, which are essential components for individual and species survival (Beamish 1978). Additionally, the reduced scope for activity in these fish limit growth and reproduction, which are also needed for survival (Fry 1971).

Shifts in the distribution of *N. celidotus* would result in a disruption to the New Zealand marine ecosystem, as *N. celidotus* is an abundant species in New Zealand waters and an important part of the food chain. Additionally, the response of *N. celidotus* to increasing temperatures is likely to be reflected in other New Zealand species and indicates we are likely to face large changes in species distribution. This could have damaging affects to New Zealand commercial fisheries, depending upon replacement by other species adapted to higher temperatures.

However, the ability of a fish to thermally acclimate to temperature fluctuations is very dependent on the size and rate of change (Kinne 1963). The current experiment involved a large temperature change that was implemented over one day; climate change incurs a much more gradual increase in temperature. Therefore, as *N. celidotus* achieved partial compensation at many organizational levels, it indicates this species does have the mechanisms to adapt to temperature change. This implies *N. celidotus* has the potential to

adapt to the gradual increase in temperature that would be experienced with climate change. If this was the case *N. celidotus* would most likely remain in New Zealand waters, but more work has to be done on this before conclusions can be made.

Acknowledgements

On reflection of the past 18 months it is evident that this thesis would never have existed if it were not for the help, guidance and input from a large number of people and my sincere thanks goes out to each and every one. I am proud of what I have achieved and owe a great deal of that to you all.

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Appendix 1: Resting Oxygen Consumption over the Acclimation Period

An additional experiment was conducted during the current study to investigate changes in resting oxygen consumption at day 10, 20 and 28 of the acclimation period, in fish acclimating to 8 and 24°C. Unfortunately, constraints arising from time-consuming main experiments prevented adequate data collection in this experiment. However, the results as presented here suggest the fish undergo thermal acclimation.

Methods

All methods stated in section 3.2 on resting oxygen consumption procedures were also carried out in this experiment, except the acclimation period in the closed-box respirometer was only 60 minutes. This may result in oxygen consumption slightly above resting levels. Additionally, feeding was ceased at day 14 of the acclimation period; hence, oxygen consumption at day 10 and 20 was most likely elevated from resting levels due to digestive and absorptive processes. At day 10 and day 20 of the acclimation period three fish were randomly selected from the acclimation tanks for experimentation. Data from day 28 comes from the resting oxygen consumption of the main experiment (section 3.3.1).

GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA) was used for statistical analysis and graphing. A one-way analysis of variance (ANOVA) with

Bonferroni's Multiple Comparison post-hoc test was used to compare the means and associated standard error of the different acclimation days.

Results

The oxygen consumption of the 24°C acclimated fish was much greater than the 8°C acclimated fish, but statistical analysis found no difference between the oxygen consumption on the different acclimation days, within the groups. However, as the sample size was small (N=3) these results cannot be conclusive.

Observation of the data show a slight change in the oxygen consumption in the direction expected by thermal compensation, in both groups between day 10 and 20. This change is increased in both groups between day 20 and 28.

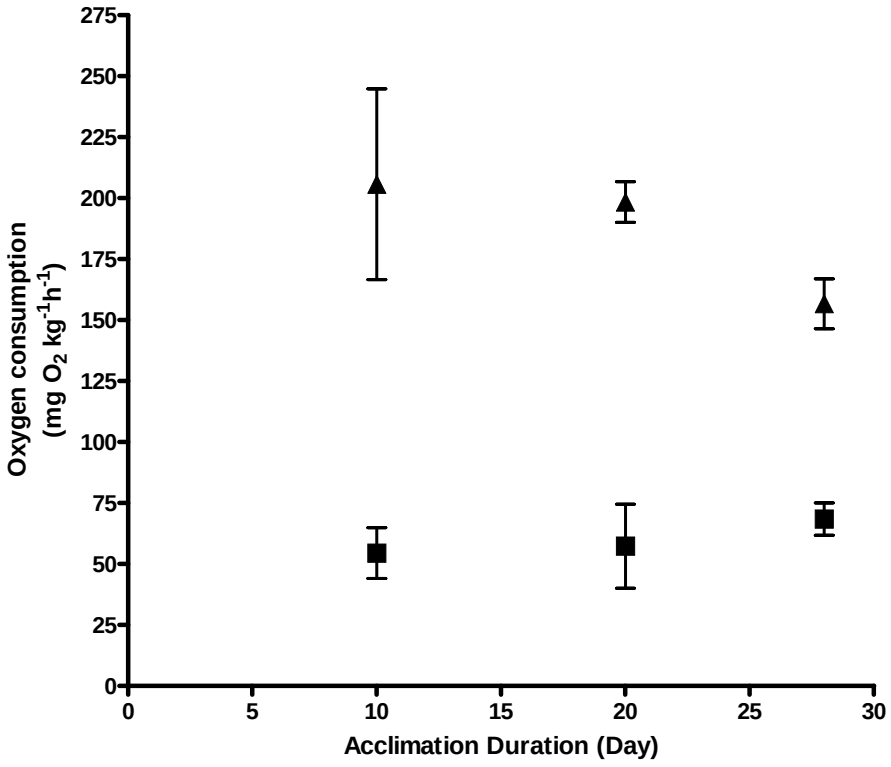

Fig. A.1 The resting oxygen consumption of fish acclimated to either 8 (■) or 24°C (▲) at day 10, 20 and 28 of the acclimation period.

Discussion

The results of this experiment are not conclusive as the sample size is too small for statistical analysis and oxygen consumption may be raised above resting levels on day 10 and 20 due to influences of feeding and inefficient acclimation time to the respirometer. However, the results suggest both acclimation groups are undergoing thermal acclimation to achieve optimal levels of resting oxygen consumption.