THE GLOBAL IMPACTS OF CLIMATE CHANGE ON FISH

A Thesis submitted for the degree of Doctor of Philosophy

By

Natalie E. Crawley

Institute for the Environment

Brunel University

February 2013

Abstract

Climate change is a global issue and the effects on fish populations remain largely unknown. It is thought that climate change could affect fish at all levels of biological organisation, from cellular, individual, population and community. This thesis has taken a holistic approach to examine the ways in which climate change could affect fish from both tropical, marine ecosystems (Great Barrier Reef, Australia) and temperate, freshwater ecosystems (non-tidal River Thames, Britain).

Aerobic scope of coral reef fish tested on the Great Barrier Reef was significantly reduced by just a 2°C rise in water temperature (31, 32 and 33°C, compared to the current summer mean of 29°C) due to increased resting oxygen consumption and an inability to increase the maximal oxygen uptake. A 0.3 unit decline in pH, representative of ocean acidification, caused the same percentage loss in aerobic scope as did a 3°C warming. Interfamilial differences in ability to cope aerobically with warming waters will likely lead to changes in the community structure on coral reefs with damselfish replacing cardinalfish.

Concerning Britain, there is evidence of gradual warming and increased rainfall in winter months over a 150 year period, suggesting that British fish are already experiencing climate change. It was evident from an analysis of a 15 year dataset on fish populations in the River Thames, that cyprinid species displayed a different pattern in biomass and density to all the non-cyprinid fish population, suggesting that there will be interfamilial differences in responses to climate change. Using a Biological Indicator Approach on the three-spined stickleback, *Gasterosteus aculeatus,* a 2°C rise in water temperature resulted in a stress response at the cellular and whole organism level. A 6°C rise in temperature resulted in a stress response at the biochemical level (higher cortisol and glucose concentrations), cellular level (higher neutrophil: lymphocyte ratio) and whole organism level (higher ventilation rate and lowered condition factor, hepatosomatic index and growth). *G. aculeatus* is considered to be temperature tolerant; therefore these results indicate that climate change may prove to be stressful for more temperature-sensitive species. This study has demonstrated that climate change will have direct effects on fish populations, whether they are in temperate regions such as Britain or in tropical coral reefs, but with strong interfamilial differences in those responses.

Declaration

The work described in this thesis was carried out in two sections: between October 2007 to March 2008 on Lizard Island on the Great Barrier Reef under the supervision of Philip Munday from James Cook University, Townsville, Queensland and between March 2009 and September 2012 at the Institute for the Environment, Brunel University, Uxbridge, Middlesex, United Kingdom. The work was conducted independently under the supervision of Professor John Sumpter and also of Dr Andrew Johnson (Centre of Ecology and Hydrology, Wallingford, United Kingdom). None of the data used in this thesis have been submitted by me or anyone else for any other degree.

Acknowledgements

There are many people that I need to thank for their support and encouragement whilst undertaking this research. First and foremost, thank you to my supervisors Prof. John Sumpter and Dr Andrew Johnson for their time, feedback and for funding the study, in particular thanks to John for the advice and guidance in the final stages. Thanks also to the other students and researchers in the Institute for the Environment: in particular Dr Jayne Brian, Dr Kugathas Subramaniam, Dr Luigi Margiotta-Casaluci, Dr Tamsin Runnalls and Dr Catherine Harris, whose advice and enthusiasm was always freely given and assistance on sampling day was greatly appreciated. I am indebted to Julie Walker, Sue Toogood and the late Steve Pash, for their expertise in looking after my sticklebacks and support in carrying out the *in-vivo* study. Steve was so sympathetic and caring, his kindness won't be forgotten. Many thanks also to Ioanna Katsiadaki from CEFAS for rearing the sticklebacks used in this study. Thanks to Dr Chris Parris in Biosciences within the School of Health Studies for advice and training on using a flow cytometer and to Dr Allan Tucker for his input and excellent tuition on Bayesian Networks. I am also hugely indebted to James Cook University in Townsville, Queensland, in particular, Dr Philip Munday and Prof Geoffrey Jones. I am extremely grateful to Prof Göran Nilsson from the University of Oslo, Norway, for funding the study on coral reefs and his use of equipment. Thanks to the Lizard Island Research Station for their use of laboratories and excellent hospitality. Thanks to Sara Horne and Prof. Sue Collins for granting me research leave and making this research possible and Dr Mick Allen for feedback on a draft thesis. I owe a huge thank you to the following members of the New Researchers Group in the Department of Education at Brunel: Geeta Ludhra, Dr Gwen Ineson, Dr Hilary Coole, Hilary Dodman, Dr Jacqueline Hebron, Alison Silby, Cathy Gower and Anne Chappell. Thanks for their understanding, patience, and flexibility with my working hours; but most of all for their interest shown in my research, never ending encouragement and their friendships. I have special gratitude to Dr Deborah Jones for her constant support and excellent advice and for always being there to set me on the right track. Heartfelt thanks to all my friends and family who have patiently listened to me and helped me along this journey. Lastly, thanks to Adam for being my motivation to finish and for his constant support. I couldn't have done it without any of you. Thank you.

Contents

List of Figures

List of Tables

Chapter 1. Introduction

1.1 Climate Change

Climate change is one of the biggest threats to ecosystems and yet despite much scientific research and media interest, the responses of ecosystems remain largely unknown. The Intergovernmental Panel for Climate Change (IPCC) defines climate change as:

'A change in climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and that is in addition to natural climate variability observed over comparable time periods'. (IPCC, 2007).

Human activity, particularly the burning of fossil fuels for energy, has escalated since the industrial revolution. The burning of fossil fuels produces vast quantities of carbon dioxide (CO_2), which is also a naturally occurring greenhouse gas. Without CO_2 our climate would be around 30°C colder (FSBI, 2007) and so at natural levels, greenhouse gases are essential for life as we know it. However, since man's dependence on fossil fuels, CO_2 concentration in the atmosphere has increased from 280ppmv (parts per million by volume) to 385ppmv (Soloman *et al*., 2008), with all greenhouse gas emissions having increased by 70% between 1970 and 2004 (IPCC, 2007; Guinotte & Fabry, 2008). This concentration of CO_2 is not thought to have been experienced in the last 650,000 years. Not only is the amount of greenhouse gases in the atmosphere increasing, the rate of increase is also the fastest seen at any time in the last 10,000 years (FSBI, 2007; IPCC, 2007). Greenhouse gases such as CO_2 and methane (CH_4) affect the absorption, scattering and emission of radiation within the Earth's atmosphere and as a result are changing our climate. Today there is evidence from all continents and most oceans that natural systems are being affected by regional changes in climate, such as gradual warming over time and ocean acidification (EEA, 2012; IPCC, 2007). Predicting the consequences of climate change is one of the biggest and most important challenges facing scientists. Models have been used to predict the effects of different scenarios of CO_2 emissions,

which depend largely on population growth and economic development. At present there is a movement to become 'greener' and more eco-friendly, with other sources of energy such as renewable and nuclear energy receiving more attention. However, even with a complete cessation of CO_2 emissions, changes in the climate will still be seen for the next 50 years due to the inertia in the climate system (FSBI, 2007; IPCC, 2007).

By applying the Atmosphere-Ocean General Circulation Model (AOGCM), the IPCC has defined four main scenarios for climate warming, as described in the Special Report for Emissions Scenarios (SRES) (IPCC, 2007). These four scenarios (A1, A2, B1 and B2) take into account the possible changes in lifestyle, economy and technology over the next 100years. A1 describes a world where there is rapid population and economic growth, with the sub-scenario A1F1 describing a global community reliant on fossil fuels, resulting in the greatest warming. A2 describes a heterogeneous world with population expansion combined with slow economic growth and technological development. B1 describes a convergent world with high population growth with the economy shifted to service and information industries. B2 describes a lower population growth rate with emphasis being on local solutions to economy and environmental sustainability. Figure 1.1 shows that even under predictions of relatively lower emission scenarios (B1 & B2) there will still be increases in temperature, whereas the highest increases in temperature would occur in the A1F1 scenario (IPCC, 2007). The IPCC has not attributed any likelihood to the above scenarios. Predicitions on the exact nature of how climate will vary are difficult due to the inherent uncertainity in population growth and how society will function and therefore uncertainity in future carbon emissions.

Air temperature is the primary controlling factor governing water temperature in rivers and oceans. Air temperature has increased by approximately 0.06°C per decade over the last century (Daufresne *et al.*, 2003), with 2 main periods of warming between 1910-1945 and 1976 onwards (IPCC, 2007) with the decade 2002-2011 being 0.8°C warmer than pre-industrial levels (EEA, 2012). Models for future air temperature predict a rise of between 1.1-6.4°C globally (EEA, 2012) resulting in an increase of 2-4°C in Britain (EEA, 2012; FSBI, 2007) and a 1-3°C in tropical regions such as the Great Barrier Reef over the next 50-100years (Lough, 2007).

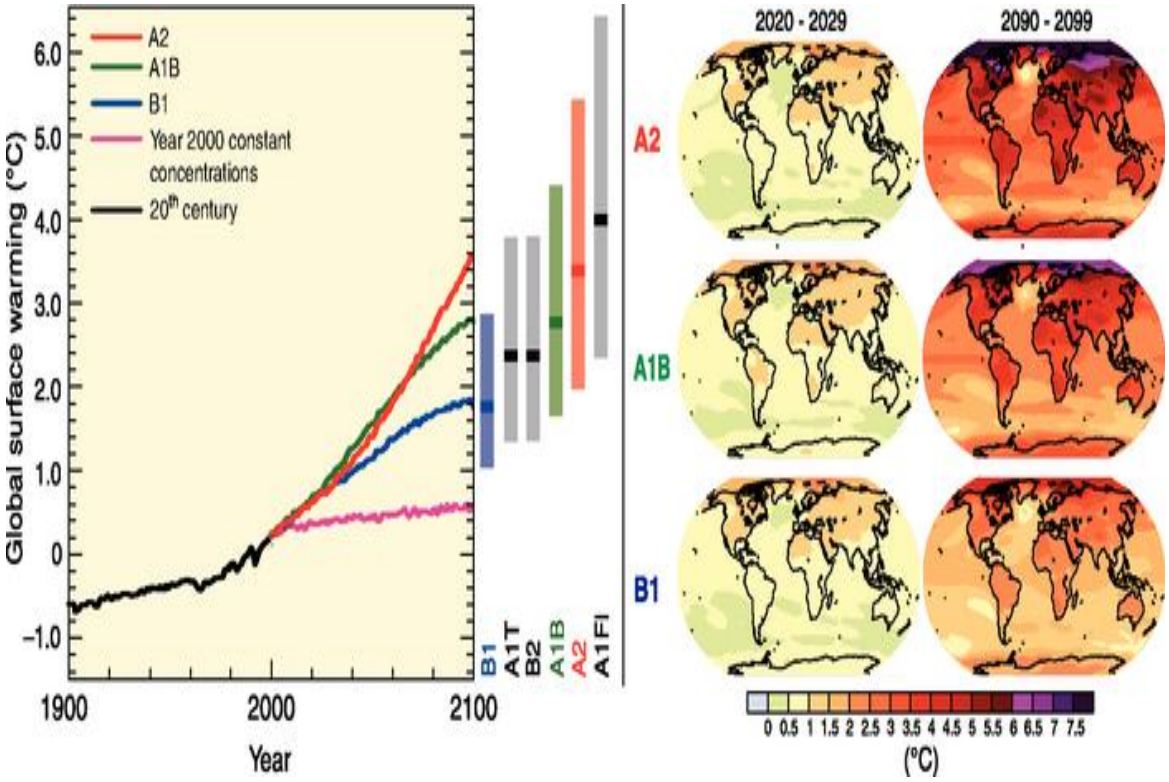


Figure 1.1. Predicted global surface temperature increases for the early (2020-2029) and later (2090-2099) 21st century relative to the period 1980-1999 under three SRES emissions scenarios (A2 [top], A1B [middle] and B1 [bottom]) based on the Atmosphere-Ocean General Circulation Models (AOGCMs) (IPCC, 2007).

Climate change is a truly global issue which will impact all oceans and landmasses. However, some regions will be more severely affected than others and some ecosystems will be more sensitive to changes in their environment. Changes in climate are predicted to be more pronounced towards the poles and high latitudes (FSBI, 2007; Rombough, 1997) and so it is anticipated that Britain will be significantly affected (FSBI, 2007). The main effect will be a warmer climate caused by the build-up of greenhouse gases trapping the heat energy from the sun. There is already strong evidence that the climate of Britain is changing. Central England was 0.5°C warmer in the 1990's compared with the 1961-1990 average and temperatures are predicted to continue to rise (Graham & Harrod, 2009). Research on the ecological effects of climate change in Britain has focused on terrestrial animals, and shows a general movement into northerly latitudes (Crick *et al*., 1997) and phenological changes such as earlier breeding seasons (Crick *et al*., 1997), earlier migration timing in birds (Crick, 2004) and earlier first appearances of butterflies

(Roy & Sparks, 2000). Fish, however, will be exposed to different environmental parameters to land-based animals. Freshwater fish are constrained by their environment (Daufresne *et al*., 2003) and so cannot move to different locations if conditions become unfavourable where they are, which marine fish can do, at least to some degree. It is thought that the impact of climate change will be greater on freshwater species than marine, especially those in northerly latitudes (FSBI, 2007). Fish inhabiting rivers are already under pressure from an array of factors such as pollution from urbanisation, chemical contaminants, sewage effluent and blocked migratory channels caused by weir construction. Climate change will exacerbate the current anthropogenic stresses (IPCC, 2007; Mulholland *et al*., 1997), making responses of fish more complex and difficult to predict. Few studies on the consequences of climate change have focused on freshwater fish, possibly due to the complex nature of such a study, but it is in these environments where the impact could be most dramatic. The responses of freshwater fish to these higher temperatures and other climatic changes are largely unknown. Given that freshwater fish are predicted to fair worse than marine species, it is important to investigate and understand the implications, primarily of warming waters, on freshwater species inhabiting British rivers.

Similarly to Britain, there has already been a warming of 0.4°C in the average sea-surface temperature around the Great Barrier Reef (GBR) off the coast of Australia (Lough, 2007). Tropical regions, such as the GBR, will experience less dramatic temperature changes than British rivers; however the fish that live in these regions have evolved under a steady environmental regime. Whilst rivers are a very dynamic environment, coral reefs only grow in very specific conditions of ocean chemistry, salinity and temperature. These conditions have not changed dramatically in the last half a million years (Hughes *et al*., 2003) and therefore the organisms living here are not adapted to cope with large fluctuations in environmental conditions (Guinotte & Fabry, 2008). Consequently, it is possible that although the environmental changes will be smaller, the responses may be of a similar magnitude to those of fish in temperate regions.

1.2. Predicted changes to the reef environment

The Great Barrier Reef (GBR) is a World Heritage Site that is protected as part of the Great Barrier Reef Marine Park. The Marine Park has a total area of 350,000km^2, with over 2900 separate coral reefs (Wachenfeld *et al*., 2007). The GBR is extremely important in terms of its cultural and socio- factors and has been used by the aboriginal people earlier than recorded history. Today, the GBR brings in huge amounts of money to the Australian people. Tourism on the GBR brings in $6.1billion annually; commercial fishing contributes $119 million and recreational fishing a further $640 million annually, accounting for over 64,000 jobs in the marine park area (Wachenfeld *et al*., 2007). Therefore, aside from the obvious ecological point of view, a loss of both coral and fish diversity would drastically reduce the income to the area and so it is important to understand how climate change may alter the reef biome. There are many anticipated changes to this region, all of which will pose a significant stress on the ecosystem. A change in river flow, which may alter the sediment load onto the reef, has been predicted. The frequency and intensity of tropical cyclones is anticipated to increase, with more category 5 cyclones. These intense cyclones are extremely damaging to anything in their path, including coral reefs, and can destroy vast areas of reef that have taken thousands of years to develop. Sea-level rise is another potential threat to coral reefs, as corals need to be in well-lit surface waters in order for photosynthesis to take place. However it is thought that the GBR is less vulnerable to moderate sea-level rise than other stressors, since the rate of coral growth is currently higher than the predicted rises in sea-level (Hoegh-Guldberg *et al*., 2007a). Warming of sea-surface water temperature is possibly the biggest threat, along with ocean acidification, both of which threaten to weaken the actual structure of the reef and therefore remove habitat for all the other reef organisms, such as fish. Increases in the atmosphere CO_2 concentration were responsible for the Paleocene-Eocene Thermal Maxima (PETM) that occurred 55 million years ago (Guinotte & Fabry, 2008). At this time, a rapid release of CO_2 led to warming and ocean acidification that was thought to be the main driver in the mass extinction of coral reefs. It has taken global coral reefs millions of years to recover, yet the anthropogenic inputs of CO_2 to the levels seen in the PETM are set to occur within the next 300 years. Therefore it is important to understand how the reefs of today will cope with the predicted changes in climate.

1.2.1. Temperature

The temperature fluctuations over a normal annual cycle on the Great Barrier Reef are between 22°C in winter in the south and 29°C in summer in the north (Lough, 2007). This variation is very small compared to the mean annual range of temperatures that a temperate freshwater fish might encounter, e.g. 5-18°C (Johnson *et al*., 2009). In lower latitudes (i.e. the north) of the GBR, such as Lizard Island, the mean annual temperature is 28.9°C but the annual range is only 4.8°C (Gardiner *et al*., 2010). Based on the 1961-1990 average, there is a predicted increase of 1.1-1.2°C in SST on the GBR by 2050. Along the coast of Queensland, the number of extreme days (air temperature above 33°C) is set to increase from 16 to 59 days annually (Lough, 2007).

Corals in particular are very sensitive to changes in temperature, particularly to increases in temperature. Although hard, stony structures, coral is in fact an animal and it has a symbiotic relationship with dinoflagellate protists (*Symbiodinium*), also commonly called zooxanthellae (Hoeugh-Guldberg *et al*., 2007b). The zooxanthellae provide the coral with sugars, amino acids and lipids through photosynthesis. In return they receive a rich supply of inorganic nitrogen and phosphorus from the host, in what would otherwise be a low-nutrient environment. However, in times of stress, such as when temperatures surpass the tolerance zone for corals, the endosymbiotic relationship between the animal and the zooxanthellae breaks down, resulting in a loss of the symbionts. Since it is the zooxanthellae that is pigmented and gives the corals their colour, the expulsion of the symbiont leads to what is known as coral bleaching (Figure 1.2). Without the zooxanthellae, the coral will eventually die and the skeletal structure that remains will slowly break down.

Figure 1.2. Photograph of staghorn coral with has undergone extensive coral bleaching due to warming waters on the Great Barrier Reef.

Mass coral bleaching has been reported for the last 70 years. However, as waters have warmed over recent decades, corals have been pushed closer to their upper thermal limits (Hoegh- Guldberg *et al*., 2007b). As a result, these mass coral bleaching events are occurring ever more frequently and over larger areas, and have been reported with only a 1-2°C increase in surface waters (Hoegh-Guldberg, 1999). The most widespread coral bleaching event took place in 1997-1998 and was thought in large part to be due to warmer waters due to an El Niño event (Nystrom *et al*., 2000) and resulted in mortality of 16% of the world's reefs (Hughes *et al*., 2003). If the stress event is short-lived, corals have demonstrated the ability to recover their symbiotic relationship with the dinoflagellates and regain pigmentation. However, if the stress is as chronic as it predicted with climate change, corals are unlikely to recover on a mass scale. There has been some speculation as to whether corals can adapt by changing the composition of the types, or rather clades, of symbiodinum that they hold in their tissues. So far, seven clades of zooxanthellae have been recognised and some have greater tolerance to warmer waters. If corals did have the ability to take up more resilient clades of symbionts, then it is thought that mass coral bleaching events would be reduced. However, corals are long-lived species and

therefore it is generally accepted that they will not be able to evolve quick enough to the changes in climate and associated warming waters (Hughes *et al*., 2003). A great deal of research to date on climate change and the coral reefs of the world, in particular those of the Great Barrier Reef has focussed on the corals and coral bleaching. This is not surprising given that it is the coral that is, quite literally, the bedrock of these ecosystems. Since the coral is the substrate and structure of the reef, decreases in coral cover and complexity affect the whole ecosystem, and the health of the reef is often estimated by the percentage of bleached corals. With climate change, the occurrence of mass coral bleaching events is predicted to increase as waters warm.

However, increases in temperature are likely to adversely affect many more organisms on the reef. The coral reef ecosystem is made of several related biomes, for instance mangroves and seagrass beds (Guinotte & Fabry, 2008). These biomes are important nursery grounds for many reef fish and so a loss of habitat here as well as on the main reef itself will likely impact many reef fish. Given that coral reefs and their associated fish have evolved in a very steady environment, it is likely that they have smaller thermal ranges than temperate fish. It is also thought that the recent warming has already pushed many species closer to their upper thermal limit and so even small additional increases in temperature may prove to be stressful for a whole range of coral reef fish.

1.2.2. Ocean Acidification

The atmosphere and surface ocean are tightly linked, and therefore increases in atmospheric CO_2 will also result in increases in oceanic CO_2, with concomitant declines in ocean pH. Given that a whole unit decrease in pH is equivalent to a 10-fold increase in acidity (Guinotte & Fabry 2008), this phenomenon is termed ‘Ocean Acidification’. Since the beginning of the industrial revolution, increases in atmospheric CO_2 concentration are occurring at a rate 100 times faster than has been seen in the last several million years, and this has been altering ocean chemistry (Feely *et al*., 2004). Over the last 250 years, it is thought that the pH of the surface oceans has dropped by 0.1units (Guinotte & Fabry, 2008) and it is predicted to

decline up to 0.25units by 2050 and 0.4units by 2100 (IPCC, 2007). Although this change might sound insignificant, it is in fact considerable, given that the oceans chemistry has been stable for the last 400, 000 years (Hughes *et al*., 2003). At present, anthropogenic production of CO_2 is approximately 5.0-7.6Gt C yr^{-1} and the oceans absorb about 30% of this CO_2 (Feely *et al*., 2004; Kurihara *et al*., 2004). This makes the oceans one of the most important carbon sinks. Oceanic absorption of CO_2 from fossil fuels may result in larger pH changes over the next several centuries than any inferred from the geological record of the past 300million years (Caldeira & Wickett, 2003). By the end of the century, atmospheric CO_2 is expected to have reduced ocean pH from 8.0 to 7.8 pH units. Past oceanic pH has been reconstructed based on the levels of isotopic boron levels in fossilised carbonate shells in marine organisms which fluctuate with ocean pH. Over the last 300 million years, the oceans pH has never been shown to be less than 0.6 units below today's levels, therefore these relatively small decreases may be extremely significant for many marine organisms. There have been some extreme predictions of a pH decline by 1.4 by 2300 (Orr *et al*., 2005). However, it is likely to be less, due to changes in temperature, weathering and sedimentation, which may act as buffers (Caldeira & Wickett, 2003). It is the capacity of the oceans to buffer changes in ocean chemistry thus far that has meant that the threats of ocean acidification were long overlooked.

Once CO_2 is absorbed into the oceans, the carbon becomes involved in a series of complex reactions and is controlled by the Biological Pump (Figure 1.3). The Biological Pump is the movement of CO_2 from the atmosphere to the deep ocean floor though a series of biological processes. The metabolism of organic (photosynthesis and respiration) and inorganic (precipitation and dissolution of calcium carbonate, $CaCO_3$) carbon absorbed by the oceans are the two major biological processes controlling the biogeochemical carbon cycle of marine ecosystems (Feely *et al*., 2004). Carbon is drawn into the deep ocean by the passive export of organic carbon and carbonates by gravity or the vertical migration of zooplankton. Due to the pycnocline (a boundary layer created by differences in density between the surface and deep waters), carbon is accumulated in the deep ocean and unable to exchange gases into the atmosphere. However, as the ocean warms, the stratification between the deep and surface water will increase, which will reduce the downward flux of carbon into the deep ocean. At the same time, the

atmospheric CO_2 absorbed by the ocean is increasing and is trapped in the surface waters (Kurihara *et al*., 2004). The absorption of carbon in ocean surface waters could increase the dissolved inorganic carbon concentration by 12%, and simultaneously cause the carbonate ion concentration to decrease by 60%.

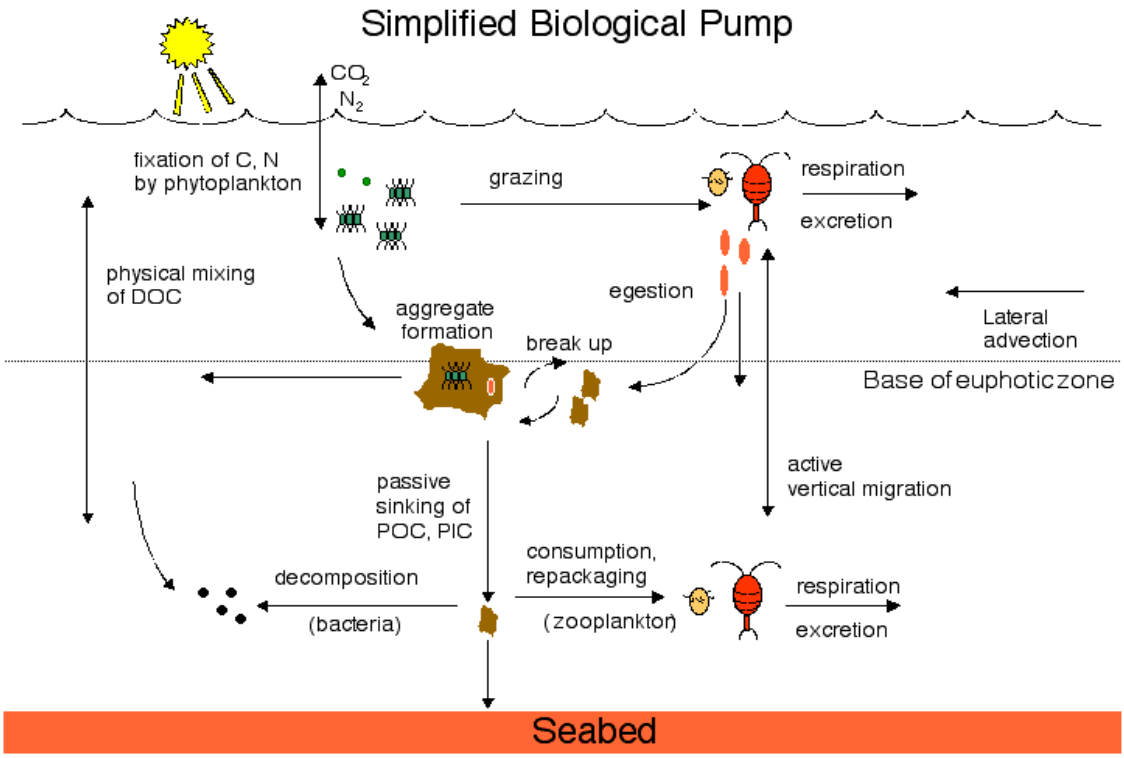


Figure 1.3. Diagram of the Biological Pump depicting transport of carbon from surface water to deep waters. http://www.msrc.sunysb.edu/octet/biological_pump.html

Under normal pH (around 8. pH units), bicarbonate is the predominant form of carbon. However, as the pH of the water decreases, there is a shift towards free CO_2 being the predominant form of carbon (Knutzen, 1981). Therefore as pH declines there is also a decline in the carbonate ion concentration. Carbonate ions are extremely important, as they are used to make calcium carbonate ($CaCO_3$), which is used for skeletal structures by many marine organisms, such as corals, plankton, echinoids and also for otoliths in fish. The carbonate of calcified tissue mainly occurs in 2 crystal forms: calcite and aragonite. Under normal conditions calcite and aragonite are stable in surface waters, since the carbonate ion is in a supersaturated state.

Animals using calcium carbonate will be most affected by increases in atmospheric CO_2 concentration (Caldeira & Wickett, 2003; Shirayama & Thornton, 2005), as calcification is controlled by the saturation of seawater with aragonite (Reynaud *et al.*, 2003). The main aragonite producers are reef-building corals and planktonic pteropod and heteropod molluscs (Feely *et al*., 2004). The effects of elevated CO_2 and therefore declines in carbonate saturation values will have profound impacts on calcification rates for many species of $CaCO_3$ shell-forming organisms. The consensus is that the calcification rate will decrease by 11-32% by 2100 (Gattuso *et al*., 1998; Leclercq *et al*., 2002). Recent studies have shown that dissolution of carbonate tests (i.e. when the carbonate shells dissolve) of plankton occurs at a much shallower depth than first thought, and 60-80% of dissolution occurs in the top 1000m. Dissolution of $CaCO_3$ will probably increase as the waters become increasingly under-saturated over time (Feely *et al*., 2004), and marine animals that utilise calcium carbonate will become more vulnerable.

Corals have so far received more attention than other organisms due to their high dependence on calcium carbonate and low tolerance to temperature changes. Calcification rates of scleratinian corals are predicted to decrease by 50% by 2100 (Reynaud *et al*., 2003) as a result of a decrease of aragonite saturation state (Ω) (Leclercq *et al*., 2002). Corals have also displayed little ability to acclimatize and so are at great risk from global increases in pCO_2 (Leclercq *et al*., 2002). Although pH will affect coral reefs, it is thought that coral skeletons are better protected against acidification than other carbonate skeletons and shells of animals. Photosynthesis of zooxanthellae makes the daytime microenvironment less acidic and the outer layer of living polyps protects against leakage of calcium (Glas *et al*., 2012). Despite this, coral reef ecosystems are negatively affected by the increases of both temperature and pCO_2. However, under all scenarios of climate change, the increased CO_2 will likely reduce the oceans aragonite saturation value to below 3.0, which is not thought to be high enough to prevent a net dissolution, and therefore a breakdown of the reef structure (Hoegh-Guldberg *et al*., 2007a). Corals have received the most attention so far, but they will not be the only marine organisms to be affected increased atmospheric CO_2 (Orr *et al*., 2005).Ocean acidification is therefore a real threat to the structure of the reef and all the organisms that depend upon the reef for shelter or food.

1.3. Predicted changes to the river environment

Where rivers are concerned, alterations in water temperature, flow rate and dissolved oxygen content are all likely as a result of warmer air temperature and altered rainfall patterns. The residual inertia in our climate systems means that changes will be seen over the next half a century even if emissions of CO_2 stopped now, and this will surely impact ecosystems and biodiversity (FSBI, 2007). Therefore we can expect to see changes in the fish communities of rivers over the next 50 years (FSBI, 2007). The question is which species will adapt and what will be the implications of these changes to mankind.

Warmer climates and altered precipitation patterns will change the river systems in England. Alterations in river temperature, dissolved oxygen concentration and flow rate pose the greatest challenges and threats to the organisms inhabiting the river. These three properties are related and so may act synergistically, or changes in one may confound changes in another.

1.3.1 Temperature

Water temperature is positively correlated with air temperature and with a predicted increase of 2-3.5°C in air temperature in the south-east of Britain (FSBI, 2007); the River Thames is likely to experience warming this century. Water temperature predictions have been produced in the past from a simple linear relationship with air temperature (Eaton & Scheller, 1996). However, due to evaporative cooling at temperatures higher than 25°C, an S-shaped relationship is now employed (Mohseni *et al.*, 2003; Webb & Walsh, 2004). The S-shaped function means that when higher than 5°C, water temperature will increase almost linearly until 25°C, but past 25°C, water temperatures plateau and are unlikely to continue to rise with increasing air temperature.

Under a high CO_2 emissions scenario, there could be a global rise in atmospheric temperature of 6°C by 2080 (IPCC, 2007). However, a rise of 4°C by 2080 could occur in the south-east of Britain (FSBI, 2007), with the temperature of

the River Thames predicted to increase by 3.5°C or more (Webb & Walsh, 2004). These temperature increases will be seasonal (Mohseni *et al*., 2003), with a predicted rise of 1.6°C in summer and 1.8°C in winter by as early as 2050 in the south-east of Britain (Arnell, 1998). Increases as high as 3°C in winter would bring the current winter temperature to 8°C and an increase of up to 5°C in summer would give a mean temperature of 22.5°C by 2080 (Johnson *et al*., 2009). All these water temperatures are below the 25°C threshold, and so river temperature increases are thought to be linearly related to air temperature.

Additional or confounding factors such as precipitation, groundwater inputs, riparian cover and domestic and industrial effluents will also lead to variations in river water temperature. Precipitation is predicted to decrease by an average of up to 15% annually in Britain (FSBI, 2007). Winter precipitation rates will likely be higher, but there will be a marked reduction in summer rainfall, and so summer flows may decline by as much as 50% in the south-east (Johnson *et al*., 2009). Waters may warm further with the additional risk of droughts. It may prove difficult to assess the effects of reduced summer precipitation due to the buffering effect of groundwater inputs, which are cooler than direct runoff from rain (Arnell, 1998). Therefore, areas just downstream from springs may provide localised areas of cooler water, which may be essential in the warmer summer months for fish. Conversely in the winter, the groundwater input will be warmer than the average river temperature and so provide warm water refuges (Mohseni *et al*., 2003). However, groundwater is also likely to increase in temperature as part of climate change, and so cold-water summer refuges may shrink (Mohseni et al., 2003; Shuter & Meisner, 1992). Riparian cover provides shading and thus cooling effects on rivers. This would help to ensure that rivers do not warm further, particularly with the added risks of decreased cloud cover and increases in solar radiation (FSBI, 2007), which in the summer may increase by 8-17% in the south-east (Arnell, 1998).

The climate in Britain is governed by several ocean current and circulation patterns. The North Atlantic Oscillation controls the strength and direction of westerly winds, strongly influencing weather, particularly in winter (FSBI, 2007). The North Atlantic Current, a continuation of the Gulf Stream, brings warmer water from the equator to the coast around Britain, creating a warmer weather system than there would be otherwise. Although the Gulf Stream is wind driven, the North

Atlantic Current is driven by the Thermohaline Circulation (THC), also known as the Meridonal Overturning Circulation (MOC). There are fears of drastic cooling in Britain and Europe if there were changes in these circulation patterns. However, changes to the MOC are considered to be very unlikely (IPCC, 2007). Conversely, the Gulf Stream may weaken, but this is still unlikely to cool Britain, as the predicted increase in temperature associated with the increase in greenhouse gases far exceeds the cooling power of a weaker Gulf Stream (FSBI, 2007; Graham & Harrod, 2009).

The increase in temperature will dramatically alter the climate in Britain. In Britain, spring temperatures are likely to occur earlier in the year, possibly by 1-3 weeks by 2050 (FSBI, 2007), with large implications for all manner of wildlife, from flowering plants, migrating birds and spawning fish. Summers will become hotter and drier (FSBI, 2007). The frequency of high temperature extremes will increase (EEA, 2012), with heat waves in May and July becoming more frequent and causing droughts (FSBI, 2007). Winter weather will be milder and last for a shorter period of time, with more rain, fewer very cold winters (EEA, 2012) and reduced snow fall (FSBI, 2007).

1.3.2 Dissolved oxygen concentration

Oxygen solubility in water is strongly temperature dependent (FSBI, 2007), so as temperature increases with climate change, the solubility and availability of oxygen is reduced (Arnell, 1998). The rate of de-oxidation is more sensitive to increasing temperature than is the rate of re-oxidation (Arnell, 1998), therefore oxygen is lost faster than it is replaced. Dissolved oxygen concentrations are also influenced by biogeochemical oxygen demands (BOD) (Arnell, 1998). Effluent which contains high BOD is likely to further reduce the dissolved oxygen concentration in rivers (Arnell, 1998). With population increases predicted for the future, this will place additional stress onto Sewage Treatment Works (STWs) to ensure effluent discharged into rivers is of an acceptable standard. This, coupled with warmer waters, could potentially reduce the availability of oxygen to levels which are detrimental to invertebrate and fish health.

The response of fish to reduced oxygen concentrations varies between species and also with different life stages (FSBI, 2007). Reductions in oxygen solubility will likely have multiple effects, such as on behaviour, reproductive success, predation risk and habitat use (FSBI, 2007). Raised temperature not only means water holds less oxygen, but it has a two-fold effect by increasing the metabolic rate of fish, thereby increasing demand for oxygen in the tissues (Pörtner & Knust, 2007). This is known as the Temperature-Oxygen Squeeze (Rombough, 1997). This mismatch between supply and demand is the first mechanism to affect an animal's tolerance to raised temperature, with implications for their growth and reproductive success (Pörtner & Knust, 2007).

1.3.3 River flow

River flow is principally controlled by the amount of water that an area receives, i.e. the amount of rain that falls. Currently, there are no long-term trends in the amount of annual precipitation that Britain receives (FSBI, 2007), making future predictions difficult. Predictions for precipitation vary depending on which model and scenario is used, and also human changes in habitat use may affect flows (Sefton & Boorman, 1997). Sefton & Boorman (1997) anticipated a 10% increase in annual rainfall in the south-east of Britain and up to a 20% increase in the north and west. Arnell (1998) predicted a 20% reduction in rainfall in the south-east. A more recent study predicts a 15% decline in annual rainfall in Britain (FSBI, 2007). What is generally accepted now is that there will be a marked change in seasonality of precipitation, with winter rates higher, and a marked reduction in summer rainfall (Arnell, 1998; FSBI, 2007; Johnson et al., 2009). Since there will be less cold extremes in UK winters, there will be less snowfall and snowmelt, possibly by up to as much as an 80% reduction (IPCC, 2007). This, combined with higher rates of evapo-transpiration in warmer weather, may lead to declines in flow (Mulholland *et al*., 1997). Summer is likely to fair much worse than winters, with runoff reduced by as much as 50% in the south-east due to reduced rainfall, increased evapo-transpiration and increased storage in soils (Arnell, 1998; Arnell & Reynard, 1996). This will result in reduced flow rates of rivers in the south and east of England,

which are already flowing through ‘dry’ areas very susceptible to reduced rain (Arnell & Reynard, 1996). Even under wetter scenarios, flow rates in rivers are likely to decrease (Johnson *et al*. 2009). The south-east and the River Thames in particular will have lower flows than at present in all seasons, except winter when there may be an increase (Johnson *et al*., 2009; Sefton & Boorman, 1997). High flow events are also likely to decrease in all seasons, with their summer and autumn frequencies declining by as much as 50% (Johnson *et al*., 2009).

Lower flow rates will lead to water staying in the river longer, resulting in longer residence times (FSBI, 2007; Johnson *et al*., 2009). Waters will warm more due to reduced flushing, exacerbating the problems of warmer air temperatures and further reducing oxygen solubility (FSBI, 2007). Pollutants, contaminants and nutrients will also build-up (Johnson *et al*., 2007), and so alterations in primary production and BOD will be expected (FSBI, 2007), thereby increasing the risk of eutrophication in British rivers. A lower flow rate therefore poses great threats to the wildlife inhabiting an already temperature-stressed environment. With less water available, habitats will be lost and so competitive interactions between fish may increase and lead to possible elimination of weaker species (Mulholland *et al*., 1997).

Alterations in the seasonal flow patterns may interrupt food chains (Nunn *et al*., 2003) and migratory patterns. Due to reduced flows causing multiple effects, it has been suggested that flow rates are more important than temperature in controlling the fish populations and biomass (Arnell, 1998, Nunn *et al*., 2003). However, the two factors are related to one another because a reduced flow rate will lead to higher water temperatures.

‘Second-order’ effects of climate change, such as changes in agriculture and vegetation type, will influence river flows, as they may change soil moisture and alter loses through evaporation (Sefton & Boorman, 1997). The underlying geology of a river catchment will also influence how climate change alters a flow regime (Sefton & Boorman, 1997), and therefore each catchment will be individual and responses unique.

1.3.4. Big Events

If climate model predictions are correct, Britain should brace itself for not only ‘wetter-milder winters’ and ‘ warmer-drier summers’, but also for more severe weather changes. It is anticipated that there will be an increase in intensity and frequency of extreme weather events (IPCC, 2007) such as flooding, heat waves and droughts. Whilst these events already occur in Britain, they may become part of the ‘normal’ climate. Despite the fact that little is known about how freshwater fish will adapt to the general climate predictions of a warming world, even less is known about how they will respond to extreme weather events (Lake, 2003). Likewise, in Australia, it is anticipated that there will be an increase in intensity of tropical cyclones and extreme warm days, which are likely to be damaging to the corals, which in turn affects the rest of the reef ecosystem. Tropical cyclones off the coast of Australia appear to have decreased in frequency from the 1970s to present, however their intensity and destructive power has increased. Tropical Cyclone Ingrid (2005) and Tropical Cyclone Larry (2006) were category 4 and 5, respectively, and caused large amounts of damage (Lough, 2007). Due to their close timings, there was less time for reefs to recover in between (Hughes *et al*., 2003). Tropical cyclones are predicted to be more intense with time, with greater maximum wind speeds and greater rainfall, which coupled with higher sea level could led to greater storm surges, affecting both reefs and land (Lough, 2007).

1.4. Responses of Fish to Climate Change

1.4.1 Individual level responses

The impacts of climate change will affect fish at all levels of organisation: biochemical, cellular, individual, species, population, community and ecosystem (Figure 1.2). The responses will be varied, depending on life stage, species, and previous acclimation history. Most studies to date have examined the effects of warming at the species and population levels (Shuter & Meisner, 1992; Webb & Walsh, 2004); however, there is a distinct lack of information on the molecular and physiological responses. Temperature is the key parameter controlling many biological and behavioural processes and therefore considered of paramount importance in view of climate change (Magnuson & Destasio, 1997). While it is thought that warming will not produce water temperatures that will exceed the thermal tolerances of most fish (FSBI, 2007), prolonged raised temperature may still elicit a stress response. Stress can ultimately result in reduced reproductive output and susceptibility to disease (Adams, 1990). Studies have shown that even small increases in temperature can have detrimental effects on fish, such as on embryonic development (Rombough, 1997). The temperate three-spined stickleback, *Gasterosteus aculeatus*, when exposed to an increase in water temperature of 4°C, displayed a 60% reduction in population biomass (Moran *et al*., 2010). Whilst this study was central in demonstrating that even robust, temperate fish can be negatively affected by warming, it did not address the mechanisms behind this decline in biomass.

Dhabhar (2002) defines stress as:

A constellation of events comprised of a stimulus (stressor) that precipitates a reaction in the brain (stress perception) which subsequently activates physiologic fight or flight systems in the body (stress responses).

Physiological stress responses are controlled by the Hypothalamus-Pituitary-Interrenal Axis (HPI). When a fish is exposed to a stress, the hypothalamus in the brain produces the hormone, Corticotrophin Releasing Factor (CRF), which travels via nerves to the pituitary gland. Special cells of the pituitary gland then synthesize

and secrete a second hormone, Adrenocorticotrophic Hormone (ACTH), which travels to the interrenal tissue of the kidneys via the systemic blood system (Iwama *et al.,* 2004). From this point, there are primary and secondary responses which lead to whole organism changes. These could also be termed biochemical, cellular and whole organism responses, all involved in allowing the organism to cope with the stress and regain homeostasis (Adams, 1990). The first or the Primary Stress Responses are concerned with the release of the stress hormones cortisol and epinephrine. Special proteins called Heat Shock Proteins (HSPs) are also rapidly synthesised at this stage (Iwama *et al.,* 2004). The increased levels of cortisol in the blood lead to a Secondary Stress Response, an increase in plasma glucose level, to provide additional energy for protection against the stress (Silbergeld, 1974). These responses also lead to observable whole-animal changes, such as increased heart rate, breathing rate, altered behaviour patterns (Adams, 1990) and metabolic rate (Pörtner & Knust, 2007). Stress is adaptive in the short-run, but can be harmful when it is long lasting, with chronic stress lasting for weeks to months (Dhabhar, 2002). A stress response is an energy-draining process and chronic stress can mean energy is diverted away from processes such as growth and reproduction (Rice, 1990; Schrek, 1990; Thomas, 1990) and can lead to individuals being susceptible to disease (Zarate & Bradley, 2003).

Fish are routinely exposed to biotic (e.g. predation and competition) and abiotic (e.g. fluctuations in temperature, flow patterns, dissolved oxygen concentration, chemicals) stressors in the aquatic environment. One of the biggest threats to aquatic ecosystems today is thermal stress as a result of climate change. Most investigations on the effect of temperature stress have focused on acute stress at temperatures higher than predicted by climate change models (Brian *et al*., 2008; Currie *et al*., 2008; Perez-Casanova *et al*., 2008). Whilst informative of acute stress responses, they provide little ecologically significant data on how fish will respond to climate change. Fish are more likely to be exposed to chronic, sub-lethal levels of thermal stress which may still affect ultimate survival (Lucentini, *et al*., 2002).

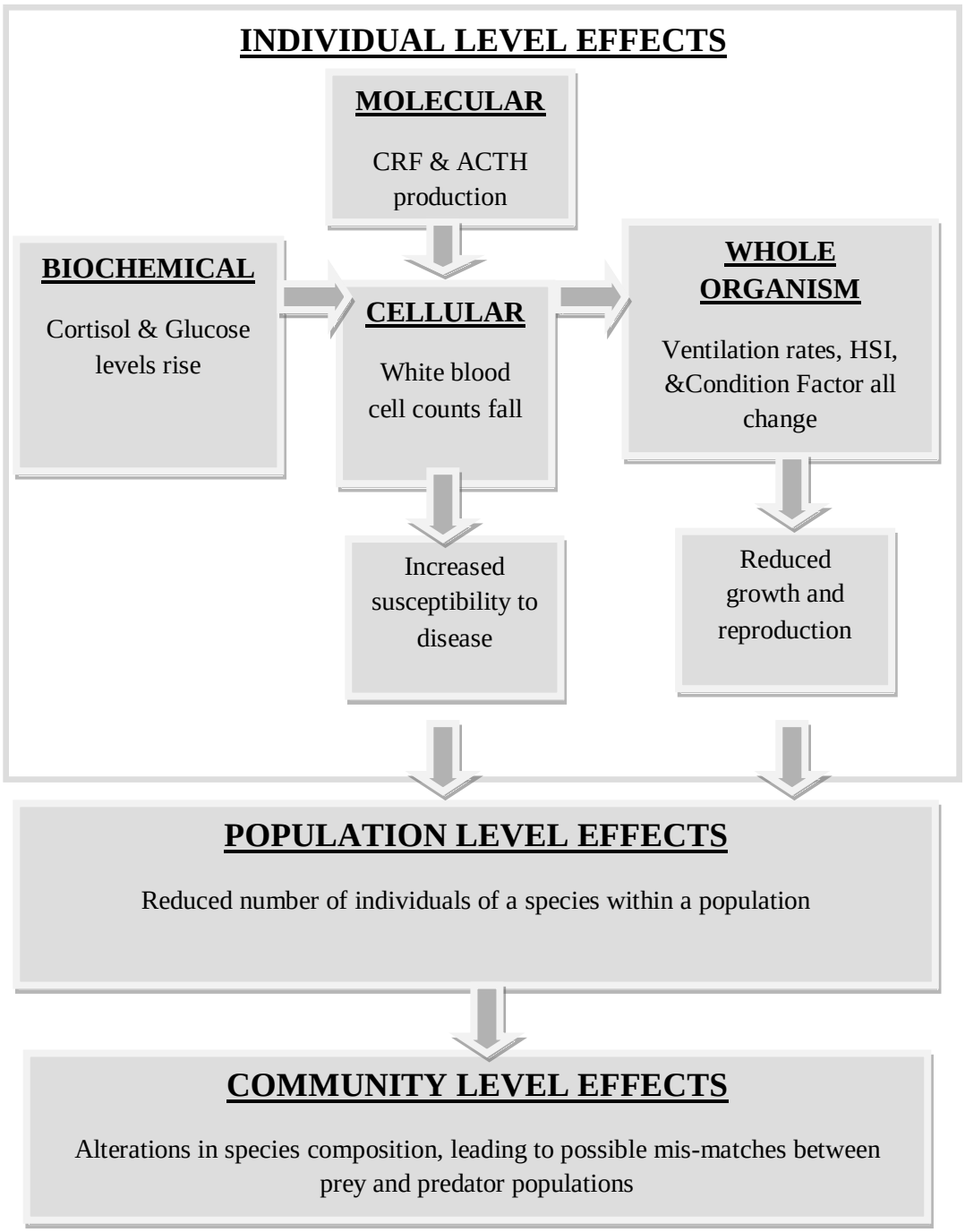


Figure.1.2 Diagrammatic representation of a stress response from the individual level through to the community level.

1.4.2. Species level responses

The effects of climate change have already been observed across the planet, with a study by Parmesan and Yohe (2003) demonstrating that 87% of phenological changes are occurring in the direction predicted by climate change. For example, in the northern hemisphere there has been a general movement of terrestrial animals into more northerly latitudes (Crick *et al*., 1997) and phenological changes such as earlier breeding seasons (Crick *et al*., 1997), earlier migration in birds (Crick, 2004) and earlier first appearances in butterflies (Roy & Sparks, 2000). Studies have also focused on the effects of climate change on the marine environment. There has been a rapid loss of coldwater species in the North Sea, such as the Atlantic cod, with warming waters; although fishing pressures on this species in particular is also a determining factor (Dulvy *et al*., 2008). However, research also shows that two-thirds of fish in the North Sea have shifted either in their depth or latitude with warming (Dulvy *et al*., 2008). This movement into deeper water is at a mean rate of 3.6m decade^{-1} (Dulvy *et al*., 2008), which is comparable to upward altitude movements of terrestrial animals (Parmesan & Yohe, 2003). Therefore similar responses to a warming world are being seen in the oceans as well as on land, providing greater confidence that fish in rivers may also be susceptible to climate change. However, coral reef fish and British freshwater fish have received little attention and so the responses remain largely unknown. Since freshwater fish are constrained in their environment, the consequences could be more dramatic (Daufresne *et al*., 2003). Climate change effects in freshwater ecosystems have been studied for the last 20 years in North America (Mohseni *et al*., 2003; Mulholland *et al*., 1997), and whilst some of the responses may be similar to what we may expect in Britain, there will probably be large differences due to the effects of the Gulf Stream. Recent attempts to understand the implications of climate change in Britain (Graham & Harrod, 2009; Johnson *et al*, 2009) have been instrumental in predicting general consequences on resources and providing preliminary predictions for a few key species (e.g. salmon, brown trout and roach). By and large though, British fisheries have scarcely been studied. Little is known about whether there has yet been any response to a warming world and what implications changes in climate will hold for rivers and the fish therein. Changes in the river environment, such as river flow rates, higher temperatures and reduced oxygen availability may all act as

stressors for fish. The effects may directly impact fish at the population level, for example high flow rates displacing larvae downstream and even out of the river into the estuary. Or the effects may be sub-lethal and more subtle, resulting in a stress response which may take quite some time to lead to declines at the population level. It is highly important to understand how likely it is that climate change will act as a stressor, and what the consequences of any climate induced stress responses will be.

1.4.3 Community Level Responses to Climate Change

Changes to habitats induced by climate change are likely, both for rivers and for coral reefs. On coral reefs, in some way or another, all fish are dependent on the coral, either as a food source, for protection or for supporting the smaller fish that larger fish prey upon. Some fish are obligate coral dwellers, some feed on the live coral and others prefer to settle into live coral, e.g. by hiding in amongst the branches for protection. Some species of fish are thought to be particularly vulnerable to bleaching events, with butterflyfish (Chaetodontidae), cardinalfish (Apogonidae) and gobies (Gobiidae) the most vulnerable (Munday *et al*., 2007). However, declines in community structure are evident even for those fish that are not dependent on coral in any way and therefore climate change, through bleaching effects, is likely to have negative effects on the assemblage of fish found on reefs. This is even before the increases in temperature have any effect on the fish directly and therefore fish living on coral reefs are thought to be particularly sensitive to climate change. Not only is their substrate and habitat going to be reduced, but the fish themselves have evolved in a highly thermally stable environment and are also thought to be living near their upper limit and therefore may be very sensitive to even small increases in temperature.

Where rivers are concerned, riparian cover and catchment characteristics will play a part in regulating the effects of climate change (Webb & Walsh, 2004). Shadier parts of rivers may not warm as much as uncovered areas and so could be used by fish as cool water refuges in waters that are otherwise too warm. However in general, cold water species such as trout, salmon and grayling (Webb & Walsh, 2004) face a dismal outcome. Habitats that are currently suitable for cold water

species may be lost (Mohseni *et al*., 2003) and distribution ranges may shrink (FSBI, 2007). As river temperatures increase, even with some localised cooling from riparian cover, essential summer cold water refuges will shrink (Shuter & Meisner, 1992). Warmer waters may have negative effects on egg incubation and fry size, leading to increased overwinter mortality for some species (Webb & Walsh, 2004). Therefore it is highly likely that cold water species will experience unfavourable conditions with climate change, and reductions in abundance and diversity are expected to be seen. Whether any declines due to climate change are already occurring in British rivers is currently unknown.

However, the majority of fish in Britain are thought likely to respond positively to warmer waters. Most fish species are well within their thermal limits (Arnell, 1998) and so for some species climate change may be beneficial. It may be that there will be an expansion in suitable habitat for cool and warm-water fish as river temperature increases, particularly in winter months (FSBI, 2007; Mohseni *et al*., 2003). Climate change may allow fish to spawn earlier in the year (Webb & Walsh, 2004), thus lengthening their growing season and so reducing overwinter mortality in the first year of life (Shuter & Meisner, 1992).

The literature suggests that the River Thames may still be inhabited by fish under various climate change scenarios, but the population composition may change (Johnson *et al*., 2009). Only under extreme scenarios is the River Thames predicted to become too stressful for nearly all species (Webb & Walsh, 2004). Research conducted in the Rhône River, France, showed that as river temperature increased, northern cold-water species, such as chub and barbel, were replaced by more southern thermophilic species such as bleak and dace (Daufresne *et al*., 2003). It may be that similar changes have already occurred in the River Thames, but as yet this possibility has not been studied (Johnson *et al*., 2009). It is likely though that climate change will favour cyprinid and percid species such as perch, roach, bream and carp, while salmonid species will be lost (FSBI, 2007). Survival will depend on species ability to adjust to the new temperature and flow regimes, and with time some species may be able to adjust genetically to increase their thermal tolerance (Webb & Walsh, 2004).

1.4.4. Application of Bayesian Networks

In order to predict how well fish in an ecosystem, for example the River Thames, will respond to climate change, it is important to understand the complex relationship between the physical environment and the fish, but also to include and appreciate the interactions between fish species themselves (Milns *et al*., 2010). Understanding the myriad of connections is problematic, to say the least. While many studies have focused on trying to identify the principal controlling factors, such as temperature or flow rate, the techniques of using traditional statistics may be missing key interactions.

Bayesian Networks (BNs) are a relatively new technique but have proved to be highly successful for analysing ecological data sets (Dose & Menzel, 2004; Marcot *et al*., 2001; Milns *et al*., 2010). They may possibly have a greater power than traditional statistical techniques, such as Principal Component Analysis (PCA) and Multiple Linear Regression, which cannot easily make use of incomplete data sets (Dose & Menzel, 2004). Obtaining complete, long-term data sets is often very problematic for ecological systems. BNs also understand and utilise variables that may be auto-correlated, for example water temperature and dissolved oxygen concentration, which is usually prohibited in linear regression and PCA (Shaw, 2003).

A BN is a model which is created based on the probable links between multiple variables, providing a wealth of information about the connections in complex data sets. BNs utilise Ockham's razor, which is the principle that when explaining 'a thing', no more assumptions should be made than necessary (Oxford English Dictionary). That is to say that the simplest explanation is usually the correct explanation. Using all the data available, a BN will rank the probability of each variable affecting another, and produces a model which uses the minimum number of variables necessary to explain the data (Dose & Menzel, 2004). The data set is tested rigorously by mathematical algorithms until a model is learnt that explains the data in its simplest terms. The resulting graphical network therefore only relates nodes (with each variable represented as a node) that are probabilistically and statistically related to each other by a causal dependency. Each node or variable can be either discrete (e.g. seasons) or continuous (e.g. biomass and density of fish).

Arrows connect each node, with the direction of the arrow indicating the direction of causality.

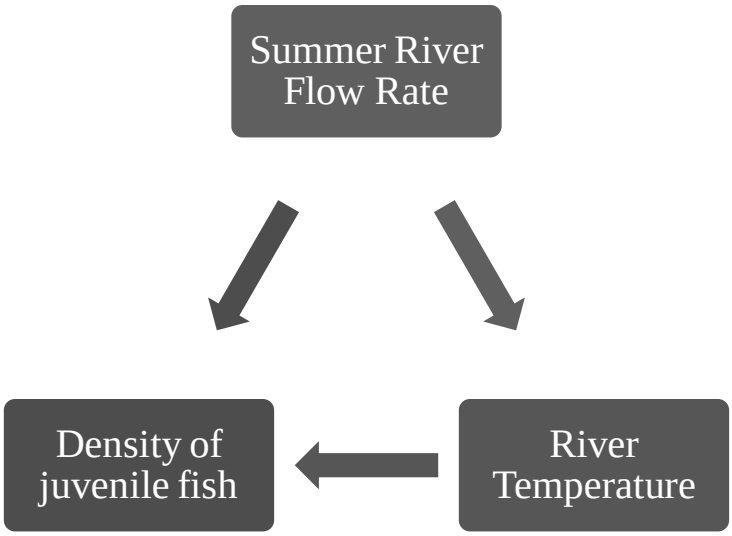


Figure 1.3. Diagrammatic representation of a graphical Bayesian Network displaying three nodes (summer river flow rate, river temperature and density of juvenile fish) and arrows, which show the direction of relatedness.

For example (Figure 1.3), summer river flow rate can be high or low. A high river flow rate can cause the river temperature to be lowered. High summer flow rates can also cause a reduction to the density of juvenile fish due to the flushing effects of high flows on small fish that are not strong enough to swim against the current. Low river temperatures can also result in a reduction in the density of juvenile fish by reducing growth rates and therefore making them more susceptible to predation and increasing the chances of overwinter mortality.

Given that these networks provide information on the cause and effect relationships in a dataset, they are useful in their application of future predictions based on learnt models of past events. These networks have recently been successfully applied to various ecological data sets; for example, in effectively identifying known relationships between birds and their habitats in the Peak District National Park (Milns *et al*., 2010). In this study, 37 bird species, 6 vegetation groups, altitude, slope and path distribution data were applied to a Bayesian Network. The resulting network correctly identified the known relationships of habitat types and

birds and also the interspecific relationships between birds. Furthermore, the model provided novel insights into complex relationships between bird species that were not previously known. This study by Milns *et al*. (2010) showed the potential for using BNs to understand and interpret the complex interactions in ecosystems, and therefore their potential for understanding interactions between fish species and their future changing physical environment.

Because the networks identify causal relationships, they allow predictive analysis of a data set. For example, once a network is learnt for a given data set, it can be manipulated to represent the forecasted physical conditions, for example, 'wet and mild' winters and 'hot and dry' summers. The BN should then be able to predict, through confidence based on known relationships, likely outcomes on fish biomass and density, from whole community to species-specific effects. It can also take into account the dependencies and interactions between different species, which may also affect their likely responses to climate change.

1.5. Confounding factors

Fish are exposed to a wide range of factors that may alter their biomass and diversity. Population changes in fish may occur due to factors which are unrelated to direct effects of climate change (e.g. temperature, pH and flow rates), but rather due to factors such as food availability, re-stocking populations, pollution events, disease outbreaks, extreme storm events, fishing pressure and changes in nursery habitat.

Commercially important coarse fish species, such as roach, bream, rudd and dace, are commonly re-stocked in the River Thames (Johnson *et al*., 2009) to support the angling societies which serve nearly four million people in Britain (Davies *et al*., 2004). Atlantic salmon has been absent from the River Thames for many years, due largely to the heavy pollution since the industrial revolution. But salmon is viewed as a 'prize fish', both in terms of catch and also for eating, and so anglers are keen to see it is re-established in the River Thames (Davies *et al*., 2004). Despite the River Thames being cleaner, several attempts to re-populate the River Thames with

Atlantic salmon have failed (Wheeler, 1969) because of migration obstruction from the presence of weirs. Chub and roach are also farmed commercially for restocking (Davies *et al*., 2004), since they are important sports fish. This steady restocking may mask true patterns in abundance and diversity of fish, making it difficult to see which species are coping with the changes to their environment and which ones are not.

Sewage Treatments Works (STW) strip wastewaters of harmful chemicals, so that they are at safe levels before water is released into the river system. However, mistakes can be made, with devastating effects of the fauna and flora. When carp were exposed to raw sewage, all fish died within 6 hours (Kakuta & Murachi, 1997). Chemical spills can have equally detrimental effects. In 2007, a bleach spill in a Thames STW devastated the local fishery along the River Wandle in the south-west of London (ENDS, 2009). This resulted in thousands of fish being killed, with an anticipated 10 years recovery period. Even without disasters such as these, decreases in quality of released water could have negative impacts on fish populations. Due to water being warmer around sewage effluent pipes, fish often congregate in these regions (Kakuta & Murachi, 1997). However, fish here may be exposed to higher levels of chemicals or raw sewage, which may present a chronic stress for fish. Physiological dysfunctions (Kakuta & Murachi, 1997) including reduced performance, lowered reproduction and lowered growth rates (Winter *et al*., 2008), may all result from poor water quality.

In 2009, Bolivia experienced an unusually cold July and August, with extreme variations in temperature between day and night. This coincided with a mass mortality event in fish, with some 6 million fish deaths, along with thousands of alligators, turtles and river dolphins (Petherick, 2010). This mass mortality was largely attributed to the effects of extreme temperature shock that may well be linked to our changing climate. However, it was also apparent that many fish had white spots on the surface of their skin, suggesting that there had been a disease outbreak (Petherick, 2010). Whether it was extreme cold that killed the fish or the disease outbreak, or whether the extreme cold left the fish vulnerable to infection is not clear. This paper highlighted, however, that at any one time there may be multiple stressors interacting, making it extremely difficult to pinpoint the actual variable affecting fish populations.

The Great Barrier Reef is also already subjected to a large amount of both natural and man-made disturbances. Globally there has been a decline in coral reefs by 30% and this is predicted to increase to 60% by 2030 (Hughes *et al*., 2003). The GBR is subjected to a large amount of coastal clearing which increases the sediment load to the reef. This then smothers the coral and eventually kills off patches of reef. Algal growth then proliferates and within decades a hard coral reef can be altered into an algal reef, as occurred in the Caribbean in the 1980s (Nyström *et al*., 2000). This transition was also due to over-fishing in the area, which removed key species of benthic algal feeders and so grazing was not kept in check and algal growth increased, which is also an issue off the coast of Australia. There is also a great deal of nutrient enrichment into the GBR zone which is carried into the ocean with land runoff, particularly in wet months. This eutrophication exacerbates algal growth. Despite heavy policing, there is still a large amount of uncontrolled tourism on the GBR (Nyström *et al*., 2000), which can be very damaging to the reef through degradation through improper anchoring, inexperienced divers touching the reef and engine emissions.

Natural disasters such as tropical cyclones, earthquakes, tsunamis and volcanic eruptions can be very destructive to the coral matrix itself (Nyström *et al*., 2000). Other natural problems include outbreaks of coral predators, such as Crown of thorns starfish, *Acanthaster planci*, and the rock-boring sea urchins, *Echinometra mathaei* and *Ophiocoma dentata* (Hutchings *et al*., 2007; Nyström *et al*., 2000), which can damage huge sections of the reef. Crown of thorns starfish populations are closely monitored and managed by the GBRMPA to limit outbreaks and the damage they cause, as it can take reefs 10-15 years to recover from an outbreak of *A.planci* (Hutchings *et al*., 2007). If background stress is at a minimum, natural disasters, which usually only occur in pulses (i.e. not a chronic stress), aid in reef development. However, the many man-made stresses that now occur on the reef are decreasing the resilience of reefs and so recovery times from natural stressors is longer.

Differences in abundance and diversity of fish may result indirectly from climate change or from other external factors. Therefore, relating changes in the fish populations to changes in temperature, pH, river flow or oxygen concentrations, is even more challenging. Gaining information on many of these confounding factors is

highly problematic and so often not included in data sets. These factors, even if not included, need to be taken into consideration when assigning responses to causes.

1.6. Summary

Predicting the effect of climate change on fish populations is a complex task; especially those that are so heavily impacted by anthropogenic stresses, such as the River Thames and the Great Barrier Reef. It is important to understand the implications that changes in river and ocean parameters will have on the fish populations. Changes such as warming waters, reduced ocean pH, altered flow regimes, and an increase in extreme weather events, will likely affect fish from the biochemical level through to the community level. The general supposition is that there is likely to be a shift in community structure, both in rivers and on coral reefs. In British rivers, warm-water species will likely replace cold-water species; nevertheless, most native UK species should cope with the predicted changes. On the Great Barrier Reef, fish that are already living near their upper thermal limits will likely be sensitive to warming waters and will be replaced by more resilient species. However, fish will not just be influenced by temperature, but by multiple and potentially confounding stressors. It is important to understand the interactions of multiple factors, not just temperature, but pH, flow regimes, oxygen levels, habitat accessibility and prey availability. Due to the importance of coral reefs, there has been a large amount of research which indicates that climate change is already occurring on the Great Barrier Reef and will likely cause damage to the reef structure through repeated mass coral bleaching events. However, the direct effect of climate change on the fish population is largely unknown. What is also not well understood is whether there is any evidence yet of climate changes in the River Thames and if so whether the fish have responded. Only once it has been established that climate change will not pose a significant stress on native freshwater fish can we be comfortable in stating that freshwater fisheries in the UK will not be adversely affected by the changing climate. Given that climate change is a truly global issue, it is important to take a holistic approach and appreciate that there will be many ecosystems affected. This thesis will take a comparative approach to evaluate

whether relatively small changes, particularly in temperature, will affect the physiology of fish, from both temperate freshwater ecosystems (River Thames) and tropical marine ecosystems (Great Barrier Reef). Both of these ecosystems are already subjected to a large amount of background stress. By investigating the effects of climate change from the biochemical to whole organism stress responses, and by using statistical modelling to predict the consequences at the population and community level, this thesis aims to provide key information on how we can expect these fisheries to cope and therefore provide essential guidance should they need protecting.

1.7 Aims and Objectives

The aim of the work herein was to test the hypothesis that the small changes in the physical environment predicted with climate change will have similar significant effects on fish from temperate freshwater habitats and tropical marine habitats. The thesis is split into 4 principle data chapters (chapters 2 to 5) and involves complementary studies involving analysing existing data sets using statistical modelling techniques and *in-vivo* studies.

Chapter 2: Effects of chronically raised temperature on five species of coral reef fish on the Great Barrier Reef.

- To test whether small increases in temperature realistic with climate change decrease the aerobic scope of five common coral reef fish.
    - *In-vivo* study: Measuring and comparing the resting (basal) and maximal respiration rates of fish acclimated to the 31°C, 32°C and 33°C compared to a control temperature of 29°C.

Chapter 3: Interacting effects of chronic thermal stress and ocean acidification on two species of cardinalfish on the Great Barrier Reef.

- To examine whether there are interactive effects of temperature and pH on the aerobic scope of two cardinalfish, *Ostorhinchus cyanosoma* and *O.doederleini*.
    - *In-vivo* study: Measuring and comparing the resting (basal) and maximal respiration rates for fish to determine aerobic scope of fish acclimated to different temperature and pH conditions predicted to occur as a consequence of climate change by 2100.
    - Conditions used were 29°C (control), 31°C and 32°C, each in combination with pH 8.15 (control) and 7.8 (representing ocean acidification).

Chapter 4: Recent evidence of climate change in the non-tidal River Thames

- To ascertain whether there is yet any evidence of climate change in the River Thames and what effects this has had on the fish population of the non-tidal part of the River Thames.
    - Using a 15 year historical data set of river temperature and flow rates, to establish if there is any indication that climate change is already physically affecting the River Thames.
    - Using a 15 year data set for fish biomass and density, to establish whether there have been any changes in fish population structure during that time.
    - To evaluate the use of Bayesian Network models in understanding the complex interactions in freshwater ecosystems.

Chapter 5: Biological indicators of thermal stress in the stickleback, *Gasterosteus aculeatus*.

- To investigate whether the predicted increases in water temperature as a consequence of climate change elicit a stress response in a species of fish native to the River Thames.
    - *In-vivo* study: chronic exposure of the three-spined stickleback to water temperatures at current-day summer mean (19ºC), best case scenario B1 (21ºC) and worst case scenario A1F1 (25ºC).
    - Evaluation of the stress responses using a range of endpoints: water cortisol concentrations, plasma glucose concentrations, leukocyte profile, ventilation rates, condition factor, hepatosomatic index and growth rates.

Chapter 2: Effects of chronic thermal stress on five species of coral reef fish living on the Great Barrier Reef.

Nilsson, G.E., **Crawley, N**., Lunde, I.G. & Munday, P.L. (2009). Elevated temperature reduces the respiratory scope of coral reef fishes. *Global Change Biology*, 15, 1405-1412

2.1 Statement of Contribution to Work

This work was conducted on Lizard Island on the Great Barrier Reef under the supervision of Dr Philip Munday (James Cook University) and Professor Göran Nilsson (University of Norway). I collected the fish from the reef and conducted the respirometry experiments in accordance to methodology previously used by Nilsson *et al*. (2007). I input the data and contributed to statistical analysis and discussions on the layout and content of the paper.

2.2 Abstract

The aim of this study was to determine whether small increases in temperature realistic of climate change cause a reduction in the aerobic scope of five commonly occurring coral reef fish on the Great Barrier Reef. Two species of cardinalfish, (*Ostorhinchus cyanosoma* and *O.doederleini*) and three species of damselfish, (*Dascyllus aruanus, Chromis atripectoralis* and *Acanthochromis polyacanthus*) were exposed to temperatures 31°C, 32°C and 33°C compared to the current summer mean of 29°C. Resting and maximal oxygen consumption was obtained using respirometary experiments and aerobic scope subsequently calculated. In three of the five species of coral reef fish tested, their aerobic scope was significantly reduced by as little as a 2°C rise in water temperature and all five species significantly affected by a 4°C rise in temperature. The reduced aerobic scope was due to increased resting oxygen consumption and an inability to increase

the maximal oxygen uptake. However, there were interfamilial differences, with the two species of cardinalfish being more sensitive to increases in temperature than the damselfish, demonstrated by cardinalfish experiencing a complete loss in aerobic scope with a 4°C rise in temperature. These interfamilial differences in ability to cope aerobically with warming waters could lead to a change in the community structure on coral reefs and potentially a loss of diversity.

2.2 Introduction

2.2.1. Temperature and Coral Reefs

Coral reefs need particular environmental conditions in order to grow. Typically, coral reefs develop in well-lit, nutrient poor waters, in the warm equatorial waters between 30°S and 30°N (Hughes *et al.*, 2003). Although coral reefs require warm water to grow, their existence may be under threat from the anticipated warming associated with climate change. It is expected that the average sea-surface temperatures in the vicinity of coral reefs will increase by several degrees Celsius over the coming century (Guinotte *et al*., 2003; Lough, 2007). There is already evidence of warming in Australia, for example, in Queensland, nine of the ten warmest years since 1850 have occurred between 1997 and 2006 (Lough, 2007), and a further 3°C rise is expected on the Great Barrier Reef in the next 50-100 years (Lough, 2007). Changes of only a few degrees are expected to be sufficient to result in significant impacts to individual performance, community structure and geographical distributions of corals and their associated reef fish (Munday *et al.*, 2008a).

Despite the fact that it is expected that reef fish will be directly affected by temperature increases, to date research has focussed on the corals themselves, particularly concerning outbreaks of coral bleaching with increasing temperature (Hoegh-Guldberg, 1999). Climate change, through mass coral bleaching events, will indirectly affect coral reef fish. Since nearly all fish on the reef rely on the coral matrix for shelter or food, any deterioration to their habitat will likely impact the assemblage of coral reef fish present. Much less is known about how increases in

sea-surface temperature will directly affect reef fish (Munday *et al*., 2007). Tropical reef fish have evolved in a stable environment and experience very little background variation in temperature; therefore only a small change in their stable environment could cause a disproportionate response.

2.2.2. Effects of temperature on coral reef fish.

Unlike many temperate freshwater fish, it is thought that most coral reef fish are now living close to their short-term lethal thermal limits (Mora & Ospina, 2001). However, even if not lethal, increases in temperature could still have an effect on individual performance, such as physiological condition, development rate, growth rate and reproductive capacity (Wood & McDonald, 1997). All of these factors will affect the long-term sustainability of a population of fish (Munday *et al*., 2008a).

The two functional physiological components of growth are consumption and metabolism, both of which are strongly affected by temperature (Biro *et al*., 2007). Temperature is a major factor governing growth, as it affects the rate of metabolic reactions affecting all physiological processes in ectotherms (metabolism, food intake and nutrient efficiency) (Burel *et al*., 1996). The general trend is that at higher temperatures, a smaller body size is produced. Temperature does affect growth, with higher temperatures resulting in smaller mean length, body depth and mean wet weight (Burel *et al*., 1996; McCormick & Molony, 1995; Pörtner & Knust, 2007). The size of the organisms dictates the thermal tolerance; with smaller fish having a higher tolerance than juvenile and adult fish (Burel *et al*., 1996). At larger body sizes, the effects of a lack of oxygen due to reduced solubility at higher temperatures will be more pronounced. The premature loss of aerobic scope of large eelpout individuals compared to smaller individuals indicates that specimens do not grow beyond oxygen-dependent size limits set by temperature (Pörtner & Knust, 2007). A reduction in abundance results when all size groups of a population are affected (Pörtner & Knust, 2007).

There are, however, some reports that growth rates can be increased with fluctuating temperatures. This is known as the Acceleration Effect (Jobling, 1997). To an extent, higher temperatures will increase the rate of growth, even though the

absolute growth will be smaller. It was shown that at 7 days after hatching, larvae that were reared at 25ºC were smaller than those at 28ºC. However, when sizes and weights were compared at the same developmental stage (pre-metamorphic stage, 11 days for larvae at 25ºC, and 9 days at 28ºC), the larvae reared at 25ºC were larger. Therefore although it took longer for the larvae to develop and reach metamorphosis, the larvae were larger at this ontogenetic stage (Green & Fisher, 2004).

If temperature exceeds an optimum, then effects will be seen, such as negative growth rates and lower survival. This is because with higher temperatures, metabolic rate is increased, foraging effort is increased but food conversion efficiency is reduced (Biro *et al.*, 2007; Kucharczyk *et al.*, 1997). Due to this decreased growth and also increased foraging activity, survival is reduced, and so temperature has been suggested to account more for reduced survival than food abundance does (Biro *et al.*, 2007).

At higher temperatures, metabolic processes are increased as more energy is needed to maintain body structures (McCormick & Molony, 1995; Shirayama & Thornton, 2005). Therefore there is a need for a greater oxygen supply (Taylor *et al.*, 1997) and so rates of resting oxygen uptake are a reliable measure of aerobic metabolism (Taylor *et al.*, 1997). However, in higher temperatures the oxygen solubility in water and plasma is reduced. Simultaneously the oxygen affinity of haemoglobin is reduced (Taylor *et al.*, 1997) and so it is difficult for the oxygen demand for aerobic metabolism to be met. This is known as the Temperature-Oxygen Squeeze (Rombough, 1997).

It can take days to weeks before fish attain metabolic rates characteristic of new temperature regimes, but acclimation does appear to be feasible. However at high temperatures, fish may not be able to acclimate or the build-up of an oxygen-debt from intense activity may reach critical levels (Taylor *et al.*, 1997). This greater oxygen debt at higher temperatures may limit the duration of anaerobic burst swimming, which will reduce effective foraging behaviour. Animals with higher metabolic rates, such as when temperature stressed, also become more sensitive to other environmental perturbations, such as elevated carbon dioxide (CO_2) (Shirayama & Thornton, 2005). With the projected models of higher CO_2 and temperature, it is likely that metabolic demands of coral reef fish will be increased,

likely to a point when more energy is expended than consumed. When this happens, growth rates will be slowed, which can impact other processes, such as reproduction and survival.

2.2.3. Aerobic Scope

Aerobic scope or aerobic capacity is a good measure of the maximal sustainable aerobic ability of an animal. It is a dimensionless value and is essentially the difference between the resting (basal) and the maximal oxygen uptake. The greater the aerobic scope, the more an organism is able to cope with their environment and have energy left over after essential maintenance for growth and reproduction. Therefore, a decreased capacity to perform aerobically (i.e. reduced aerobic scope) at higher temperatures is thought to be the key physiological mechanism that will determine how species will cope with climate change (Pörtner & Knust, 2007). There are at least four factors that govern the maximal rate of oxygen consumption: 1) cardiac output (determines the rate of blood flow through the gills and out to the body), 2) gill surface area, 3) oxygen carrying capacity of the blood (which is dependent on the haemoglobin content), 4) degree of downloading of oxygen from blood to tissues (Gardiner *et al*., 2010).

Whilst at higher temperatures there is an increased oxygen demand, there are limits to the capacity of the circulatory and ventilatory systems to keep pace. It is this limited capacity that will ultimately set the boundaries for whole-organism tolerance to temperature increases (Pörtner & Knust, 2007). Small increases in acute temperature are unlikely to affect fish, unless they are already at their upper thermal limit (Taylor *et al*., 1997), or if there are additional environmental stresses, such as lowered pH. If the aerobic scope is reduced by chronically elevated temperatures, this will affect all aspects of individual performance, and thus ultimately will affect the population sustainability.

Shifts in geographical location have been observed for many terrestrial species due to warming (Parmesan & Yohe, 2003). Movement to higher latitudes as waters warm have also been documented for several marine fish in many locations. For example, in North Carolina there has been an influx of tropical marine species

previously never recorded in the area, as waters have warmed (Parker and Dixon, 1998). In the North Sea, there has been a northward movement of Atlantic cod (*Gadus morhua*) and the common sole (*Solea solea*) (Perry *et al*., 2005) with warming. These geographical shifts indicate that there are many families of marine fish that are sensitive to even small increases in temperature. It is therefore hypothesised that reduced aerobic scope is the primary mechanism that controls the thermal niche for marine fishes and therefore their geographical range.

2.2.4. Common Coral Reef Fish

Five species of commonly occurring coral reef fish found at Lizard Island were used in this study. These species are all abundant on the reef, none are subject to special protection, and they are typical of coral reef fish found in coral reefs in the Indo-Pacific region. Given their wide geographic range, they are considered to be representative of reef fish found on many coral reefs. Two species of cardinalfish, *Ostorhinchus cyanosoma* and *O. doederleini* (Figure 2.1 a and b, respectively) and three species of damselfish, *Chromis atripectoralis*, *Dascyllus aruanus*, and *Acanthochromis polyacanthus* (Figure 2.1 c-e, respectively) were selected.

It was important to study several different species from different families in order to have a greater understanding of both species and familial tolerances to temperature. All chosen species are small; the average wet weights of adults were 3.05±1.4g. These species are all typically coral dwelling and due to their small size are able to hide from predators among the branches of coral, such as the staghorn coral (*Acropora spp*) or in caves (Marnane & Bellwood, 2002). As is normal for coral reef fish, all of the species except *A.polyacanthus* have a dispersive larval stage. By having such a reproductive strategy, it promotes a better gene flow between local populations, which aids in natural selection and preventing a small gene pool. *A. polyacanthus* instead has a resident larval stage, whereby the larvae remain in the same local area of the reef from which they originated. This species also displays parental care, where the parents actively guard their nests. Whilst this increases the chances of offspring survival it comes at an energetic cost to the parents. This breeding technique restricts gene flow between neighbouring

populations, but may allow each local population to adapt to changes in their thermal environment quicker (Munday *et al.*, 2008b), with each local population being suited to its particular thermal niche. By including fish with both a dispersive and resident larval stage, it may provide information as to how restrictions on gene flow may prevent or permit populations from adapting to warming waters.

Damselfish and cardinalfish are extremely common on coral reefs. However they play different roles on the reef and have employed different life strategies. *D. aruanus* are polygamous and form harems consisting of one or two males accompanied by several females. In order to keep the harem structure, they are protogynous hermaphrodites, with sex-change of females occurring to replace any lost males (Fishelson, 1998). *D. aruanus* displays high site attachment, probably due to strong predation pressure; the more isolated and site-attached the harem is, the more dominant a role sex change plays in the group (Fishelson, 1998). There is high plasticity in the damselfish family compared to other families of coral reef fish, as displayed in delays in sex-determination, sex change, varied behaviour patterns and colour patterns. This plasticity might allow damselfish to cope better with negative environmental parameters. It may also allow them to genetically change quicker and be 'ready to adapt', which may permit damselfish to adapt quicker to long-term changes in their environment (Fishelson, 1998).

The cardinalfish *O.cyanosoma* and *O.doederleini* are important species on coral reefs as they act as nutrient recyclers (Marnane & Bellwood, 2002). Being nocturnal, they move away from the reef matrix at night and feed on plankton in the water column. Both species display high site attachment, and so in the day return to the same patches of reef and through excretion, deposit nutrients onto the reef matrix which would otherwise not be there. There are generally high abundances and rapid turn-over of these cardinalfish on reefs, which suggest that they are important components of the coral reef community. Being nocturnal and migrating from the reef at night, there is the possibility that cardinalfish are exposed to a slightly different thermal regime than that of damselfish, since the reef matrix and the lagoon may vary in temperature between day and night. This may therefore have implications as to the actual thermal regimes in which they are most active, with damselfish naturally being more active in daylight hours when the water is warmer and cardinalfish more active at night when water temperatures are cooler. However,

relatively little is still known about them, with cardinalfish being one of the least-studied families of coral reef fish (Marnane & Bellwood, 2002).

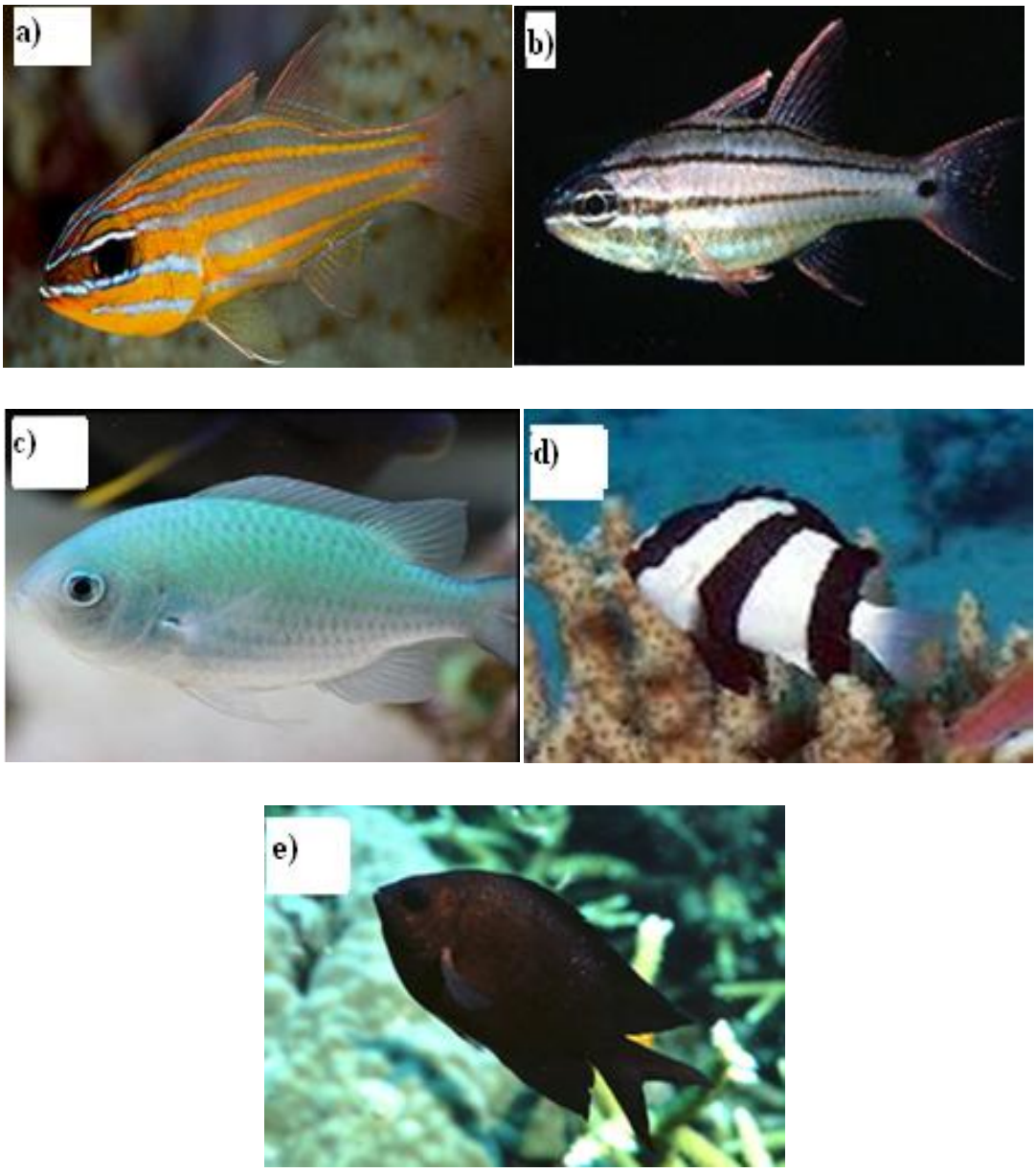


Figure. 2.1. Photograph of cardinalfish a) *Ostorhinchus cyanosoma,* b) *O. doederleini* and three damselfish c) *Chromis atripectoralis*, d) *Dascyllys aruanus* and e) *Acanthochromis polyacanthus* (Photos from: www.fishbase.com [date accessed: January 2012]).

2.2.5. Aims of study

The aim of this study was to test whether the aerobic scope of five common coral reef fish was affected by small increases in temperature at Lizard Island on the northern Great Barrier Reef, Australia. Two species of cardinalfish, *Ostorhinchus cyanosoma* and *O.doederleini*, and three species of damselfish, *Dascyllus aruanus*, *Chromis atripectoralis* and *Acanthochromis polyacanthus*, were selected for experimentation.

Resting oxygen consumption (MO_{2rest}) and maximum oxygen consumption (MO_{2max}) for each species were calculated in a closed-system respirometer at current-day average summer sea-surface temperatures at Lizard Island (approximately 29°C; Lough, 2007) and at temperatures likely to be experienced at this location over the next century (31°C, 32°C and 33°C). Maximum summer sea-surface temperature in the vicinity of Lizard Island regularly exceeds 30°C and extremes of up to 32.7°C have been recorded (Lough, 2007). Average sea-surface temperatures on the GBR are predicted to increase by up to 3°C over the next 50-100 years (Lough, 2007); therefore, 31-33°C encompasses the range of average and maximum summer temperatures likely to be regularly experienced by coral reef fishes on the northern GBR by 2100.

2.3 Methods

2.3.1 Study Site

Experimentation was carried out at Lizard Island Research Station (LIRS) on the Northern Great Barrier Reef (14°40'S, 145°28'E) (www.lizardisland.net.au). The Island is 270km north of Cairns and 30km from the Australian mainland. It is a high granite island that is surrounded by a well-developed fringing reef. The island and surrounding reefs are protected by the Great Barrier Reef Marine Part Authority. All experimentation was carried out between December 2007 and January 2008 (austral summer).

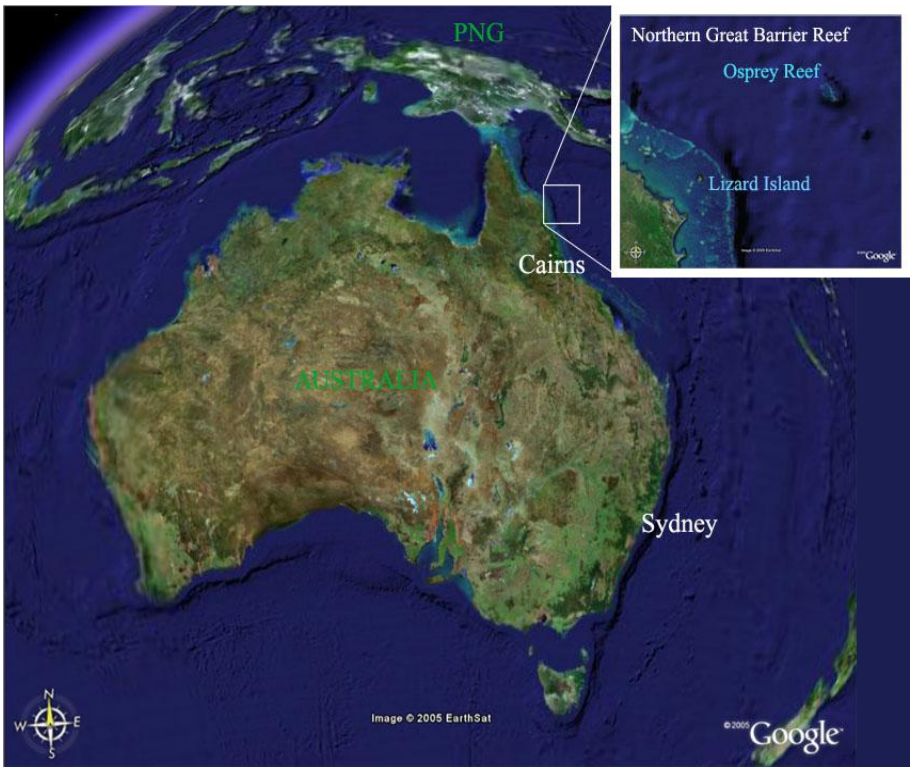


Figure 2.2. Location of Lizard Island on the Great Barrier Reef off the coast of Queensland, Australia (www.lizard.island.net.au)

Figure 2.3. Labelled map of Lizard Island group. White stars denoting areas in the lagoon reef where fish were collected by SCUBA diving. (www.lizard.island.net.au)

2.3.2 Fish Husbandry

Nine adults of *Ostorhinchus cyanosoma* (2.3±0.6g), *O. doederleini* (2.1±0.8g), *Dascyllus aruanus* (3.4±1.3g), *Chromis atripectoralis* (5.3±0.9g) and *Acanthochromis polyacanthus* (2.5±0.7g) (wet weight ranges within parenthesis) were caught by SCUBA diving in the lagoon around LIRS at a depth of between 2 and 5 meters. A sample size of nine was selected since previous similar experimentation on these species has been shown to be sufficient for significant statistical analysis (Nilsson *et al.*, 2007). Furthermore, sample sizes were constrained from being larger due to ethics and permit restrictions. Juveniles of *A.polyacanthus* were used so that all fish were the same size, as metabolism is known to be size dependent and *A.polycanthus* are a slightly larger size when adult than the other species. Fish were caught by methods previously described by Östlund-Nilsson & Nilsson. (2004). In brief, the fish shoal together within or over branching corals at a depth of 1-5m. Fish were lightly sprayed with clove oil which acts as an anaesthetic (50ml of clove oil, 40ml ethanol and 400ml seawater) allowing individuals to be

caught in a net and transferred into a plastic bag containing fresh seawater. The fish recovered within a minute of being placed in clove-oil free water. Fish were carried back to the aquarium in large plastic bags filled with fresh seawater. Fish were then immediately transferred into 50L holding tanks in a shaded outdoor aquarium with a continuous supply of fresh seawater pumped directly from the reef. Oxygen levels in the water varied between 95-100% saturation throughout the experimental period.

Fish were fed until satiation with frozen blood worms and commercial fish food (INVE, Aquaculture Nutrition pellets), but were starved for 24hours prior to measuring oxygen consumptions. All tanks were checked hourly during daylight hours (6am-7pm) and any mortality recorded.

2.3.3 Experimental Set-up & temperature regimes

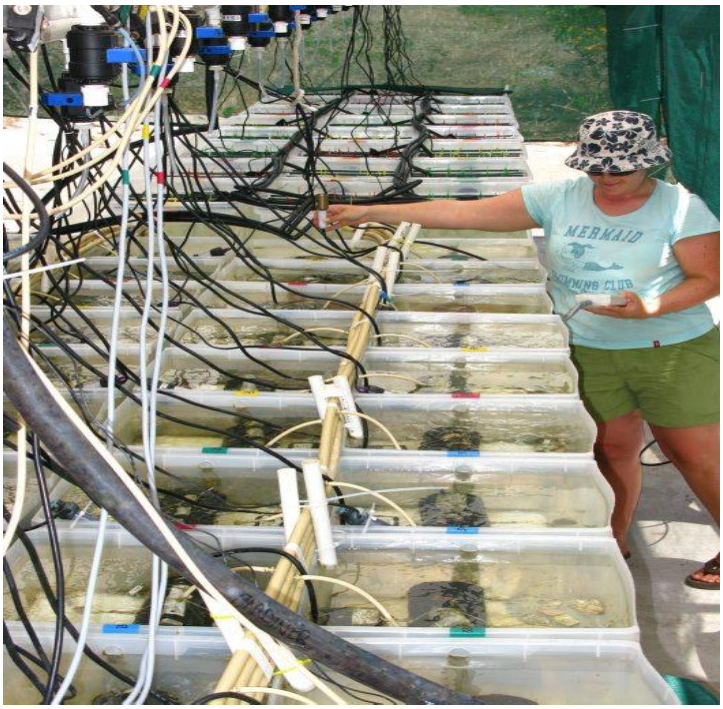


Figure 2.4. Photograph of the outdoor aquarium at Lizard Island where tanks are supplied with fresh seawater pumped directly from the lagoon.

Four 50 litre tanks were supplied with fresh seawater pumped directly from the lagoon. The control tank was kept at ambient sea-surface temperate (29°C± 0.5°C) and the other tanks were subsequently heated with aquarium heaters to 31°C,

32°C and 33°C over a period of 1-2 days. Temperatures in each tank were checked hourly between the hours of 6am and 7pm, with maximum fluctuations being ± 0.5°C. Fish were held at experimental temperatures for a period of one week prior to respirometry testing. A period longer than one week was not deemed necessary, as it has previously been reported for these species that a longer acclimation period has no effect on oxygen consumption (Nilsson *et al.*, 2007).

2.3.4 Respirometry for measuring resting oxygen consumption (MO_{2rest})

At the start of each trial, the fish were carefully transferred from the holding tanks into the respirometer, which was set up in an identical tank held at the same temperature as the acclimation tank. The respirometer was a custom-made plexi-glass chamber with an internal diameter of 80mm and held a volume of water of 500ml. Care was taken not to stress the fish when placing in the respirometer, as this will have raised their rate of oxygen consumption. Each fish was allowed to acclimate to the respirometer for 30 minutes before a plastic lid was attached (to prevent oxygen diffusing into the water in the respirometer) and recording their oxygen consumption. An oxygen electrode (OXI 340i, WTW) continuously recorded the concentration of oxygen within the chamber and recorded data directly onto a computer. Each fish was left resting in the closed chamber for a period of 30 minutes, as this time was deemed long enough to provide data on oxygen consumption.

MO_{2rest}=Concentration of oxygen consumed/ time

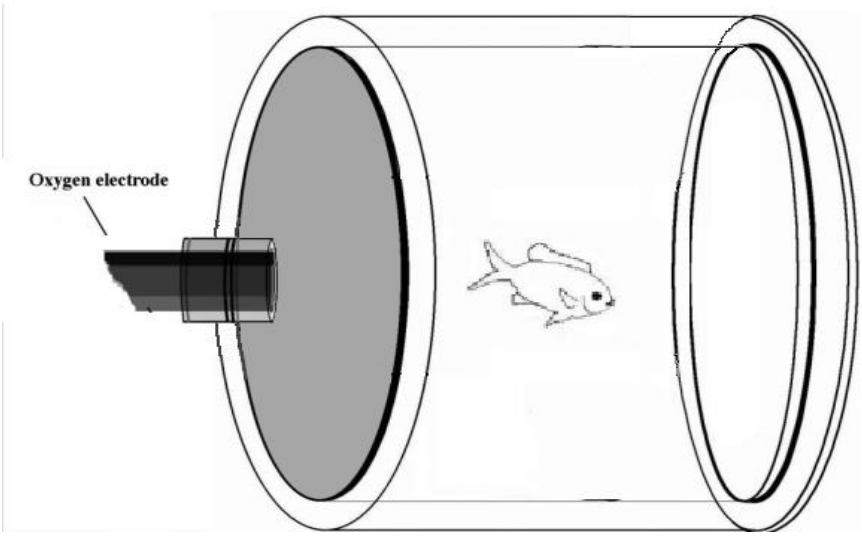


Figure 2.5 Diagrammatic representation of the resting respirometer set-up with fish enclosed in Perspex cylinder with an oxygen probe inserted at the top. Cylinder was placed on its side and the oxygen probe transmitted readings to a connected computer.

2.3.5. Swimming respirometry for measuring maximal O_2 uptake (MO_{2Max})

The respirometer chamber consisted of a Perspex cylinder (80 mm inner diameter, 500ml total water volume). The chamber could be opened at the bottom, where a petri dish was tightly fitted. The tip of an oxygen electrode (Oxi 340i-WTW) was inserted 10 mm above the bottom of the chamber. A removable wire mesh (5 mm mesh width) was positioned horizontally in the middle of the chamber. Above the mesh, a centrally placed cylinder created a circular swim chamber, and the water was set in motion by a 6 cm long stirring magnet in the compartment below the mesh (Figure 2.6). The respirometer was placed on the bottom of a temperature-controlled aquarium, below which a magnetic stirrer was placed to drive the magnet in the respirometer. The water speed was regulated with the magnetic stirrer. As soon as the water was set in motion, the fish started swimming against the current. The speed was set to a point where it was clear that the fish swam at their aerobic maximum speed. This was done by increasing the water speed to a point where the fish was barely able to maintain a steady position in the chamber, but still

displayed pectoral fin movement, as this is indicative of aerobic respiration (Gardiner *et al*., 2010). Water oxygen concentration was recorded for 10 min, during which time a linear fall in water oxygen concentration was seen. During the runs, water oxygen concentration was between 90 and 100 % of air saturation.

MO_{2max}=Concentration of oxygen consumed/ time

2.3.6 Aerobic Scope

Aerobic scope (in %) was calculated for each individual as:

Aerobic Scope (%) = 100 x (MO_{2max}-MO_{2rest})/ MO_{2rest}

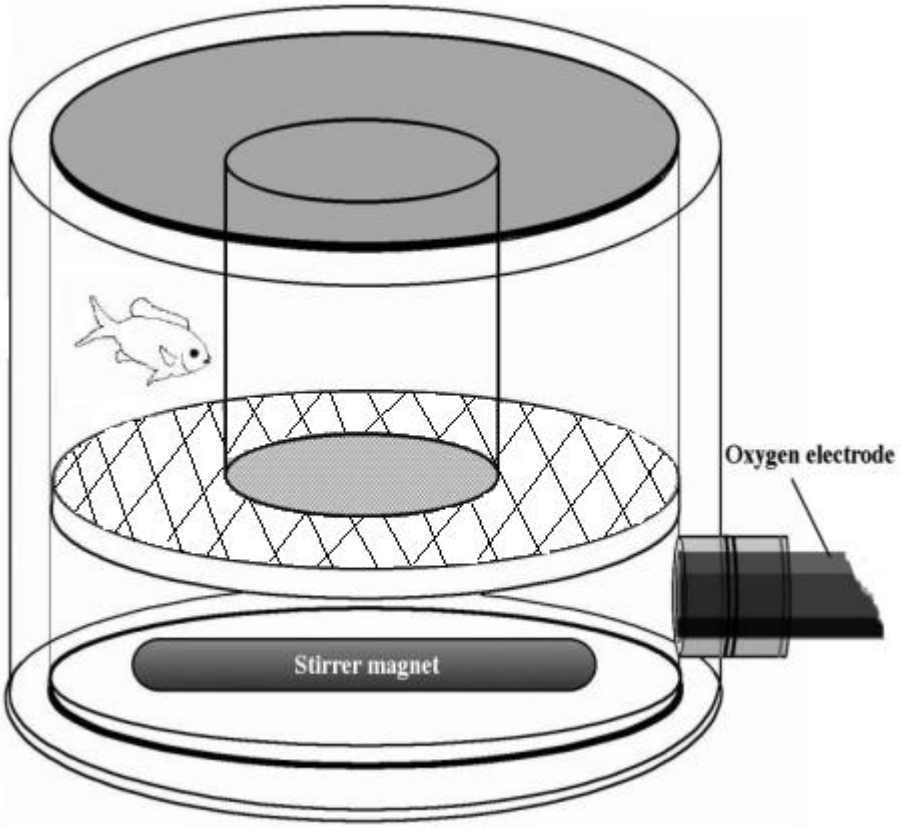


Figure 2.6. Diagrammatic representation of a Swimming Respirometer used for measurement of maximal aerobic capacity.

2.37. Statistical analysis

Means were calculated for each species in each treatment (±SEM). SPSSv15 was used for statistical analysis. Statistical significance ($p<0.05$) was tested with ANOVA followed by Tukey's post-hoc test, where results from experimental temperatures (31, 32 and 33°C) were compared with control (29°C). Where data exhibited statistically different variances (as detected by Levene's test for equal variances) a Kruskal-Wallis test with Dunn's post-hoc test was used.

2.3.8. Ethical approval and funding of study

This study followed the ethical guidelines provided by James Cook University, Queensland, Australia (see Appendix 1). The permit granted by the Great Barrier Reef Marine Park Authority (see Appendix 2, permit number GO6/ 20234.1) allowed the capture of fish. Research was funded by the University of Oslo, the Research Council of Norway, the Australian Research Council and James Cook University.

2.4 Results

Increasing the water temperature had a significant effect on the oxygen consumption of all five species of coral reef fish tested. However, there were differences in the magnitude of response between the two different families of fish, with cardinalfish more severely affected than the damselfish. There were no mortalities for the three damselfish species; however, mortalities were seen at the highest temperature (33°C) for both species of cardinalfish, with 37% mortality for *O.doederleini* and 44% for *O.cyanosoma*. Although nine individuals of each species were captured on the reef, not all were considered suitable for sampling, either due to mortalities or individuals were too weak to sustain swimming in the swim respirometer. Therefore sampling sizes ranged between 6 and 9 for the damselfish and 4 and 9 for the cardinalfish.

2.4.1. Cardinalfishes (Apogonidaes)

For *O.cyanosoma*, temperature increases of only 2°C resulted in a significant increase in resting oxygen consumption (from 238±14[SEM] $mgO_2kg^{-1}h^{-1}$ at 29°C to 393±23[SEM] $mgO_2kg^{-1}h^{-1}$ at 31°C), which increased until 32°C, when a plateau was seen (Figure 2.7). Increasing the temperature by 4°C resulted in a doubling of resting oxygen consumption. On the contrary, maximal oxygen consumption was unaffected by temperature increases until 33°C, whereby a significant decline in MO_{2max} was evident (1022±54[SEM] $mgO_2kg^{-1}h^{-1}$ at 29°C to 633±23[SEM] $mgO_2kg^{-1}h^{-1}$ at 33°C. The combination of an elevated MO_{2rest} and either a constant or a decline in MO_{2max} resulted in a significant decline in aerobic scope with increasing temperature (Figure 2.12a). The scope for oxygen uptake declined from 313± 84% at 29°C, to 165± 57%, 120± 37% and 22± 44% at 31°C, 32°C and 33°C, respectively. Therefore at the highest temperature there was almost a complete loss of aerobic capacity.

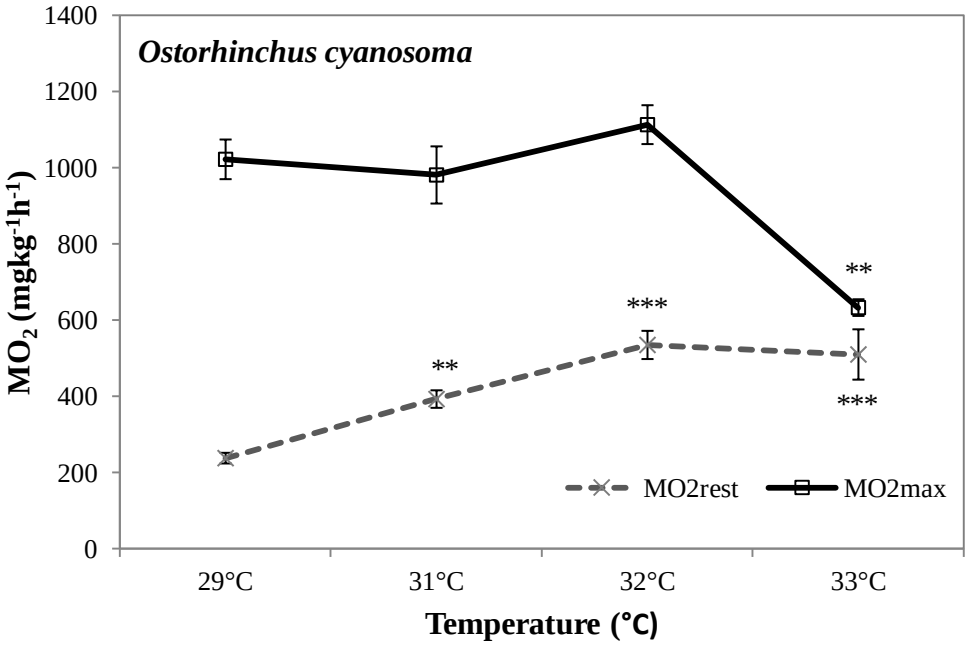


Figure 2.7. Mean (±SEM) Resting (MO_{2rest}) and maximal (MO_{2max}) oxygen consumption at each temperature for *O.cyanosoma* (MO_{2rest}: One Way ANOVA, $F(3,21)=10.93$, $p<0.001$) (MO_{2max}: One-way ANOVA, $F(3,24)=6.081$, $p<0.01$).$P<0.01$, ***$P<0.001$. Sample sizes, n= 9 (29°C), 8 (31& 32°C), 5 (33°C).**

For *O.doederleini*, again the MO_{2rest} increased with temperature from 292±16mgO_2kg^{-1}h^{-1} at 29°C to 521±017mgO_2kg^{-1}h^{-1} at 33°C. However, conversely to *O.cyanosoma*, there was no significant decline in MO_{2max} for *O.doederleini* (Figure 2.8). Despite the fact that there was no change in maximal oxygen consumption, there was still a significant decline in aerobic scope for each temperature increase when compared to the control at 29°C (Figure 2.12b). Aerobic scope at 29°C was 206±21% but fell to 131±65%, 95±34% and 34±45% at 31°C, 32°C and 33°C, respectively. Therefore, as was seen in *O.cyanosoma*, there was almost a complete loss of aerobic scope at the highest temperature of 33°C.

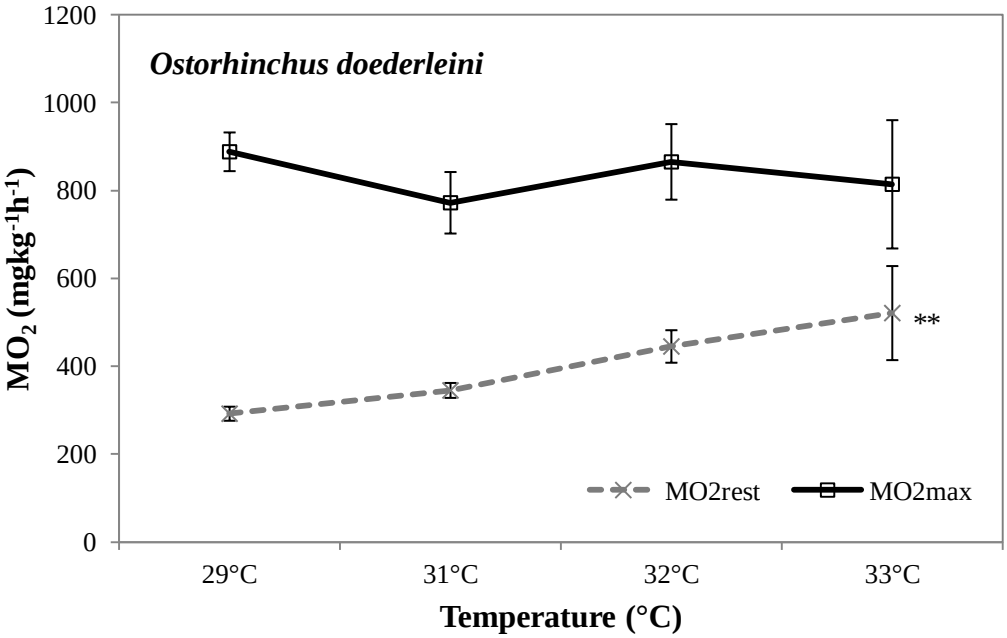


Figure 2.8. Mean (±SEM) Resting (MO_{2rest}) and maximal (MO_{2max}) oxygen consumption at each temperature for *O.doederleini* (MO_{2rest}: Kruskal-Wallis H(3)=8.96, p<0.05), (MO_{2max}: no significant differences). **P<0.01. Sample sizes, n= 6 (29°C), 9 (31°C), 8 (32°C), 4 (33°C).

2.4.2. Damselfishes (Pomacentridae)

All damselfish displayed similar responses to increases temperatures as those observed with the cardinalfish, albeit to a lesser degree. For *Dascyllus aruanus*, there was a significant rise in resting oxygen consumption as the temperature reached 31°C and 33°C; however there was no significant decline in maximal oxygen consumption with increased temperature (Figure 2.9). Whilst there was a pattern of decline in aerobic scope, with a fall from 142±57% at 29°C to 81±65% at 33°C, it was not a significant result (Figure 2.12 c; ANOVA, P=0.13).

Chromis atripectoralis displayed no significant changes to either MO_{2rest} or MO_{2max} with increased temperature (Figure 2.10). However, there was a significant decline in aerobic scope at the highest temperature, with a reduction from 300±128% at 29°C to 178±55% at 33°C (Figure 2.12d). No measurements were taken at 31°C,

as MO_{2rest} and MO_{2max} were tested at 32°C and 33°C first and since no significant changes were detected, it was deemed unnecessary to test at 31°C.

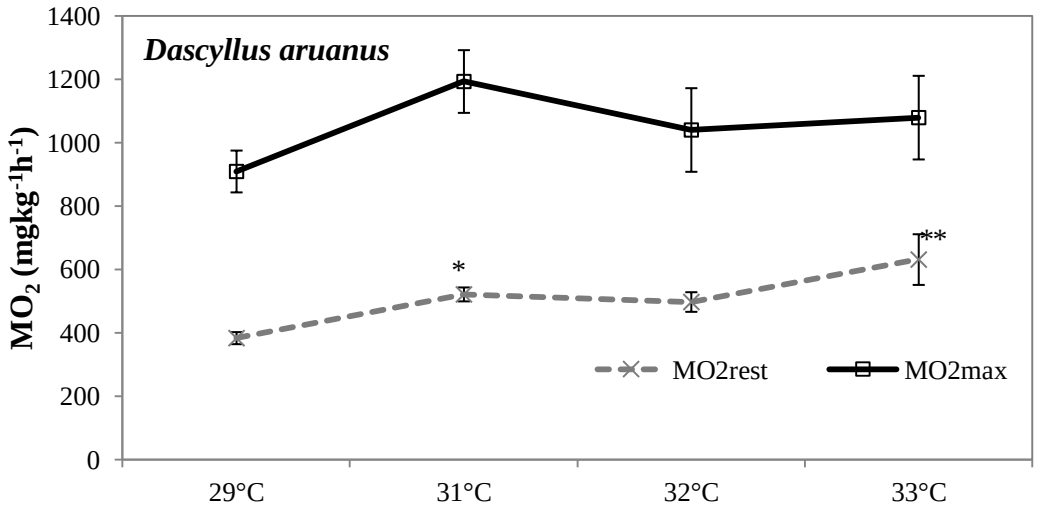


Figure 2.9 Mean (±SEM) Resting (MO_{2rest}) and maximal (MO_{2max}) oxygen consumption at each temperature for *Dascyllus aruanus* (MO_{2rest}: Kruskal-Wallis, H(3)=13.8,p<0.01), (MO_{2max}: no significance differences). **P<0.01. Sample sizes, n= 8 (29°C), 8 (31°C), 8 (32°C), 7 (33°C).

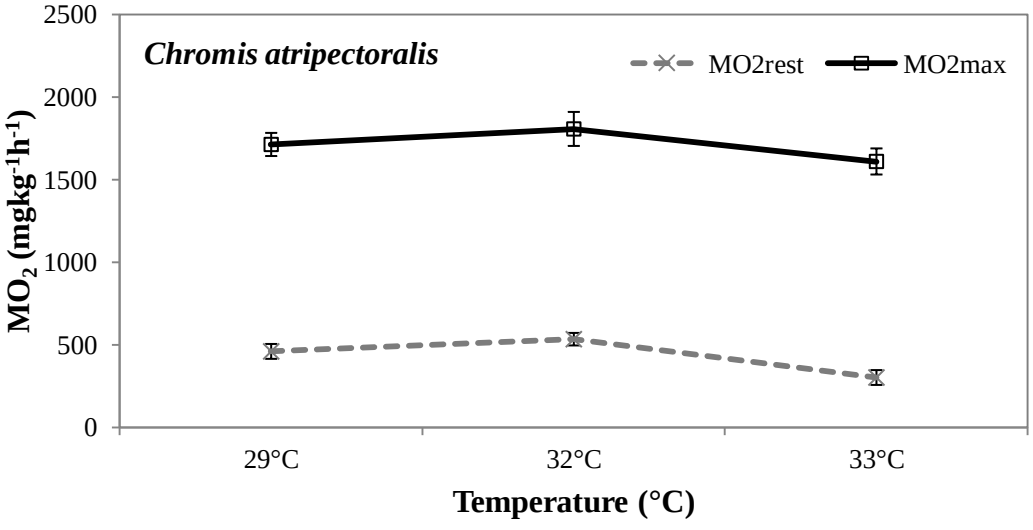


Figure 2.10 Mean (±SEM) Resting (MO_{2rest}) and maximal (MO_{2max}) oxygen consumption at each temperature for *Chromis atripectoralis* (No significant differences for MO_{2rest} or MO_{2max}). Sample sizes, n= 8 (29°C), 8 (32°C), 6 (33°C).

For *Acanthochromis polyacanthus* there was no significant effect of temperature on the maximal oxygen consumption. However, there was an increase in resting oxygen consumption for each raised temperature compared to the control of 29°C (Figure 2.11). MO_{2rest} increased from 460±25 [SEM] $mgO_2kg^{-1}h^{-1}$ at 29°C to 729±44$mgO_2kg^{-1}h^{-1}$ at 33°C. This raised resting oxygen consumption resulted in a decreased scope for oxygen uptake at each temperature compared to the control (142±42%), however the decline was as great at 31°C as it was at 33°C (81±9%). Therefore, a 2°C rise in temperature above current day summer mean resulted in the same decline in aerobic scope as a 4°C rise in temperature (Figure 2.12e).

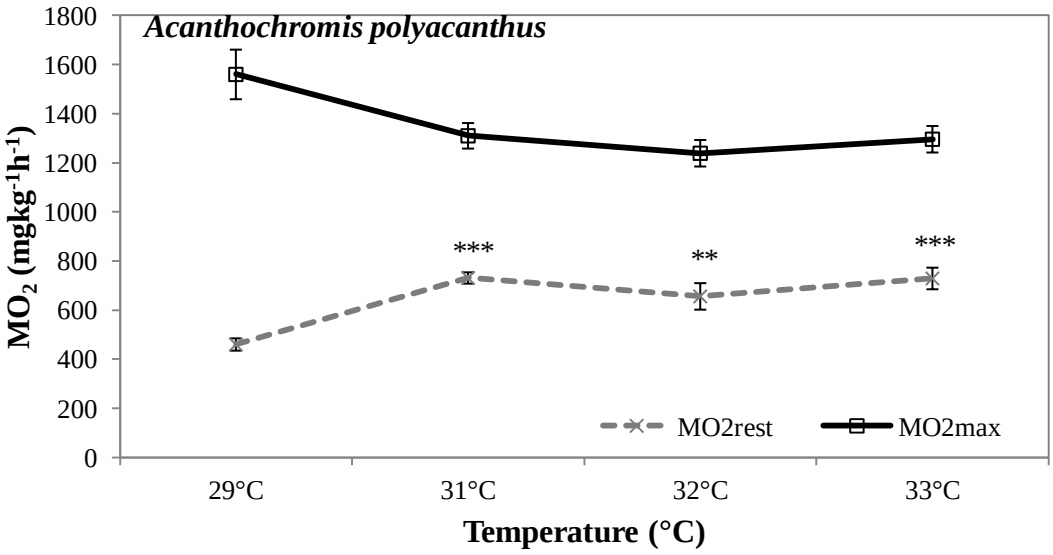


Figure 2.11 Mean (±SEM) Resting (MO_{2rest}) and maximal (MO_{2max}) oxygen consumption at each temperature for *Acanthochromis polyacanthus* (MO_{2rest}: Kruskal-Wallis, H (3)= 8.5, p<0.01, MO_{2max}: No Significance). **P<0.01, ***P<0.001. Sample sizes, n= 8 (29°C), 9 (31°C), 8 (32°C), 8 (33°C).

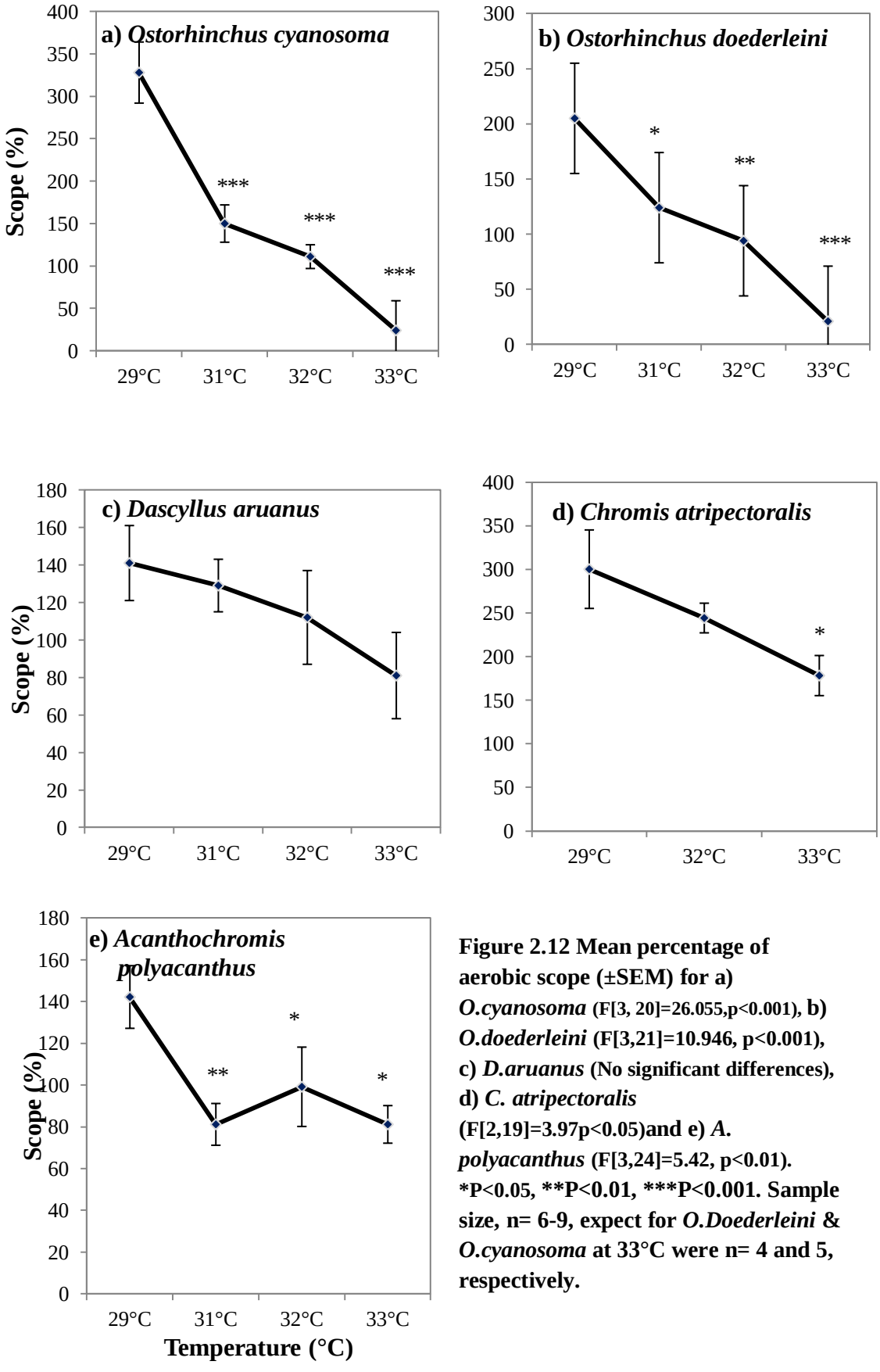


Figure 2.12 Mean percentage of aerobic scope (±SEM) for a) *O.cyanosoma* (F[3, 20]=26.055,p<0.001), b) *O.doederleini* (F[3,21]=10.946, p<0.001), c) *D.aruanus* (No significant differences), d) *C. atripectoralis* (F[2,19]=3.97p<0.05)and e) *A. polyacanthus* (F[3,24]=5.42, p<0.01). *P<0.05, **P<0.01, ***P<0.001. Sample size, n= 6-9, expect for *O.Doederleini* & *O.cyanosoma* at 33°C were n= 4 and 5, respectively.

2.4.3. Q_{10} values

Q_{10}, the temperature coefficient, is a measure of the rate of change in a biological system over an increase in temperature of 10°C. Q_{10} is calculated using the Van't Hoff equation:

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

Where T_2 is the higher temperature and T_1 is the lower temperature. Where R_2 is the rate of oxygen consumption at T_2 and R_1 is the rate of oxygen consumption at T_1.

If a reaction is independent of temperature, it will have a Q_{10} value of 1. However, if temperature does have an effect, such as most biological reactions, Q_{10} usually has a value between 2-3. The Q_{10} values for resting oxygen consumption ranged between 1.9 (*C.atripectoralis*) and 5.7 (*O.cyanosoma*) (Table 2.1), indicating temperature had a greater effect on the basal rate of oxygen consumption in *O.cyanosoma*. The mean Q_{10} for cardinalfish was 5.1, and for the three damselfish it was 3.3, suggesting that cardinalfish are more sensitive to increased temperature.

For maximal oxygen consumption, the Q_{10} values were lowest in *O.cyanosoma,* with the mean Q_{10} for cardinalfish being 1.9, compared to 2.5 for the three damselfish. In the case of MO_{2max}, low Q_{10} values indicate that the rate has lowered with temperature, therefore the rate of maximal oxygen consumption when swimming at elevated temperatures affected *O.cyanosoma* more than it did any other species tested.

Table 2.1 Q_{10} values of all five species for resting oxygen consumption (MO_{2rest}) and maximal oxygen consumption (MO_{2max}).

	MO_{2rest}	MO_{2max}
O. cyanosoma	5.7	1.5
O. doederleini	4.2	2.3
D. aruanus	4.1	3.0
C. atripectoralis	1.9	2.4
A. polyacanthus	4.0	2.1

2.5 Discussion

The general trend in all five species was that an increase in ambient water temperature induced a decrease in aerobic scope. This was due to two factors. Firstly, all species were unable to increase their MO_{2max} as water temperature increased. In fact, MO_{2max} of *O.cyanosoma* declined with increased temperature. Secondly, increasing temperature resulted in a rise in MO_{2rest} in all species, thus leading to reduced scope for oxygen uptake at higher temperatures. In three out of the five species of coral reef fish tested, aerobic scope was significantly decreased by only a 2°C increase in temperature to 31°C.

Q_{10} values have been used for nearly a century (firstly on goldfish by Ege and Krogh in 1914) to describe the relationship between metabolism and temperature (Steffenson, 2002). Ege & Krogh reported that the metabolic rate increased exponentially as water temperature increased, unless the fish has employed a physiological mechanism to counter the effects of temperature. Given that high metabolic rates lead to lower aerobic scope and therefore less energy available for growth, fish that are chronically exposed to higher water regimes are thought to physiologically adapt to prevent increases in metabolic rate, and therefore Q_{10}.Q_{10} values for biochemical reactions typically fall between 2 and 3 (Chaui-Berlinck *et al*., 2002). However in this study, values of 4.2 for *O.doederleini* and 5.7 for *O.cyanosoma* were reported. Cardinalfishes, in particular *O. cyanosoma* were more severely affected than the three species of damselfishes studied. These values are much higher than the average of 2 to 3, suggesting that these species are under some degree of thermal stress. If they are outside of their thermal comfort zone, they will have to expend more energy in order to satisfy basal metabolic needs. Whilst these values are higher than typically reported, Q_{10} values, values as high as 8 have been recorded for fish that are exposed to temperatures outside their thermal zone of tolerance (Johnston *et al*., 1991). Metabolic cold adaptation is the hypothesis that polar fish which evolved in a stable temperature environment display a resting metabolic rate that is higher than predicted from the overall rate and temperature relationship established for temperate and tropical species (Clarke & Johnston, 1999). However, although it was once widely accepted, several more recent studies have provided evidence that this theory is flawed and metabolic cold adaptation is now largely disputed (Clarke & Johnston, 1999: Steffenson, 2002). Coral reef fish,

like polar fish, have evolved in a stable environment, and are therefore mostly stenothermic, with narrow temperature tolerances (Van Dijk *et al.*, 1999). This, coupled with them being ectothermic, results in these species being sensitive to temperature increases, which is shown in the results from this study, with high Q_{10} values recorded, especially for the cardinalfishes.

Fish that are able to maintain their aerobic scope at higher temperatures are thought to have a higher thermal tolerance, and therefore are predicted to cope better with global warming (Gardiner *et al*., 2010). The two species of cardinalfish were much more sensitive to temperature increases, as they were unable to maintain their aerobic scope. A 2°C increase in temperature to 31°C, which is already experienced in the lagoon on extreme warm days, resulted in the fish losing half of their aerobic scope. An additional rise in water temperature to 33°C resulted in a loss of virtually all capacity for oxygen uptake, leading to exhaustion and in some cases death. The mortalities seen in the acclimation period at 33°C in the cardinalfishes were probably because they could no longer supply their tissues with enough oxygen for basal metabolism. It is likely that anaerobic metabolism took over, which cannot be sustained for long periods. Therefore, the fish either died due to insufficient energy supply or through lactic acidosis as a result of sustained anaerobic metabolism (Evans, 1987). A water temperature of 31°C is already experienced on warm summer days on the Great Barrier Reef, however only for short periods of time and only in the shallow water in the middle of the day. However, it is anticipated that 31°C is set to become the mean summer temperature in 50-100 years from now. The fact that aerobic scope was significantly affected at 31°C suggests that these species of cardinalfish are likely to experience difficulty in the summer months. The reduced aerobic scope may impact the ability to forage for food, to grow and also to reproduce. These factors are all likely to be hindered further as waters continue to warm and therefore the long-term viability of the populations of cardinalfish on the Great Barrier Reef is unsure. The almost complete collapse of aerobic scope at 33°C as a consequence of climate change could prove too much for this species.

The damselfish tested were less sensitive to increases in temperature, however the aerobic scope of two of the species still declined significantly, from 142 % at 29 °C to 81 % at 31 °C in *A. polyacanthus*, and from 300 % at 29 °C to 178 % at 33°C in *C. atripectoralis*. In a previous study by Munday *et al*. (2008b), both

juvenile and adult *A.polycanthus* had reduced growth and condition factor when held at temperatures just a few degrees above the average summer temperatures (in this case the mean summer temperature was 28°C and elevated experimental temperature was 31°C).The significantly reduced aerobic scope for *A.polyacanthus* with just a 2°C increase in this study therefore agrees with the study of Munday and colleagues, namely that this species may also be negatively affected by the climate change conditions anticipated in the next 50-100 years. *D.aruanus* was the only species tested to not have significant declines in aerobic scope, despite resting oxygen consumption increasing significantly with temperature. Therefore, *D.aruanus* appears to be the most thermally tolerant species of those tested, and so is likely to cope best with warming waters.

The result from this study, which compares the responses of the five species, suggests that there will be some species, or indeed some families, that have greater thermal tolerances than others. Therefore, as sea-surface temperature warms with climate change, it is possible that some families will cope better than others and that those that are highly sensitive may disappear from many coral reefs. In control conditions (29°C; the temperature currently experienced in the lagoon the fish were caught in), the cardinalfish had a much lower MO_{2rest} than the damselfish (265±31[SD] compared to 434±44[SD], respectively). This suggests that the damselfish naturally have a higher metabolic rate than cardinalfish, which is to be expected given that damselfish are considered to be one of the most active families of fish on the reef. Cardinalfishes have been well documented to be one of the least active fish on coral reefs (Gardiner *et al*., 2010). However, this could also be due to most censuses being carried out in daylight hours, when cardinalfishes would be resting since they are nocturnal. However, it may well be true the cardinalfishes are indeed less active than damselfish and therefore are not suited to the high metabolic demands that will occur with warmer waters. Therefore small increases in temperature which raise the basal metabolic rate will significantly affect these fish. Damselfish are much more active and therefore they may be aerobically fitter and potentially better suited to coping with increased energy demands, such as those induced by higher temperatures (Gardiner *et al*., 2010). Given that cardinalfish are nocturnal and these experiments were carried out in daylight hours, there is the potential that the cardinalfish were metabolically disadvantaged by the experimental

design. Being nocturnal, it is probable that the metabolic rate of cardinalfish is lower in the day when they are resting than at night when they are more active. This may be a contributing factor as to why cardinalfish appear to be more susceptible to warming waters given that they were forced to swim at a time when they would normally be resting. However, given that cardinalfish are more active at night when water temperatures are cooler, it is possible that this is to avoid the warmest waters as they are more sensitive to higher temperatures. Therefore, regardless of whether the experiments were conducted in the day or in the night, it may well be the case that cardinalfish are indeed more sensitive to elevated temperatures. It would be worthwhile repeating the study and conducting the experiments at night-time. This would allow it to be determined whether there is a difference in the respiration rates between night and day, in order to state with confidence that the reduced aerobic scope was as a result of elevated temperature and not from the experiment set-up.

Due to the apparent differences in thermal tolerance between species and indeed families of fish, it is predicted that there will be changes in the community structure of coral reef fish. In low latitude reefs, such as Lizard Island, some families, such as cardinalfish, are already evidently living near their upper thermal limit and therefore as waters continue to warm, individual performance will decline, leading to a decline in their numbers. More tolerant species such as *Dascyllus aruanus* and *Chromis atripectoralis* are likely to persist and occupy the new living spaces on coral reefs. The well known effects of warmer waters on species distribution and abundance include range shifts, population collapses, local extinctions and phase shifts (Gardiner *et al*., 2010). There is already evidence from temperate and polar regions that warming water is responsible for population collapses. For example, in the Northern Wadden Sea, a 5°C increase in the mean summer temperature led to a population crash of the eelpout (*Zoarces viviparus*). This collapse was thought to be due to a significant decline in aerobic scope and oxygen limitation (Portner & Knust, 2007). On low latitude reefs there may well be a contraction or even population collapses of cardinalfish with climate change. However, on higher latitudinal reefs (i.e. southern Great Barrier Reef), cardinalfish may well increase in numbers as the water temperature is cooler further from the equator, which could result in a net range shift of the thermally sensitive species.

The loss of some families from low-latitude reefs such as Lizard Island could have important consequences for the ecosystem if the family lost plays a significant role on the reef or in the food web (Bellwood *et al*., 2003). It is generally considered that highly diverse communities of reef fish will provide protection and resilience against environmental change. However, Bellwood *et al.* (2003) concluded that a single species, let alone an entire family, can dramatically alter the structure and functioning on reef communities. For example, the Bumphead parrotfish, *Bolbometopon muricatum,* plays a large role in bioerosion as it actually digests the reef matrix (in some cases at rates higher than calcification) and redistributes structural carbonate as sediments (forming the sandy beaches of many tropical islands). A sudden surge in *B.muricatum* numbers on the reef would lead to a loss of coral structure and an absence of them would stop the accretion of reefs and formation of sandy deposits which form of the basis of sea-grass beds and other significant ecosystems. Therefore, it is important to consider both the species and their functional roles when determining whether a loss of a species will be important (Bellwood *et al*., 2003). A loss of the cardinalfish *O.cyanosoma* and *O.doederleini* could have significant effects of the reefs. As nocturnal planktivores, they consume prey both from on the reef and from the open water. Having high site-attachment, they return each morning to the same patches of coral, and there their faecal matter and waste leads to deposits of nutrients in their local vicinity. Since the majority of their food comes from outside the reef, they replenish the nutrients on the reef and have been quoted as being nutrient recyclers (Marnane & Bellwood, 2002). Therefore a loss of these species may result in a net loss of nutrient availability on the reef for other organisms.

Just because a species has higher thermal tolerances does not secure the sustainability of the population in the face of climate change. Of the pomacentrids tested, *Dascyllus aruanus* and *Chromis atripectoralis* had the greatest thermal tolerance and therefore in this case are considered to be most likely to cope well with climate change and hence may dominant reefs. However, these species are both very closely associated with live coral for their habitat, and their populations have been known to decline rapidly following mass coral-bleaching events (Pratchett *et al.*, 2008). Whilst a 2°C increase in water temperature did not directly affect these species by reducing their aerobic scope, a 2°C increase in temperature has been

documented to cause mass coral bleaching. Therefore, declines in these more thermally tolerant species may be seen well before temperature directly affects them, due to a loss of live coral.

However, these predictions for changes in fish community structure do not take into account the possibility of acclimation and adaptation to new thermal regimes. Fish have displayed the ability to adapt and cope with short-term environmental changes (Munday *et al*. 2007), provided they are of low amplitude and periodicity (Taylor *et al*., 1997). It is also important to consider that local adaptation may play a role in responses to temperature increases. A study by Gardiner and colleagues (2010) found that individuals of the same species, in particular *D.aruanus,* from separate locations performed differently, with those from higher latitudes (i.e. One Tree Reef, which has a greater annual temperature range) coping better with increasing temperature than those from low latitude reefs (i.e. Lizard Island, which has a smaller annual temperature range). Although the aerobic scope of *D.aruanus* was not significantly affected in the present study, there was a significant increase in resting oxygen consumption. This suggests that acclimation to higher temperatures to maintain basal needs were not achievable in the timeframe given. Conversely, the MO_{2rest} was the same for *D.aruanus* at One Tree Reef and Lizard Island. However, the conspecifics on the higher latitudes reefs (i.e. One Tree Reef on the southern Great Barrier Reef) had much higher MO_{2max} at all temperatures. This suggests that the fish have physiologically adapted so that the basal metabolic demands could be met without additional requirements of oxygen and also that their maximal capacity of oxygen uptake had increased. Therefore the conspecifics which live in a cooler, more thermally dynamic system have a greater ability to increase their maximal oxygen consumption compared to those living closer to their thermal limits. The ability to adapt to different thermal regimes is essential in order to be able to cope with changes in temperature that are predicted with climate change.

There are two mechanisms by which fish can adapt to new thermal regimes; firstly by phenotypic temperature acclimation and secondly by genetic temperature adaptation through natural selection. Phenotypic acclimation, also known as phenotypic plasticity, is the change in the properties of an individual in response to a change in the environment (i.e. changes seen in the lifetime of an individual).

Genetic adaption is the natural selection of the progeny of individuals better suited to their environment and generally relates to a population of organisms.

In terms of phenotypic acclimation, fish, both as larvae and as adults, have displayed the ability to increase their gill surface area and increase the haemoglobin content in the blood to enhance oxygen uptake (Sollid *et al*., 2005), or even the shape of the mouth depending on the food available, as in the Antarctic notothenild (Eastman & Devries, 1997). Given that tropical marine organisms that are ectothermic have evolved under a steady environment, they will likely have less ability to acclimate. Despite little research being carried out to date on tropical marine organisms and acclimation, it appears that phenotypic plasticity is indeed limited in terms of adjusting the metabolic needs to prevent a reduction in aerobic scope (Donelson *et al*., 2011; Tullis & Baillie, 2005). A recent study by Donelson and colleagues (2011) tested the ability of *Acanthochromis polyacanthus* to acclimate to waters warmed 1.5°C and 3.0°C above current day summer temperatures. Whilst at +3°C *A.polyacanthus* was able to reduce its resting respiration rate (and therefore prevent a decline in aerobic scope), it came at a cost, with lowered condition factor and low growth rates. The lack of plasticity in tropical species may be related to the many costs associated with phenotypic plasticity (DeWitt *et al*., 1998). In the wider scheme, phenotypic plasticity may actually be a hindrance to longer term genetic adaptation, by reducing the number of phenotypes available for natural selection. It can also be costly to maintain the new phenotype, particularly in an ever-changing environment (DeWitt *et al*., 1998). Given that temperature is predicted to continue to increase over the coming century, fish would have to make continuous changes to phenotypes to prevent declines in aerobic scope. One week is thought to be a long enough period of time for fish to acclimate to new thermal conditions (Nilsson *et al*., 2007) and a shorter acclimation period of only 1-2 days has been reported to be necessary when warming the water (Barrionuevo & Fernandes, 1998). Therefore, if the fish tested in the study were able to acclimate to the raised temperatures, there should not have been significant differences in the resting respiration rates. In a study carried out on the tropical whitespotted bamboo shark (*Chiloscyllium plagiosum*), metabolic acclimation to higher temperature was not possible even after several months (Tullis & Baillie, 2005). Based on the literature and from the results of this study, it appears unlikely that coral reef fish

will be able to display phenotypic acclimation to climate change in their lifetime without encountering significant physiological costs.

However, adaptation over several generations through natural selection may provide more resilience against climate change and the associated warming waters. Genetic adaptation in localised areas may prove beneficial, to allow a specific population to cope with the environmental conditions that they are usually exposed to. However, if there is no genetic connectivity between populations, the effectiveness of adaptation and natural selection is reduced. In order for natural selection to work, there needs to be genetic diversity. On coral reefs this is possible, due to the fact that most coral reef fish have a dispersive larval stage and so there is connectivity between local populations, as larvae from one reef are transported by currents to adjacent reefs. The combination of equatorial populations already living at water temperatures that are likely to become average conditions on high-latitude reefs over the next 100 years, combined with high levels of gene flow among populations, provides hope that populations currently living at higher latitudes might adapt to increased water temperature (Munday *et al*. 2008b). This connectivity might also be sufficient to permit range shifts of species into new, more suitable environments. For example, if the temperature at low latitude reefs, like Lizard Island, becomes too high for species such as cardinalfish, they may though larval dispersal and connectivity migrate over generations to higher latitude reefs further south on the Great Barrier Reef that are cooler. Whilst this strategy may work for species with dispersive larval phases, it is unlikely to help species such as *Acanthochromis polyacanthus*, which do not have a dispersive larval stage. Compared to the other two species of damselfish species, *A.polyacanthus* was more sensitive to increases in temperature. Therefore, although this species may be better suited to the current thermal environment at Lizard Island, further warming may prove to be stressful for this species. The limited connectivity between adjacent reefs, and therefore reduced gene flow between populations, means that the potential to receive favourable genotypes from other populations as local environmental conditions change is reduced. Given the results from this study of a reduction in aerobic scope of a 43% arising from just a 2°C rise in temperature (29 to 31°C), this small change might be sufficient enough to cause significant declines in the populations of this species on the Great Barrier Reef. Even for species with

dispersive larval stages, it is unlikely that adaptations in aerobic capacity will be able to keep pace with increasing temperature in equatorial regions or low latitudinal reefs such as Lizard Island. Therefore, whilst there may be range shifts in some species to higher altitude reefs, there may also be a loss of some species from low latitudinal reefs in fish species already living near their upper thermal limit (Gardiner *et al*., 2010). The effect of temperature on aerobic scope provides a mechanistic explanation that can be used to predict how communities of marine fishes are likely to respond to rapid increases in ocean temperature. Whilst the values for aerobic scope may not represent the true oxygen consumption in nature, it does allow for comparisons between species to help predict which species or indeed families of fish are the most susceptible to population declines and are likely to exhibit pronounced range shifts as ocean temperatures increase.

If fish are able to adjust to the new temperature regimes, it may come at a cost, due to necessary adjustments of physiological processes. These trade-offs will have consequences for the biogeography, growth, life-styles, development, fecundity and recruitment of fish (Pörtner & Knust, 2007). Recent research has demonstrated that although warming waters may be detrimental to some coral reef species, there is hope for adaptation occurring over very short time periods of just two generations (Donelson *et al*., 2012). *A.polyacanthus*, when exposed to warmer waters, had the typical responses as shown in this study with decreased aerobic scope but also with lowered fecundity. However, although fecundity was reduced in the parental generation, the viable offspring were more thermally tolerant and displayed complete compensation, with zero reduction in aerobic scope in elevated waters (Donelson *et al*., 2012). Therefore, whilst warmer waters may negatively affect one generation of fish, these recent findings provide some hope that, as long reproduction is still possible the next generation of coral reef fish may be better suited to the warmer world into which they are born.

2.6 Conclusions

The results from this study demonstrate that increases in water temperature, predicted to occur this century with climate change, will likely have negative effects on at least two families of coral reef fish. Increasing the water temperature had a significant effect on the oxygen consumption of all five species of coral reef fish tested. However, there were differences in the magnitude of response between the two different families of fish, with cardinalfish (*Ostorhinchus cyanosoma* and *O.doederleini*) more severely affected than the damselfish (*Dasyllus aruanus*, *Chromis atripectoralis* and *Acanthochromis polyacanthus*). A 4°C warming in waters lead to a significant decline in aerobic scope in four of the five species, and a complete loss in aerobic scope in both species of cardinalfish. This reduced aerobic scope was due to an increase in resting oxygen consumption at elevated water (31°C, 32°C, and 33°C compared to the control 29°C) and an inability to increase the maximal oxygen consumption. Indeed, there was a significant decline in maximal oxygen consumption for *O. cyanosoma*. This suggests that cardinalfish may already be living at, or very close to, their thermal maximum at Lizard Island. These results also indicate that there may be interfamilial differences in the responses of fish to climate change, which may result in changes to the community structure of the reef.

Chapter 3: Interacting effects of chronic thermal stress and ocean acidification on two species of cardinal fish living on the Great Barrier Reef.

Munday, P.L., **Crawley, N.E.**, Nilsson, G.E. (2009). Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Marine Ecology Progress Series*, 388, 235-242.

3.1 Statement of Contribution to work

This work was conducted on Lizard Island on the Great Barrier Reef under the supervision of Dr Philip Munday (James Cook University) and Professor Göran Nilsson (University of Norway). I collected the fish from the reef and conducted the respirometry experiments in accordance to methodology previously used by Nilsson *et al*. (2007). Experimental set-up to incorporate lowered pH was designed in collaboration with Dr Munday. I input the data and conducted the preliminary statistical analysis. The first draft of the paper was written by me; however, the paper was subsequently relinquished to Dr Munday for final review and publication.

3.2 Abstract

The anthropogenic inputs of CO_2 into the atmosphere will not only result in warming waters, but also a lowering of pH, an effect known as Ocean Acidification. Whilst it is known that ocean acidification will have negative effects on calcifying organisms, such as corals, very little is known about the effects on the fish associated with the coral. This study compared the resting (MO_{2rest}), maximal (MO_{2max}) and aerobic scope of two species of cardinalfish at elevated temperature (31°C and 32°C compared to a control of 29°C) and reduced pH 7.8 (compared to a control of pH 8.15). The two species of cardinalfish, *Ostorhinchus doederleini and O.cyanosoma*, were shown to be sensitive to increases in temperature in chapter two. The results from this study demonstrated that even at control temperatures (29°C), a lowered pH of 0.3 units caused the same percentage loss in aerobic scope as did a 3°C warming.

Increases in temperature coupled with reduced pH further reduced the aerobic scope of cardinalfish. These results provide evidence that ocean acidification will directly affect coral reef fish, whereas until now it was considered that reef fish would be primarily impacted indirectly, through a loss of coral cover due to mass coral bleaching events.

3.3. Introduction

Understanding the responses of marine fish to changes in temperature and pH is vital in order to predict how sensitive individuals and populations are to climate change. Atmospheric levels of CO_2 have risen by 31% (since pre-industrial times) and it is this gas that is thought to be the biggest contributor to global warming. On a business-as-usual scenario, atmospheric CO_2 levels are expected to rise from the current level of 380ppm (Feely *et al*., 2004) to over 800ppm by the end of the century (IPCC, 2007). More seriously, without a significant reduction in fossil fuel emissions, the increase in atmospheric CO_2 could be as high as 2000ppm within the century (Shirayama & Thornton, 2005). This is anticipated to result in a global mean ocean temperature increase of 1.5-4.5ºC by 2100 (Rombough, 1997). Due to atmospheric CO_2 readily dissolving into the oceans, this rise could also result in a concomitant drop in the ocean pH by 0.4 units by 2100 (IPCC, 2007) or to pH 7.3 in the next 300 years (Caldeira & Wickett, 2003). Decreases in pH are already detectable (Pörtner *et al*., 2005) and it is expected that there will be effects on marine organisms in the surface waters (Pörtner *et al*., 2005). However the actual effects and implications remain largely unknown.

3.3.1. Temperature and aerobic scope

Research on temperature effects to date has focused on the ability of corals to acclimate to warmer waters, but there has been little attention on how fish will cope. Although the expected increases in water temperature are likely to be greater in temperate than tropical regions, it is thought that tropical species will respond more strongly. Coral reef fish have evolved in a thermally stable environment and

therefore are considered to have narrower thermal tolerances and to live nearer their upper thermal limit (Hoegh-Guldberg *et al*., 2007a**;** Tewksbury *et al*., 2008), as was seen in Chapter 2. It is known that extremes in temperature can induce anaerobic metabolism, and raised temperature will accelerate the basal metabolic rate (Pörtner *et al*., 2005). Increased temperatures can also exacerbate oxygen limitation, as demand for oxygen increases with higher metabolic rates, but oxygen solubility is lower in higher temperatures, making it harder to be absorbed by marine organisms (Pörtner & Knust, 2007).

Aerobic capacity or aerobic scope is a good measure of the maximal sustainable aerobic ability of an animal and it is thought to be the key physiological mechanism that will determine how marine species will cope with climate change (Pörtner & Knust, 2007). Whilst it has previously been stated that small increases in temperature are unlikely to affect the aerobic scope of fish (Taylor *et al*., 1997), the results in chapter two of this thesis present contradictory evidence. The aerobic scope of four commonly occurring coral reef fish (*Ostorhinchus doederleini, O.cyanosoma, Acanthochromis polyacanthus* and *Chromis atripectoralis*) declined with increases in water temperature of between 2-4°C. These results suggest that at least four species from two families of coral reefs are already living near their thermal maximum. However, aerobic scope is expected to be affected by more than just temperature increases; for example, from acid stress resulting from ocean acidification. In particular, the two species of cardinalfish tested which were most sensitive to temperature could be increasingly threatened by confounding factors such as low pH.

3.3.2. Ocean Acidification

It is known that as additional CO_2 dissolves into the ocean, the pH is lowered, a process known as Ocean Acidification. Decreased pH is thought to confound the effects of higher temperatures on the aerobic capacity of marine organisms (Pörtner & Farrell, 2008). Therefore understanding the ways climate change may impact marine organisms depends upon understanding the way in which temperature and pH interact to affect the performance of individuals.

Ocean pH is predicted to decline with climate change as the oceans absorb more and more CO_2. Declines in pH of as much as 0.4 units are predicted by 2100 under an A1 SRES scenario (IPCC, 2007). This would make the oceans more acidic than they have been at any point in the last 400, 000 years (Feely *et al*., 2004). This decrease in pH will likely affect all organisms that use calcium carbonate ($CaCO_3$) for their skeleton, such as corals. Many other invertebrates utilising $CaCO_3$ will be affected in much the same ways as coral, and studies carried out on plankton, sea urchins (Kurrihara *et al*., 2004) and gastropods (Shiryama & Thornton, 2005) have shown similar responses. Studies on gastropods and echinoids showed that small increases in atmospheric CO_2 could have negative consequences on growth rates (Shirayama & Thornton, 2005). In gastropods, both shell height and body mass decreased as a result of increased CO_2, indicating that it affects the physiology of organisms (Shirayama & Thornton, 2005). Reduction in pH can reduce or increase the toxicity and availability for uptake of many substances, such as metal ions, which can be toxic to marine organisms.

3.3.3. Low pH and fish

Low pH can affect many biological processes, such as enzyme function, the ability of haemoglobin to carry oxygen and transport of body electrolytes. Freshwater fish can withstand greater fluctuations in pH than marine fish, due to the dynamic system in which they live. It is thought that most freshwater fish can tolerate extremes of 5-9 pH units (Fromm, 1980), however marine fish live in a more stable environment and so their lower tolerance limit is a pH of 7.0 (Michaelidis *et al*., 2005). Fish in acidified waters may experience impaired ionic regulation, since they are not as able as crustaceans at regulating their internal pH (Allan & Maguire, 1992), with increased permeability to H^+ and Na^+ across the gills (Fromm, 1980). Low pH can also affect reproduction, with females producing less viable eggs or even ceasing spawning all together, although this happens only at extremely low pH.

As with thermal stress, high pCO_2 can cause a decrease in cardiac output in fish, which reduces the oxygen delivery to the muscles (Pörtner *et al*., 2005). Without sufficient oxygen supply to the muscles and tissues, energy will be derived

from unsustainable anaerobic metabolism, and ultimately growth and reproduction will be slowed. Acutely elevated CO_2 may result in increased ventilation by fish and other water breathers, to counteract the accumulated CO_2 in the tissues from anaerobic metabolism resulting in hypercapnia (decreased pH of the blood due to high CO_2 levels) (Evans, 1987). However, this is only viable as a short-term response and has limited effects. On longer timescales, it is known that invertebrates have the ability to display metabolic depression in similar ways to hibernating animals (Pörtner *et al.*, 2000). Metabolic depression is an adaptive biological process to preserve energy by lowering body temperature, reducing breathing rate and/or a lowered metabolic rate. By decreasing their energy turnover, it allows them to survive longer in stressful environments. However, metabolic depression has not been shown for fish, and there is some thought that metabolism may even be stimulated by hypercapnia (Pörtner *et al.*, 2005).

Aerobic scope is a good indicator of the health of an organism as it provides an indication as to the amount of excess energy an individual has for growth and reproduction. It is hypothesised that CO_2 affects several mechanisms, such as metabolism, that are also affected by thermal stress (Pörtner *et al.*, 2005). For these reasons, if given the choice, fish will actively avoid water with low pH or high CO_2, a behaviour termed 'avoidance homeostasis' (Fromm, 1980). However, with climate change and ocean acidification, this may not be possible, as it will affect large areas not just isolated patches of water.

In most studies where pH has been manipulated, it has been though the introduction of acid (e.g. hydrocholoric acid, HCl) not of CO_2. This produces significantly different results than when pH is lowered by bubbling CO_2 into the water. Furthermore, due to their wider tolerances, the effects of pH have been studied largely on freshwater fish rather than marine fish, particularly tropical marine species. Therefore, there is inadequate knowledge of pH tolerance and responses to acidification by marine organisms and coral reef fish (Caldeira & Wickett, 2003; Knutzen, 1981).

3.3.4. pH and gill structure

The gill epithelium serves many purposes; aside from being the site of gaseous exchange, it is also the site for ionic-regulation, nitrogenous waste excretion and acid-base regulation. Through the reversible hydration/ dehydration reaction of CO_2 with an acid (hydrogen ions, H^+) or base (bicarbonate, $HC0_3^-$), the fish gill is able to maintain the acid-base balance:

$$\mathbf{CO_2 + H_2O \quad H^+ + HCO_3^-} \quad \text{(Perry \& Gilmour, 2006)}$$

In times of elevated CO_2, there is an increase in the hydrogen ions which lowers the pH, both of the water and also of the plasma of fish. In order to compensate for this acidosis (lowered plasma pH by the increase of H^+ ions), a series of enzymatic changes takes place to increase the plasma and cellular bicarbonate levels. This in turn results in an increase in CO_2, which can then be passively removed via the gills (Fromm, 1980; Michaelidis *et al.*, 2007; Perry & Gilmour, 2006). This regulation of H^+ and $HC0_3^-$ is believed to be closely linked to Na^+ and Cl^- ions, respectively, and therefore disruption of internal pH is often coupled with imbalances in ion regulation (Evans, 1987; Fromm, 1980; Perry & Gilmour, 2006). There are two mechanisms by which the acid-base balance is controlled. Firstly, there is respiratory compensation, whereby hyperventilation removes the excess production of CO_2; however in fish this process is thought to be limited (Perry & Gilmour, 2006). The second and more prominent mechanism is metabolic compensation across the gills. Although it is accepted that metabolic compensation across the gills and adjustments in bicarbonate levels in plasma are responsible for maintaining internal pH balance, the actual mechanism involved in this process are not clearly understood (Perry & Gilmour, 2006).

It has long been documented that aquatic acidification can have detrimental effects on the structure and physiology of the gill, which then affect the acid-base balance mechanisms. These include separation of the epithelial layers of the secondary lamellae on the gill filaments (Figure 3.1), swelling of the secondary lamellae (Evans, 1987), or an increase in the number and morphology of chloride cells (Evans, 1987; Hirata *et al*., 2003). These structural changes may be as a result of general stress rather than pH alone, or due to the failure of gill cellular

osmoregulation (Evans, 1987). These structural alterations can lead to acute acidification of the plasma and ultimately death (Hirata *et al.*, 2003).

It has long been documented that water with low pH causes excess mucus production on the gills which coagulates and also causes the gill membranes themselves to stick together. This mucus film hinders the diffusion of oxygen across the gills into the blood, and has been termed 'coagulation film anoxia' (Westfall, 1945). Therefore, even if there is sufficient oxygen present in the surrounding water, the fish may still experience hypoxic conditions, as it is unable to transport the oxygen across the gills and into the blood. This mucus secretion, not only on the gills but also on the surface of the body, may be adaptive by reducing the epithelial ionic permeability, but the role it plays is unclear.

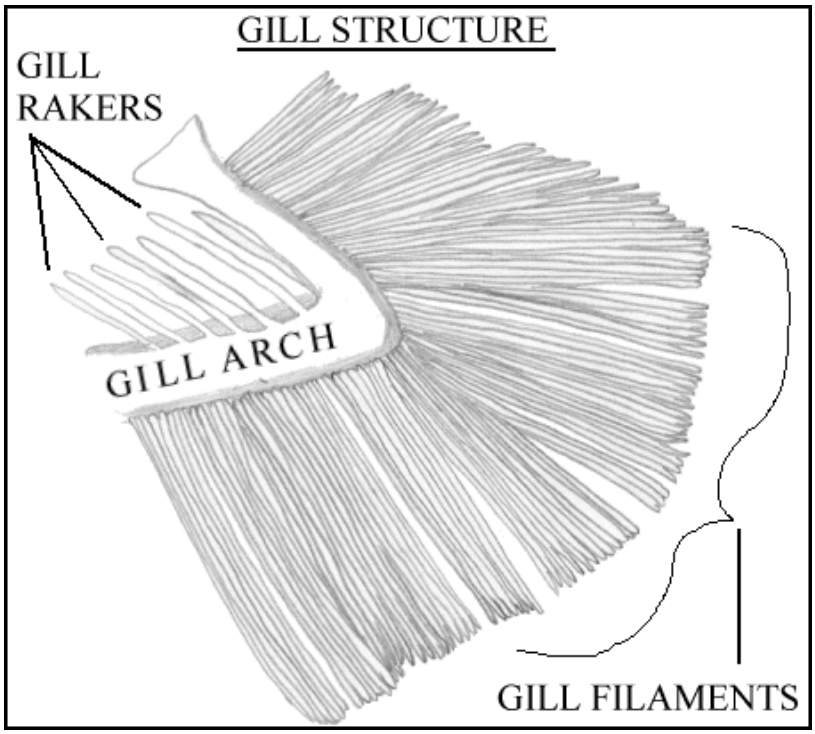


Figure 3.1. Structure of a healthy gill.

3.3.5. Synergistic relationship between temperature and pH

Despite the known synergistic relationship between increased temperature and decreased pH (Kirrihara *et al.*, 2004; Reynaud *et al.*, 2003; Shirayama &

Thornton, 2005), it has little been studied. There are no data yet on how climate change projections will affect tropical marine fish species, as most studies have focused on freshwater or temperate marine fish (McCormick & Molony, 1995). Increases in temperature are thought to induce a poleward or high-latitude shift in distribution of ectothermic animals such as fish, but there has been little focus on the effects of CO_2 and pH on fish (Pörtner & Knust, 2007), and there are no data yet as to the interacting effects of temperature and pH on marine fish. The relationship between temperature and pH clearly needs addressing, particularly in terms of climate change. It is apparent that when elevated CO_2 and temperature are combined, the effects on marine biota are likely to be far more pronounced (Shirayama & Thornton, 2005).

3.3.6. Ostorchinchus cyanosoma & O. doederleini.

Cardinalfish (Family: Apogonidae) are an extremely diverse and common family of coral reef fish. Regardless of this, there is still relatively little information available about their ecology and biology (Gardiner & Jones, 2005). Chapter two of this thesis (Nilsson *et al*., 2009) demonstrated that there were strong inter-familial differences in how fish coped with increasing water temperature. It was shown that the cardinalfish, *Ostorchinchus cyanosoma* & *O.doederleini*, were more sensitive to temperature increases than the three damselfish species tested (*Dascyllus aruanus, Chromis atripectoralis* and *Acanthochromis polyacanthus*) and therefore that there might be changes in reef fish community structure with warming waters. Additionally, it has also been reported that cardinalfish actively select live coral cover for protection and this is predicted to decline with climate change. Given that cardinalfish appear to be sensitive to temperature, it was considered interesting to investigate whether their aerobic scope is further reduced by ocean acidification. Cardinalfish are small in size and during the day spend a lot of time in large groups amongst the matrix of the coral. Being nocturnal, they will leave the shelter of the reef matrix at night and feed on plankton in open water. Due to a cessation of photosynthesises at night (which utilises CO_2 and produces O_2) and a continuation of respiration (which utilises O_2 and produces CO_2) by reef organisms, a drop in oxygen levels and pH at night is often experienced within the reef matrix (Nilsson *et*

al., 2007). Species which shelter in the reef at night are therefore exposed to lowered pH on a nightly basis, whereas cardinalfish which migrate, albeit only short distances from the reef matrix, avoid the lower pH. Therefore, it is hypothesised that these two species of fish, which are extremely sensitive to temperature increases, will not be able to tolerate even moderate declines in ocean pH.

3.3.7. Aims of Study

The aim of this study was to examine the interactive effects of temperature and pH on the aerobic scope (capacity for oxygen uptake) on two cardinalfish, *Ostorhinchus cyanosoma* (Figure 2.3 a.) and *O.doederleini* (Figure 2.3 b.). These two species have previously been shown (see results in Chapter 2) to be severely affected by increased temperature (Nilsson *et al*., 2009). This study compared the resting and maximal metabolic rates of the two species of cardinalfish examined in chapter two at present day pH (pH 8.15) to those acclimated to a lowered pH (pH 7.8) at temperatures predicted to occur as a consequence of climate change by 2100.

3.4. Methodology

3.4.1. Experimental Design

All experiments were carried out between December 2007 and January 2008 at Lizard Island Research Station (LIRS; www.lizardisland.net.au) on the Northern Great Barrier Reef (14°40'S 145°28'E), Australia (Figure 2.2 and 2.3). For the control pH dataset (average pH 8.15, range 8.02-8.21), results from chapter two for the two species of cardinalfish, *Ostorhinchus cyanosoma* and *O. doederleini* were used. This was to prevent additional removal of fish from the coral reef, as it was deemed crucial to minimise disruption to this fragile ecosystem. A sample size of nine individuals per species for each water temperature treatment was selected to be in agreement with methodology form chapter two. Nine individuals were previously deemed suitable, since previous similar experimentation on these species has been shown to be sufficient for significant statistical analysis (Nilsson *et al*., 2007). Furthermore, sample sizes were constrained from being larger due to ethics and permit restrictions.

For the comparison to lowered pH, adults of comparable size of *O. cyanosoma* (2.3±0.5g), *O. doederleini* (2.18±0.77g) were collected for experimentation. Fish were caught by SCUBA diving in the lagoon near LIRS using a hand net after lightly anaesthetizing them with clove oil, as previously described (Östlund-Nilsson & Nilsson, 2004). Fish were kept in a temperature-controlled indoor aquarium, which was continuously supplied with water pumped in directly from the ocean. A 12 hour light: 12 hour dark cycle was controlled using fluorescent lighting to mimic the natural light conditions. The water oxygen level varied between 95 and 100 % of air saturation.

Experimentation was first carried out on *O.doederleini* and then *O.cyanosoma,* following the same procedure as follows. Nine individuals were placed in each one of four 50L tanks (water temperature of 29°C). Control individuals were kept at the ocean temperature, with a mean of 29°C ±0.5°C. Other fish were kept in identical aquaria where the water temperature was increased with aquarium heaters to 31, 32 or 33°C (max daily variation was ± 0.5°C) over a period

of 1-2 days. The tanks were supplied with seawater adjusted to pH 7.8 (range 7.75-7.85) to simulate future ocean acidification. pH was adjusted by the standard method of bubbling additional CO_2 into a reservoir tank (Leclercq *et al*. 2002, Michaelidis *et al*. 2005), which then supplied equilibrated seawater to each of the test aquaria. pH in the 60 L reservoir tank was regulated with an automated pH-controller (Tunze Aquarientechnik, Germany) connected to an electronic solenoid valve. A laboratory-grade glass pH probe continuously monitored pH in the reservoir. The solenoid injected bubbles of CO_2 into a diffuser (Red Sea Reactor 500) at the bottom of the reservoir tank whenever the pH rose above 7.8. The diffuser rapidly dissolved CO_2 into the seawater and also served as a vigorous stirrer. The equivalent atmospheric concentration of CO_2 for the pH treatment was estimated by sealing replicate tanks in which the pH of the water had been adjusted and then measuring the increase in pCO_2 in a narrow space above the water surface with an infrared CO_2 probe (Vaisala, Finland). The estimated concentration of CO_2 in the pH treatment was between 1000-1050ppm. This value matches closely with other studies that have estimated CO_2 concentrations of approximately 1000ppm for a 0.4 unit decline in pH using similar methodology (Havenhand *et al*. 2008).

Fish were acclimated for one week prior to respirometry testing. They were fed daily to satiation with frozen blood worms and fish pellets, but starved for 24 h before determination of resting rate of O_2 consumption. All experiments were carried out between 08.00 and 18.00 h. Each fish was only used for one treatment and released back to the reef after experimentation.

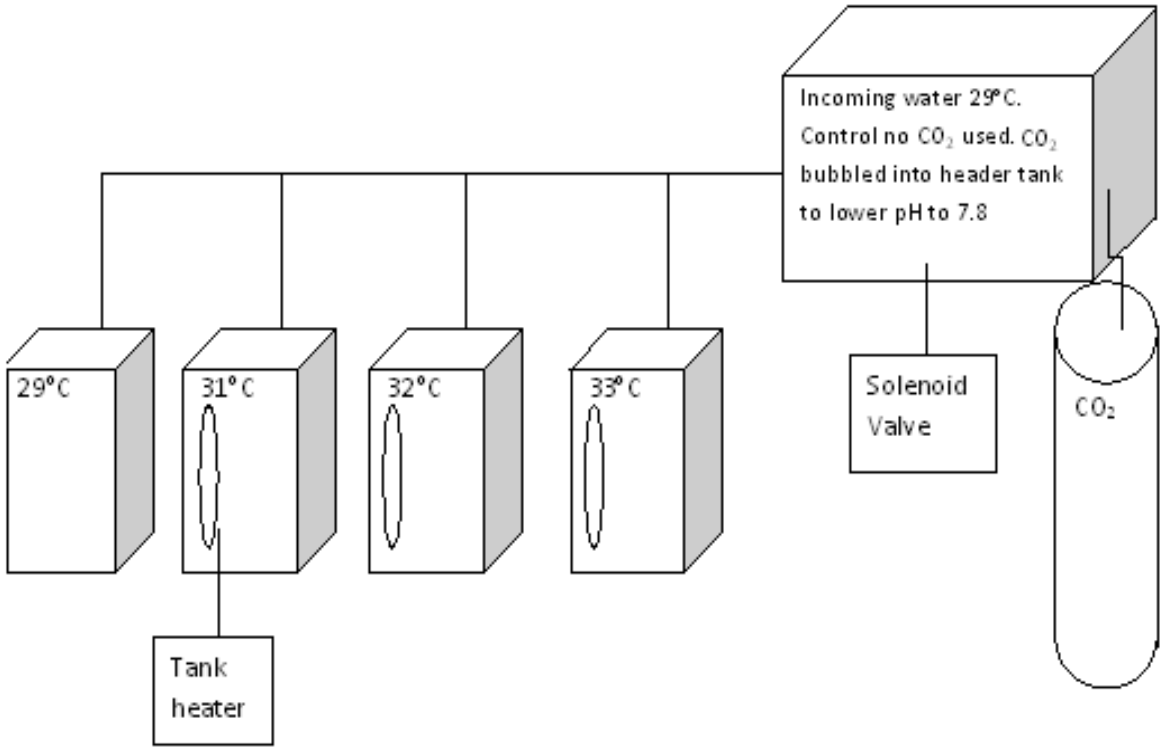


Figure 3.2. Diagrammatic representation of the experimental layout for the low pH treatment. A tank for each temperature regime (control: 29, and then individually heated to 31, 32 and 33C°) was supplied with seawater from header tank lowered to pH 7.8 by addition of CO_2 to simulate ocean acidification.

3.4.2 Respirometry for measuring resting rate of O_2 consumption (MO_{2Rest})

The method used has been previously described in chapter 2. Fish were placed individually in a respirometer and allowed to acclimate for 1 hour with water flowing through the respirometer (Figure 2.5). After an acclimation period and the fish were calm, the chamber was closed (a 750ml plexi glass cylinder with 80mm inner diameter) and the oxygen levels continuously recorded with an oxygen electrode (OXI 340i from WTW, Germany) for 30-40 minutes. The respirometer was submerged in the same temperature-pH conditions as the fish were acclimated to. All recordings were carried out at oxygen levels between 70 – 100 % of air saturation. The fish settled within minutes; trials with longer acclimation periods (24 hours) did not further reduce the rate of O_2 uptake.

3.4.3. Swim respirometry for measuring maximal O_2 uptake (MO_{2Max})

The method used has been previously described in chapter 2. The respirometer chamber consisted of a Perspex cylinder (80 mm inner diameter, 500ml total water volume) (Figure 2.6). The chamber could be opened at the bottom, where a petri dish was tightly fitted. The tip of an oxygen electrode (Oxi 340i- WTW) was inserted 10 mm above the bottom of the chamber. A removable wire mesh (5 mm mesh width) was positioned horizontally in the middle of the chamber. Above the mesh a centrally placed cylinder created a circular swim chamber, and the water was set in motion by a 6 cm long stirring magnet in the compartment below the mesh. The respirometer was placed on the bottom of a temperature-pH controlled aquarium, below which a magnetic stirrer was placed to drive the magnet in the respirometer. The water speed was regulated with the magnetic stirrer. As soon as the water was set in motion, the fish started swimming against the current. The speed was set to a point where it was clear that the fish swam at or just above the aerobic maximum speed. This was done by increasing the water speed to a point where the fish was barely able to maintain a steady position in the chamber but pectoral fin movements were still evident. Water oxygen concentration was recorded for 10 min, during which time a linear fall in water oxygen concentration was seen. During the runs, water oxygen concentration was between 90 and 100 % of air saturation.

3.4.4. Aerobic Scope

Scope for oxygen uptake is given in % and was calculated as:

$$\textit{Aerobic Scope} = 100 \times (MO_{2Max} - MO_{2Rest}) / MO_{2Rest}$$

Resting and swimming oxygen consumption of 6-8 fish was tested at each combination of temperature (29, 31 and 32°C) and ocean acidification (control seawater [pH 8.15] and pH 7.8). Respirometry was not possible at 33°C due to high mortality rates in the ocean-acidification treatments.

3.4.5. Gill samples

During the acclimation period, it appeared that fish held in the highest temperature and low pH treatment had developed swollen gills. The opercula covering the gills was raised exposing the gill filaments, however, this was not seen in those at control temperatures. Whilst not a planned section of the methodology, out of interest, gill samples were subsequently taken from individuals that had died in the acclimation period. A gill filament sample was removed from individuals of *Ostorhinchus cyanosoma* within 4 hours of death. Samples were obtained from individuals held at 32°C (pH 7.8) and 33°C (pH 7.8) and compared to a sample taken from a control (29°C and pH 8.15). Firstly, the operculum was removed with scissors to expose the gills. Gill edges were taken by forceps which were held parallel to the edge of the gill filament and scissors used to excise a cross section of the gill, to contain the gill arch, rakers and gill filaments. The sample was then placed on a microscope slide and a cover slip carefully placed on top. Given that only one sample was taken as a preliminary investigation, no fixative was used. The gill specimen was then placed under an Olympus light dissection light microscope which had a high resolution digital camera and monitor attached.

This was pilot research that was conducted primarily out of interest and therefore the results are not quantitative (no repeat samples or scale available), and consequently should be taken only as preliminary results. The results from light microscopy were not included in the final publication.

3.4.6. Statistical analysis

All values are means ± SEM. SPSS v15 was used for statistical analysis. Statistical significance ($P<0.05$) was tested with a Two-Way ANOVA. When statistically different variances were seen in the data, Tukey's Post-Hoc test was applied to compare temperature and pH to the controls, as it is more powerful than Newman-Keuls Multiple Comparisons Test. Tukey's also reduces the chances of making a Type 1 Error (incorrectly rejecting a true null hypothesis). Where data exhibited statistically different variances (as detected by Levene's test for equal

variances), the values were square root transformed prior to analysis to obtain normal distribution. This was the case for resting oxygen consumption for *Ostorhinchus cyanosoma*.

3.4.7. Ethical Approval

This study followed the ethical guidelines provided by James Cook University, Queensland, Australia (see Appendix 1). The permit granted by the Great Barrier Reef Marine Park Authority (see Appendix 2, permit number GO6/ 20234.1) allowed the capture of fish.

3.5. Results

3.5.1. Mortalities

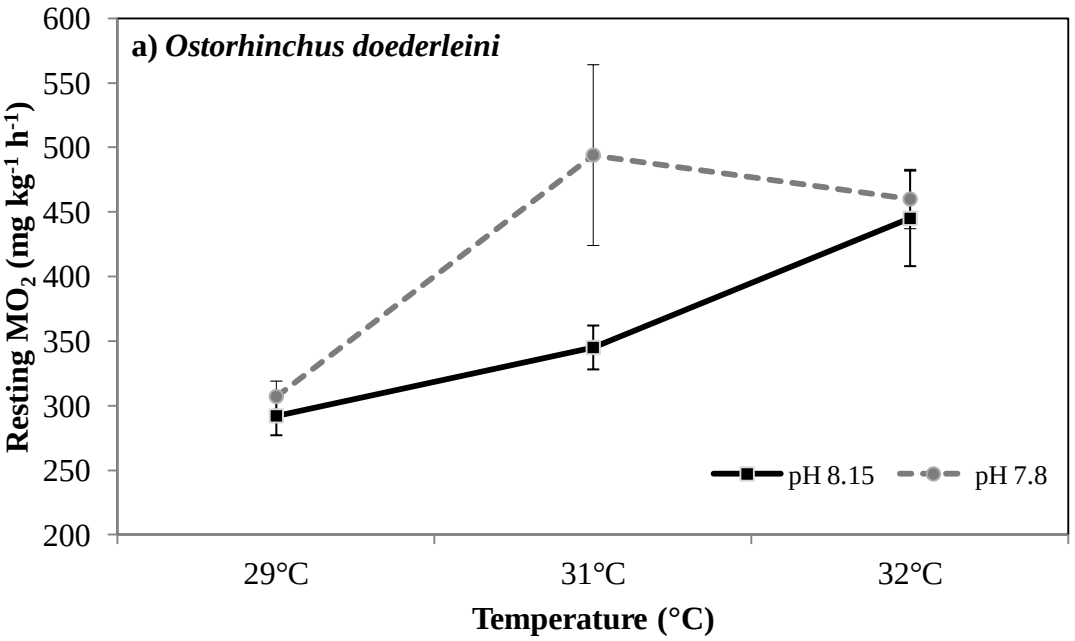
For both species of cardinalfish, temperature and pH affected the consumption of oxygen and the aerobic scope. Although the experimental design included fish at 33°C at both pH 8.15 and 7.8, it was not possible to conduct respirometry at 33°C due to high mortality rates. There was over a third mortality rate at 33°C, regardless of pH for both species and 100% mortality in *O. doederleini* at a lowered pH 7.8 (Table 3.1). All the *O.doederleini* held at 33°C and pH 7.8 died in the first 48hours, as did three of the *O.cyanosoma*. This is in contrast to those held at control pH, where mortalities were seen up to day five of the acclimation period. Therefore, when pH was lowered, particularly in conjunction with raised water temperatures, mortalities were seen much earlier into the experimentation.

Table 3.1. Number and percent (in parenthesis) of individuals of *O. doederleini* and *O. cyanosoma* from Lizard Island that died when kept at 8 combinations of water temperature (29, 31, 32, 33°C) and pH (8.15 and 7.8) for one week.

Temperature(°C)	Species and seawater pH			
	O. doederleini		*O. cyanosoma*	
	8.15	7.8	8.15	7.8
29	0	0	0	1 (11.1)
31	0	0	0	0
32	0	4 (33.3)	0	1 (11.1)
33	3 (37.5)	13 (100)	4 (44.4)	3 (33.3)

Even in control pH water, there were high mortality rates at the highest temperature of 33°C (3 out of 8 for *O. doederleini* and 4 of 9 for *O. cyanosoma*). This suggests that 33°C is near the upper thermal limit for both species at Lizard Island. Of those fish that did survive at 33°C, results from the swim test were unreliable as most were unable to sustain swimming for any length of time before complete exhaustion. Therefore the results only included data from three of the test temperatures, 29°C, 31°C and 32°C and both control (pH 8.15) and low pH (7.8).

3.5.2. Resting Oxygen Consumption



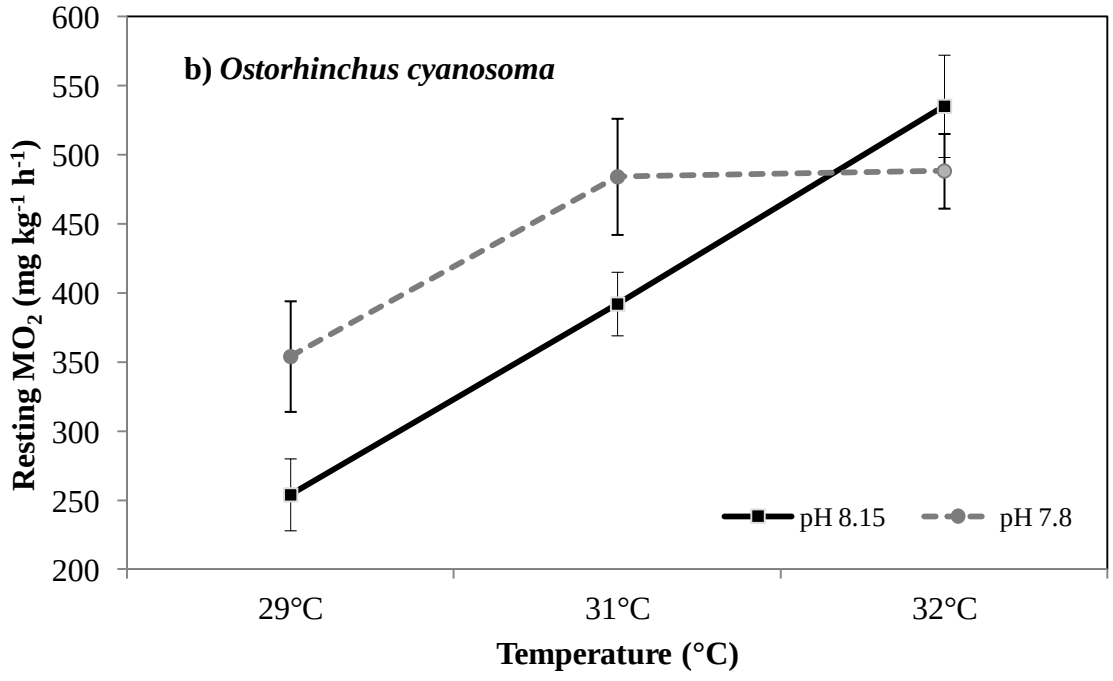


Fig 3.3. Mean resting MO_2 (±SEM) at 6 combinations of water temperature (29, 31, 32°C) and seawater pH (control 8.15, 7.8) for a) *Ostorhinchus doederleini* (n=6-9) and b) *O. cyanosoma* (n=8-9). There were significant effects of temperature ($p<0.001$) and pH ($p<0.05$) but no significant interaction between Temp x pH for *O.doederleini*. There were significant effects of temperature ($P<0.001$), pH ($p<0.01$) and Temp x pH ($p<0.05$) for *O. cyanosoma*.

Resting oxygen consumption (MO_{2rest}) for both species increased as temperature was raised and also as the pH was lowered. In acidified water, both species displayed an asymptotic relationship as water temperature was increased, that is to say there was a significant increase in MO_{2rest} from 29°C to 31°C, but no further increase in MO_{2rest} as the temperature continued to rise to 32°C (Figure 3.3). In the control individuals that were not exposed to CO_2 acidification, this asymptotic relationship did not occur and resting MO_{2rest} continued to increase with each temperature increment.

In *O.doederleini* at 29°C, the MO_{2rest} was the same regardless of pH treatment. However, as the temperature was increased to 31°C, those held in acidified water had a significant increase in MO_{2rest} which did not further increase with temperature (Figure 3.3a). This suggests that at lower temperatures, *O. doederleini* is unaffected by low pH but as temperature increases, low pH becomes stressful. Yet MO_{2rest} was as high in fish only stressed by high temperatures of 32°C as in those stressed by both high temperature and low pH.

O.cyanosoma displayed a different response. Those held in acidified water at 29°C had a significantly higher MO_{2rest} than those held in control pH (8.15). However, this was not seen at 31°C or 32°C. *O.cyanosoma* therefore showed a significant interaction between temperature and pH in resting oxygen consumption (Figure 3.3b; Table 3.2).

There were no significant differences for maximal/ swimming oxygen consumption MO_{2max} for *O. doederleini* (Table 3.2). The only significant effect seen was a decrease in MO_{2max} in *O.cyanosoma* for those individuals that were held at 32°C and pH 7.8 when compared to those held at 32°C in control pH water (Figure 3.4b). However, the effect of pH was not significant at 29°C or 31°C, suggesting that maximal uptake of oxygen consumption is not affected by temperature or pH.

3.5.3. Maximal Oxygen Consumption

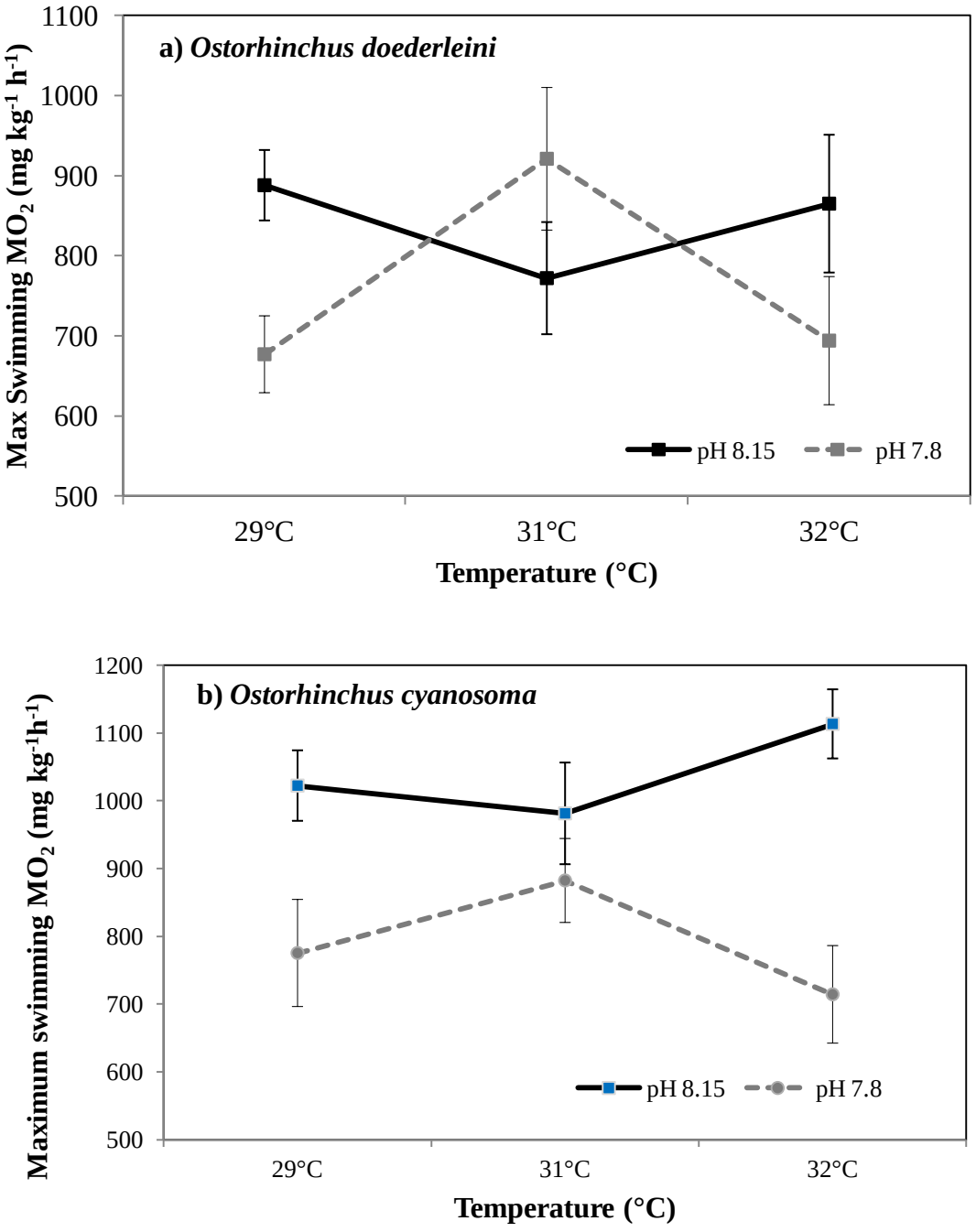


Fig 3.4. Maximum swimming MO_2 (±SEM) at 6 combinations of water temperature (29, 31, 32°C) and seawater pH (control 8.15, 7.8) for a) *Ostorhinchus doederleini* (n=6-9, except pH 7.8 & 32°C where n=3) and b) *O. cyanosoma*. (n=8-9) There were no significant effects of temperature, or Temp x pH (p=0.056) for *O.doederleini*. There were no significant effects of temperature or Temp x pH, but significant effect of pH (p<0.001), for *O. cyanosoma*.

3.5.4. Aerobic Scope

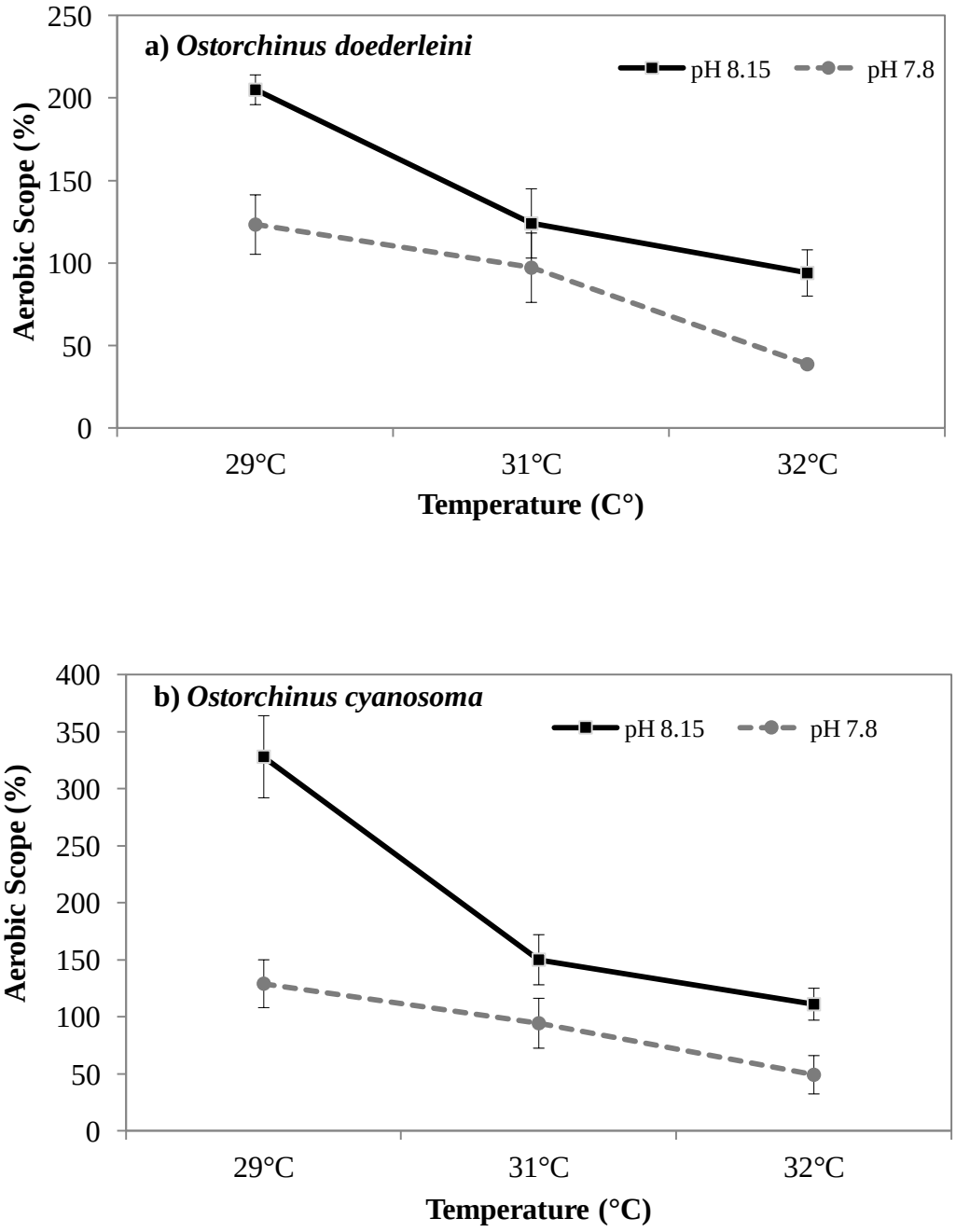


Figure 3.5. Mean aerobic scope (±SEM) at 6 combinations of water temperature (29, 31, 32°C) and seawater pH (control 8.15, 7.8) for a) *Ostorhinchus doederleini* (n=6-9, except pH 7.8 & 32°C where n=3) and b) *O. cyanosoma* (n=8-9). There were significant effects of temperature ($p<0.05$) and pH ($p<0.005$), but no significant interaction between Temp x pH, for *O.doederleini*. There were significant effects of temperature ($P<0.05$), but no significant effect of pH or Temp x pH, for *O. cyanosoma*.

Aerobic scope declined with both increasing temperature and decreasing pH. However, there was no significant interaction between temperature and pH (Table 3.2). This reduction in aerobic scope was due to the increase in resting oxygen consumption (Figure 3.3) and the fact that maximal oxygen consumption was not increased (Figure 3.4).

When temperature values were considered alone, there was a significant reduction in aerobic scope when the temperature was increased from 29°C to 32°C. The aerobic scope of *O. doederleini* declined by 36% and for *O. cyanosoma* it declined by 32% (*O. doederleini*: 483[±42] to 307[±56] mg O_2 $kg^{-1}h^{-1}$, *O.cyanosoma*: 609[±46] to 410 [±49] mg O_2 $kg^{-1}h^{-1}$).

A similar decline was also true when pH was considered alone. For *O.doederleini* kept in acidified water (pH 7.8), they had a 33% lower aerobic scope than those kept in control water (pH 8.15) (control water: 497[±35], acidified water: 330 [±40] mg O_2 kg^{-1} h^{-1}). A 47% reduction in aerobic scope was seen for *O.cyanosoma* when pH was considered alone (control water: 661[±39], acidified water 348[±38] mgO_2 kg^{-1} h^{-1}).

These results show that lowering the pH by just 0.3 units has similar effects on aerobic scope as increasing the temperature by 3°C on these two species of coral reef fish. Despite these significant effects when temperature and pH are considered separately, there was no significant interaction between temperature and pH in *O. doederleini* (Table 3.2). Within temperature treatments, there was no significant effect of pH. However, those fish held at control pH and 29°C did have a significantly higher aerobic scope than those at pH 7.8 and 33°C. Therefore, the aerobic scope of those held in conditions predicted to occur with climate change had a significantly lower aerobic scope, due to raised resting oxygen consumption.

O. cyanosoma appears to be the more sensitive species, with higher scope in control pH at 29°C and 32°C, but with the greatest reductions in scope as pH was lowered. *O. cyanosoma* had a slightly higher aerobic scope in the control experiments. However, both species had the same minimum values when at lowered pH and the highest temperature (*O. doederleini*: 195 [±91] mg $O_2$$kg^{-1}h^{-1}$, *O. cyanosoma*: 226 [±68] mg O_2 $kg^{-1}h^{-1}$).

Table 3.2. Results of an ANOVA for resting oxygen consumption (MO_{2Rest}), maximum swimming oxygen consumption (MO_{2Max}) and Aerobic Scope (MO_{2Max} - MO_{2Rest}) of individuals of *O. doederleini* and *O. cyanosoma* from Lizard Island at 6 combinations of water temperature (29°C, 31°C and 32°C) and pH (8.15 and 7.8). * denotes significant results, NS indicates results were not significant.

	O. doederleini				*O. cyanosoma*			
	df	**MS**	**F**	**p**	**df**	**MS**	**F**	**p**
MO_{2Rest}								
Temp	2	91689	8.89	<0.001 *	2	135.3	28.67	<0.001*
pH	1	45752	4.43	0.042 *	1	35.41	7.5	0.008*
Temp x pH	2	18838	1.83	NS	2	19.26	4.08	0.02*
Error	35	10317			43	4.72		
Swimming MO_2								
Temp	2	35634	1.08	NS	2	4786	0.13	NS
pH	1	74527	2.26	NS	1	750201	20.86	<0.001 *
Temp x pH	2	104294	3.16	NS	2	89444	2.49	NS
Error	33	32968			43	35970		
Aerobic Scope								
Temp	2	82801	3.35	0.047 *	2	160580	4.39	0.018 *
pH	1	239081	9.68	0.003 *	2	46822	1.28	NS
Temp x pH	2	36335	1.47	NS	2	46822	1.28	NS
Error	33	24707			43	36501		

3.5.5. Gill Structure

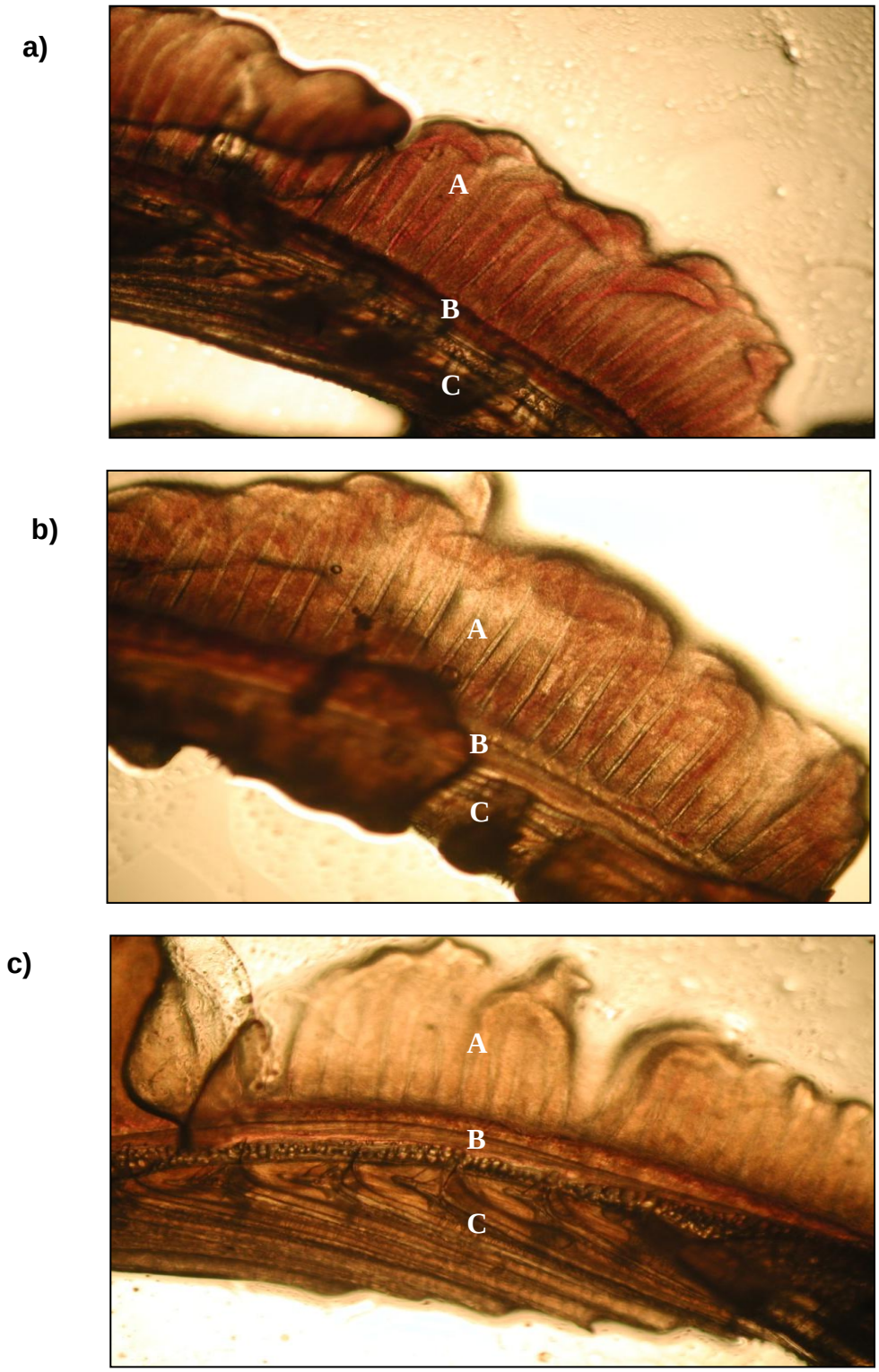


Figure 3.6. Photographs of gill samples as viewed using a light microscope connected to a high resolution camera. Samples taken from individuals of *O. cyanosoma* within four hours of death. Samples from individuals held at a) control (29°C, pH8.15), b) 32°C and pH 7.8, c) 33°C and pH 7.8. A) gill filaments (primary lamellae), B) gill arch, C) gill rakers. Scale approximate only.

Given that acid stress is known to cause deterioration in the structure of the gills, samples of gill tissue were taken from *O. cyanosoma* and observed under a light microscope. In the fish held at the highest temperature of 33°C and pH 7.8, there was clear degradation of the gill filaments and potentially reduced blood supply (Figure 3.6c) when compared to the control fish held at 29°C and pH 8.15 (Figure 3.6a). Although the gills from a fish held at pH 7.8 and 32°C still had a good structure of the lamellae of the gill, the oxygen carrying capacity of the blood may have be hindered as demonstrated by the paler colour (Figure 3.6b). It was not until the temperature is increased further, to 33°C, that the actual structure of the gills was compromised. Over 30% of the individuals held in these experimental conditions died within a week, and of those that survived, it was not possible to carry out experiments as they were unable to do achieve maximal swim rates due to complete exhaustion very early on. Clearly, the physiology of these individuals was compromised at high temperatures and low pH and the gill images presented here support that.

3.6. Discussion

Aerobic Scope, and therefore the capacity for aerobic function, affects all aspects of individual performance. This study has demonstrated that increased temperature and lowered pH, both anticipated to occur with climate change, reduced the aerobic scope of two commonly occurring coral reef fish, *Ostorhinchus cyanosoma* and *O. doederleini*.

As has been previously described in chapter 2, temperatures realistic with those expected with climate change caused a significant reduction in aerobic scope in all fish tested, particularly in cardinalfish. Additional to this finding, it was found that CO_2-acidified water caused similar reductions in aerobic scope. Even without warming, a lowered pH of 0.3 units (equivalent to an increase of~ 1000ppmv atmospheric CO_2, as is anticipated to occur in the coming century) caused the same percentage loss in aerobic scope as did a 3°C warming. Increases in atmospheric CO_2 over the coming century will cause reductions in ocean pH and increases in ocean temperature similar to those tested here. These results suggest that with climate change, and the associated warming of seawater and reduction in pH, some marine fish will have reduced capacity to manage aerobically. Thus, individuals will be forced to rely more heavily on anaerobic respiration, which is not sustainable in the long-term. These reductions in aerobic scope were due to increased resting oxygen consumption coupled with either no increase or a decrease in maximal oxygen consumption. That is to say, individuals need to respire more at rest in order to satisfy basal metabolic demands, but are unable to increase the upper limits of respiration regardless of the body's need for additional oxygen, e.g. such as is needed in burst swimming. This inability to increase maximal oxygen consumption, or indeed a reduction in maximal oxygen consumption, means that fish are likely to reach the point of exhaustion earlier, which has implications on foraging behaviour and predator avoidance.

The reductions in aerobic scope shown in this study are important, given that aerobic scope is thought to be the key physiological parameter determining how marine fish will cope with climate change. It is changes in aerobic scope that will determine alterations in species distribution limits and range shifts (Pörtner & Knust

2007). These results suggest that not only will warming waters affect the aerobic scope, but reductions in ocean pH could play an equally important role, or even further worsen the effects of temperature. Therefore, the atmospheric levels of CO_2 predicted to occur by the end of this century could significantly impact the ability of some marine fish by reducing their capacity for aerobic activity.

It has been reported that the method by which pH is lowered (by addition of an acid, e.g. HCL, or by bubbling CO_2) can have different effects on the physiology of fish (Fromm, 1980). Whilst it is evident that reduced pH affected the aerobic scope of both species, it is not clear from this study whether this was due to the lowered pH or from the CO_2 that was bubbled into the system itself. CO_2 diffuses readily across the gill membrane and therefore quickly reduces the pH of the blood and tissues (Ishimatsu *et al*., 2005; Pörtner *et al*., 2005), a process known as hypercapnia. When the blood and tissues becomes more acidic due to lowered pH or CO_2 (acidosis), the fish will try to compensate for this and re-establish their acid-base balance by accumulating bicarbonate ions and active ion transport (Claiborne *et al*., 2002). However, this will come at a physiological cost, particularly if it is prolonged. Furthermore, the accumulated CO_2 may actually reduce the ability of the blood to transport oxygen, which may be particularly detrimental to species or life stages with high metabolic demands (Pörtner *et al*., 2005), for example larvae and juveniles. However, this may also apply to times of high metabolic cost, such as at higher temperatures or burst swimming, as in this experiment. If the swimming was too strenuous and aerobic capacity was not possible, then there is the chance that fish will switch to anaerobic metabolism. This could have led to further decreases in blood pH, due to the lactic acid accumulation which occurs in anaerobic respiration (Fromm, 1980). All swimming was believed to be aerobic as the speed was set such that it did not exceed the maximum the fish could swim against, whilst there were still pectoral movements evident, which indicated aerobic respiration (Gardiner *et al*., 2010). Regardless of the speed of swimming, when temperatures reach the thermal maximum for a species, it is known to induce anaerobic metabolism in the mitochondria (Pörtner *et al*., 2000). Consequently, there is always a possibility that those fish, particularly those fish under the highest temperature and lowest pH, may have switched to anaerobic respiration earlier in the swim test. Whatever the reason for reduced aerobic scope observed here, this experiment has demonstrated that

prolonged CO_2-induced acidification of the ocean could affect the aerobic capacity of some marine fish, and therefore could affect the viability and sustainability of local populations of fish.

The reduced aerobic scope displayed by both species in acidified water was due to the fact that resting oxygen consumption increased (Figure 3.3), whilst maximal oxygen consumption either remained stable or decreased (Figure 3.4). However there was a larger percentage decline in *O. cyanosoma* than in *O.doederleini.*

Hypercapnia in invertebrates often results in metabolic depression, although this has not been shown in fish. Conversely, in fish, hypercapnia usually has no effect on resting oxygen consumption (Ishimatsu *et al*. 2005; Pörtner *et al*., 2005). This suggests that, provided no exercise is required, fish are able to cope with lowered pH. However, in this study both species displayed small increases in resting oxygen consumption with lowered pH. Given that the fish were acclimated to lowered pH for a week, this suggests that the fish were not able to physiologically adapt and therefore there was some energetic cost involved in the acid-base compensation for these two species.

Conversely to resting oxygen consumption, maximum oxygen consumption during the swim test was unaffected for *O. doederleini* and declined for *O.cyanosoma* in CO_2-acidified water. This decline in MO_{2max} for *O.cyanosoma* concurs with previous findings for other fish that have been exposed to acidified seawater, as summarised by Ishimatsu *et al*. (2005). However, in that study by Ishimatsu *et al* the levels of CO_2 used were far greater than anticipated with climate change and therefore greater than the levels used in the current study. Given that *O.cyanosoma* displayed similar responses to a smaller decrease in pH, this suggests that *O.cyanosoma* is particularly sensitive to hypercapnic conditions. The combination of increased resting and decreased maximal oxygen consumption explains why *O.cyanosoma* had the greater decline in aerobic scope (47%) compared to *O.doederleini* (33%) in acidified water.

It is thought that fishes are relatively tolerant to a wide range of pH. This stems from research that has been conducted on both temperate and tropical freshwater fish (Ishimatsu *et al*., 2005; Pörtner *et al*., 2005). These species live in an

environment where pH fluctuates daily and therefore the species are adapted to cope with large natural variations in acidification (Freda & McDonald, 1988; Ishimatsu *et al*., 2005). For example, the rainbow trout, *Oncorhynchus mykiss*, lives in both freshwater and seawater and can tolerate pH values from 6 to 9 without any physiological effects (Randall & Brauner, 1991). There are even specialist fish, such as the Osorezan dace, that have adapted to live in lake waters with the pH as low as 3.5units (Hirata *et al*., 2003). In contrast, coral reef fish have evolved in a relatively stable pH environment and so are probably more sensitive to ocean acidification, even if it is relatively small.

Most other studies on the effects of hypercapnia on marine fish have used pH levels far lower than anticipated to occur with ocean acidification. However, a study on the gilthead seabream (*Sparus aurata*) used declines in pH of 0.75units (8.05 to 7.3) through the addition of CO_2 (Michaelidis *et al*., 2007). The results from this study showed that there was a change in enzyme activity with hypercapnia representative of a shift from aerobic to anaerobic activity. Anaerobic respiration, if sustained, results in further decreases in blood pH due to lactic acid build-up produced as a bi-product of anaerobic metabolism. Due to the inefficiency of anaerobic metabolism, the authors suggest that a long-term switch in metabolic pathways from aerobic to anaerobic will result in changes in physiological processes such as growth and reproduction, therefore affecting individual performance and ultimately population dynamics. Despite the fact that the reduction in pH was twice that used in the present study, it nevertheless demonstrated that moderate levels of hypercapnia can still affect the physiology of marine fish. A decrease in pH to 7.3 is still within the predictions of climate change for the next 300 years, therefore this study still provides ecologically significant data, albeit at the extremes of what is anticipated. The changes in metabolic pathways seen in the study by Michaelidis *et al*. (2007) might explain the reductions seen in aerobic scope in *O.cyanosoma* and *O.doederleini* in this study, if aerobic capacity was hindered severely enough to cause a switch to anaerobic metabolism.

Despite coral reefs having far less fluctuations in pH than freshwater and temperate ecosystems, there is still some diel variation. Over the course of this study the pH of the seawater in the lagoon around Lizard Island ranged from 8.08 to 8.21, with pH values highest in the early morning and usually lowest at night time.

Therefore it is possible that organisms that shelter within the reef matrix at night for protection from predators may be exposed to elevated CO_2 and reduced pH, potentially as low as 8.0 to 7.8. However, these low values are never long-lasting, due to regular tidal flushing in the shallow lagoon and photosynthesis by reef algae and corals in the day-light. The two species of cardinal fish tested here are nocturnal planktivores and so do not usually shelter in the reef matrix at night; therefore it is not likely that they are exposed to the low pH values used in this study. Even if they do sometimes seek shelter at night, they would normally only be exposed for a short-time (hours), rather than a week as tested here, which may explain why they were adversely affected by chronically low pH.

Although only preliminary evidence, Figures 3.7a-c show that there was a degradation of the gill structure with increasing stress of elevated temperature and lowered pH. In the sample at the highest temperature (33°C) and low pH (7.8), there was evidence of the separation of the secondary lamellae on the gill filament. This is a typical response to pH stress. However, this separation was not evident at low pH and a temperature of 32°C, suggesting that low pH only causes significant stress at higher temperatures, symptomatic of an additive relationship between temperature and pH. It is also worth noting that the gill lamellae are paler in colour in more stressful environments, even at 32°C. This could be indicative of lower haemoglobin content, as pH stress is known to cause a breakdown of haemoglobin (Chezhian *et al*., 2011). Due to the importance of the gills in the acid-base balance and oxygen transport, significant alterations to the structure and functioning of the gills are very likely to have implications for the aerobic capacity of individuals.

At the highest temperature of 33°C, all individuals of *O.doederleini* died when the pH was lowered. However, even at control pH, there was high mortality at 33°C, with 37% of *O.doederleini* dying. The current summer mean seawater temperature in the lagoon at Lizard Island is 29°C, but maximal temperatures already exceed 30°C and a high temperature of 32.7°C has previously been recorded in the lagoon (Lough, 2007). Given that sea-surface temperatures are predicted to increase by 1 to 3°C over the coming century as a consequence of climate change, temperatures of 33°C in the lagoon will be experienced with greater frequency. Acute mortality to these extreme temperatures is not likely to be the primary threat to the majority of coral reef species. It is more probable that these increases in

temperature will reduce the capacity for aerobic function, which will then affect all aspects of individual performance, namely feeding, growth and reproduction. It is a reduction in these aspects that will ultimately threaten population sustainability, at temperatures lower than the lethal limits.

In chapter two, it was shown that *O.cyanosoma* and *O.doederleini* were more sensitive to temperature increases than the 3 species of damselfish tested (Nilsson *et al*., 2009). It is therefore possible that these two species are more sensitive to environmental perturbations, e.g. lowered pH, than other species and not representative of coral reef fish in general. Fish that shelter within the reef matrix at night e.g. damselfish (Pomacentridae) and surgeonfish (Acanthuridae), may be more tolerant to low pH. However, currently they are only exposed for a period of a few hours each night and therefore it is not known how they would respond to continued chronic low pH. Whilst further testing would be needed on many other species to compare the responses, this study has highlighted that not all species may be able to cope with even relatively small amounts of ocean acidification, particularly in conjunction with warming waters.

For species such as those tested here that are sensitive to hypercapnia and temperature, changes in their geographic distribution may be seen. *O.doederleini* & *O.cyanosoma* are not usually found at very low latitude coral reefs (i.e. near the equator) and Lizard Island is close to their latitudinal extent and geographical range (Gardiner *et al*., 2010). It is likely that ocean acidification will therefore further limit the low latitude locations where this species can persist. Latitudinal shifts in species distributions will likely be common for many species, both marine and terrestrial. There is already evidence for many species displaying latitudinal shifts (Parmesan & Yohe, 2003). Thus far, there are limited data available to assess whether this will occur for these species. If there is a loss of species such as cardinalfish from low latitude reefs, due to their role as nutrient recyclers (Marnane & Bellwood, 2002) there could be a decline in the nutrients available for other organisms. This could therefore have knock-on effects for many other reef fish and invertebrates, leading to a decline in diversity.

3.7. Conclusions

To date, the focus of most research on the effects of ocean acidification in tropical waters has focussed on coral reefs and other calcifying organisms (Caldeira & Wickett, 2003; Michaelidis *et al*., 2005; Shirayama & Thornton, 2002). Tropical fish are not considered to be of great concern where low pH is concerned. However this study has demonstrated that at least two species of tropical coral reef fish, *Ostorhinchus cyanosoma* and *O. doederleini,* are negatively affected by low pH by reducing their capacity for aerobic activity. The reduced aerobic scope displayed by both species in acidified water was due to the fact that resting oxygen consumption increased, whilst maximal oxygen consumption either remained stable or decreased. A lowered pH of 0.3 units (equivalent to an increase of~ 1000ppmv atmospheric CO_2, as is anticipated to occur in the coming century) caused the same percentage loss in aerobic scope as did a 3°C warming. In both studies described in chapter two and three, *O.cyanosoma* was more sensitive than *O. doederleini*. The results from this study support other recent findings that ocean acidification coupled with warming waters poses a significant physiological challenge to marine organisms (Pörtner *et al.*, 2005; Pörtner & Farrell 2008). Given that aerobic capacity underpins the sustainability of populations and their distribution ranges, these results indicate that ocean acidification and warming waters may pose a significant stress to some tropical marine fish. As with temperature increases, it is not yet known whether there is the capacity for fish to acclimate to permanent acidification over generations, however this is thought to be limited (Gardiner & Jones, 2005). The results from this study, and that of chapter two, make it apparent that climate change could have negative impacts on these species both indirectly (though loss of habitat) or directly (through reduced aerobic scope). Therefore, it is likely that over the coming century there will be a loss of some species, such as cardinalfish, in tropical coral reefs. Whilst other fish species may cope with changes, it is probable that the coral reefs of tomorrow will be less diverse and potentially less productive environments.

Chapter 4. Recent Evidence of Climate Change in the Non-Tidal River Thames and the responses of Fish

4.1 Abstract

Climate change is predicted to have its greatest effects in the northerly latitudes, such as Britain. There is evidence that many terrestrial organisms are responding to a warming climate, however, very little is known about how freshwater fish may respond. This study has provided evidence that there is already gradual warming in Thames region and an increase in precipitation in winter months, based on a 150 year data set. Over the more recent past, there have been a number of extreme weather events, such as record high summer temperatures and high flood events. The fish inhabiting the non-tidal River Thames have displayed different responses to the changes in their physical environment. All cyprinid species, such as roach, dace and bream displayed a similar pattern in density and biomass over a 15 year time period. However, this pattern was not followed by non-cyprinid species, such as perch, pike and the European Eel. This study also investigated the potential for Bayesian Networks to be applied to complex ecological datasets relating to aquatic habitats. The Bayesian Networks were able to correctly identify key relationships in the data and also indicated that cyprinid species may benefit from the warm-and-dry summers that are predicted to become typical with climate change. Therefore Bayesian Networks may be a useful tool in predicting the impacts of climate change on freshwater ecosystems. The results from this study suggest that there may be interfamilial differences in the responses of freshwater fish to climate change, with some families coping better than others, which may ultimately lead to a decline in species diversity.

4.2 Introduction

Understanding the ways in which climate change may affect fish populations is extremely important and yet very complex. Despite the widespread evidence of climate change in many regions across the globe, it is still prudent to evaluate an area

under investigation in order to ascertain whether climate change is already occurring there. Before one can predict the effects at the population level, it is first wise to evaluate whether the fish populations have responded to any changes in the climate. Based on that knowledge, one can begin to predict the ways that future warming and other associated changes in climate may impact an ecosystem. In terms of the river environment in Britain, this might be indications of warming waters with time, or altered flow patterns. It has been documented that the northerly latitudes such as England, and in particular the south-east of England, are set to experience the most climate change (FSBI, 2007). The specific prediction for the River Thames region is that it could experience a rise in air temperature of more than 3.5°C by 2080 (Webb & Walsh, 2004). Central England has been reported to have already warmed by 0.5°C in the 1990's compared to the 1961-1990 average, with the 1990s being a period of warming (Graham & Harrod, 2009). Since the 1990's it has also been reported that there has been an increased prevalence of warm-and-wet summers (Nunn *et al*., 2010), although general climate models predict summers will become warmer and drier (FSBI, 2007). Other indicators of climate change other than general warming are: wetter-and-milder winters, earlier onset of spring and overall reduction in flow rates.

Scientists have long understood that temperature has a major influence on the ecology and physiology of fish. However, more recently there have been debates over whether it is flow or temperature that plays a greater role in fish recruitment in rivers (Nunn *et al*., 2007b). Flow rate is important for several reasons. Firstly, high flow rates, such as flood events, can remove larvae and weak swimming juveniles from the river system. Low flow rates increase the residence times (R_t) of rivers, leading to an accumulation of nutrients and contaminants in the water, thus reducing water quality (Johnson *et al*., 2009). Slow flowing rivers are also likely to warm quicker due to reduced flushing, further increasing the temperature, which may be particularly significant in summer months. Additional factors such as the concentration of available oxygen in the water are also likely to have confounding effects, with warmer waters having a lower saturation of oxygen (Pörtner & Knust, 2007; Rombough, 1997). This, coupled with the higher metabolic demands at higher temperatures, can lead to the Temperature-Oxygen Squeeze, exacerbating the effects of warmer waters (Pörtner & Knust, 2007).

Graham & Harrod (2009) carried out a comprehensive literature search into the effects of climate change on freshwater fish populations in Britain and reported that very little is known. However, a study in the Rhone River, France, indicated a gradual displacement from 1979 to1999 of northern species by southern, thermophilic species such as chub and barbel (Daufresne *et al*., 2003). The River Thames no longer supports any cold water species, such as the Atlantic salmon or the brown trout (Webb & Walsh, 2004), and is now dominated by cool and warm water species such as the pike, perch, roach, bleak and bream. Warmer waters may prove to be beneficial for some warm-water species such as bleak and bream, with the onset of spring arriving earlier providing a longer summer for greater growth and a shorter period of winter food restrictions (Shuter & Meisner, 1992). However, there have been suggestions that climate change may make the River Thames stressful for all species currently inhabiting the river (Webb & Walsh, 2004). This hypothesis is supported by the results included in Chapter 5 of this thesis, whereby chronically elevated temperatures proved stressful for even a hardy species, the three-spined stickleback, *Gasterosteus aculeatus*.

4.2.2 Species Level Responses to Climate Change

The ability to adapt to the predicted changes will vary depending on species; there will be some winners but also some losers (FSBI, 2007). This section will detail how climate change may potentially affect some key freshwater fish species in Britain.

4.2.2.1 Brown Trout (Salmo trutta)

Currently, the distribution of brown trout is limited by temperature in Britain and will only decrease with climate change, as trout are cold water species. Even under scenarios of low warming, trout stocks will decline due to significant decreases in habitat availability and also summer temperatures being too high for growth and development (Webb & Walsh, 2004). Trout no longer naturally occur in

the River Thames (Webb & Walsh, 2004), but are present in small numbers due to restocking efforts for commercial angling (Johnson *et al*., 2009). Brown trout has a high economic value in the UK, and therefore decreases in their populations could prove to have significant local economic impacts (Arnell, 1998).

Brown trout are typically found in clean, well-oxygenated waters (Wheeler, 1969). Spawning takes place over the winter months between October and December (Davies *et al*., 2004), when the water temperature is between 5-10°C. Winter river temperatures in the UK are unlikely to rise above 8°C (Johnson *et al*., 2009). However, the warmer waters are predicted to adversely affect spawning and embryo development (Webb & Walsh, 2004). The warmer winter temperatures could potentially increase growth in the winter months, but higher summer temperatures and the increased risk of drought will probably prevent increases in overall growth (Weatherley *et al*., 1991) and lower survival. Brown trout are long lived species, living up to 13 years in Britain, which will reproduce many times in a life time (Davies *et al*., 2004), but it is thought that cold water species such as trout and the Atlantic salmon will face difficulties in a warming world.

4.2.2.2. Atlantic salmon (Salmo salar)

The iconic Atlantic salmon, the benchmark fish of healthy rivers and considered the 'King of Fishes' by anglers, faces certain pressure with climate change. The Atlantic salmon has already been subjected to many anthropogenic stresses, such as pollution caused from urbanisation and the construction of weirs which block their migratory paths (Davies *et al*., 2004). Climate change will only likely exacerbate rather than alleviate these pressures, with predictions for salmon looking very dim. Although salmon is not currently limited by temperature in the UK, warmer waters will decrease populations by affecting spawning and egg incubation (Webb & Walsh, 2004). There has already been a contraction in the southern populations of salmon, suggesting they are not adapting quickly enough to changes (FSBI, 2007). The River Thames, for example, is thermally suitable for Atlantic salmon, but they are currently not present there (Johnson *et al*., 2009; Webb

& Walsh, 2004). However, this could also be as a result of urbanisation and weir construction, rather than warming waters.

Salmon are diadromous fish which migrate from marine habitats to spawn in the freshwaters of rivers, often the river in which they themselves hatched (Davies *et al*., 2004). Having to inhabit within their life time marine, estuarine and freshwater environments, all of which will respond differently to climate change, means that salmon face many complex changes in their environment (FSBI, 2007). They require fast flowing rivers for their migration and depend on seasonal cues to initiate migration (FSBI, 2007). Shifts in seasons may cause migrations to commence when other physical parameters are not suitable, and reduced rainfall in summer could lead to earlier or later migratory runs (FSBI, 2007). This may alter the length of time they have for growth, which could prevent smoltification the following spring (transition period from freshwater to marine habitats) (FSBI, 2007). Spawning occurs between October and January (Davies, *et al*., 2004), and the juveniles stay in British rivers until aged about 3 years before migrating to the sea (Davies *et al*., 2004). After spawning, most salmon die and so generally they only reproduce once (Davies *et al*., 2004). Therefore it is important there is successful survival and development of eggs that are spawned.

Salmon require clean, well oxygenated, shallow, fast-flowing waters. They cannot tolerate water temperatures above 22-23°C without an acclimation period (Davies *et al*., 2004). Salmon are also very sensitive not only to temperature and flow rates, but also to low oxygen levels and pollution (Davies *et al*., 2004; FSBI, 2007). With water temperature set to rise, and flow rates and oxygen concentration set to decline, the Atlantic salmon faces a very dim future, particularly in the South of Britain.

4.2.2.3 Roach (Rutilus rutilus)

Roach is a eurythermal cyprinid species that have a very adaptable nature. They are generalist feeders, able to digest animal, plant and detritus (FSBI, 2007), allowing them to cope with changes in food availability. *R.rutilus* is an effective zooplanktivore and feeding efficiency increases with temperature, with maximum

efficiency at 17-19°C (Graham & Harrod, 2009). Currently roach are not limited by temperature, but by 2080 under high emissions scenarios, half the rivers in Britain could become unsuitable for roach (Webb & Walsh, 2004). However, roach are susceptible to over-winter size-selective mortality, and so as waters warm in winter, fewer mortalities could occur (FSBI, 2007). Roach are spring spawners, usually spawning in April and May when the water reaches 14°C (Davies *et al*., 2004) or 15°C (Graham & Harrod, 2009). If water temperature rises only a few degrees, it is likely that roach will spawn earlier in the year. However, more dramatic increases in temperature (8-10°C increases) may result in poor spawning success (Graham & Harrod, 2009), but these temperature increases are unlikely. With suitable environmental conditions, a large female roach can produce up to 200,000 sticky eggs which cling to vegetation (Davies *et al.,* 2004). Climate change may induce earlier spawning in the year and so lengthen the growing season (FSBI, 2007), allowing a greater size to be reached by the first winter and so also reducing overwinter mortality. Juvenile optimum temperature is around 27°C, a temperature rarely reached in many European rivers (Graham & Harrod, 2009). Therefore, warmer waters may be beneficial to juvenile growth and so roach are predicted to cope well with the anticipated changes. They are also able to withstand low concentrations of dissolved oxygen and are fairly drought resistant (FSBI, 2007). Being non-territorial, roach will migrate to more suitable environments on a daily and seasonal basis (Davies *et al*., 2004). This, combined with their generalist nature, should allow them to adapt and thrive.

4.2.2.4 Perch (Perca fluviatilis)

Perch can live in a variety of habitats, from still to fast flowing rivers (Davies *et al*., 2004) and this species is generally considered to be a good example of a cool water fish (FSBI, 2007). Perch are not currently limited by temperature in Britain. However, by 2080 under high emissions scenarios, some rivers may become unsuitable (Webb & Walsh, 2004) particularly in winter months when females are developing eggs (FSBI, 2007). Whilst warmer winter temperatures may reduce overwinter mortality in the first year of life, it may cause gonad malfunctions in females if temperatures are too high. Oogenesis (creation of ova) commences in

August and vitellogenesis (yolk deposition) continues until females are ready to spawn. Spawning occurs from April to early June, when waters reach 10°C (Davies *et al*., 2004; FSBI, 2007), with the development time of eggs being determined by water temperature (FSBI, 2007). Once eggs are hatched, the optimum temperature for growth is about 23°C (FSBI, 2007), and so warmer waters may help to increase scope for growth. Therefore fecundity should be increased with the larger body sizes, which could lead to increased survival and distribution of perch with climate change (FSBI, 2007). Roach is a strong competitor to perch, and given that roach are predicted to do well with climate change, there could be negative connotations for perch. The competitive interaction between the two species is temperature dependent and roach have been shown to depress perch populations in lakes and in laboratory studies at temperatures higher than 18°C (FSBI, 2007). Although the physical properties of the water may suggest a possible increase in population size in some instances for perch, the biotic factors such as competition with other fish species may also affect the ability of perch to cope.

4.2.2.5 Pike (Esox lucius)

Pike are solitary fish, and being non-territorial they will freely move to different locations to feed on a variety of organisms. When young, pike feed mostly on invertebrates, but will become carnivorous with development, feeding on other fish and vertebrates (Davies *et al*., 2004). Feeding ceases during the months of spawning, which usually occurs in spring, from February to May (Wheeler, 1969). If environmental conditions are optimum, development may be fast and spawning can occur after 1 year, but usually sexual maturation takes 2 to 3 years (Davies *et al*., 2004).

Pike may be able to cope well with climate change, since the species is able to survive in a variety of river flow conditions, and also is able to tolerate some pollution and low dissolved oxygen concentrations (Davies *et al*., 2004). Even under scenarios of high CO_2 emissions, habitat is still predicted to be suitable for pike (Webb & Walsh, 2004), but water temperatures higher than 29°C could prove fatal (Davies *et al*., 2004). It is unlikely that river temperature in Britain will rise as high

as 29°C, even under high CO_2 emission scenarios, and so fatalities of pike caused by thermal stress are unlikely.

4.2.2.6. Chub (Leuciscus cephalus)

Mature chub are often solitary and inhabit deeper waters of middle to lowland reaches, where there is moderately fast flowing water (Davies *et al*., 2004). Chub will feed on a variety of food, including small fish, frogs and even crayfish when chub are fully grown. Berries and fruit that fall into the river will also be consumed by chub, and insect larvae are the food preference for juveniles (Davies *et al*., 2004). Young chub will often be in competition with young salmon and trout for food (Wheeler, 1969), but since these species are unlikely to cope well with climate change, this should reduce competition pressures for developing chub.

Males reach sexual maturity first, at about 3 to 4 years, and females at 4 to 5 years of age. Once sexually mature, spawning takes place in clean running water in May to June when the water temperature reaches 15°C (Davies *et al*., 2004; Wheeler, 1969). If there is a late spring, chub have been shown to delay spawning until the environmental conditions are right (Wheeler, 1969). However, with climate change, it is more likely that spring will be advanced and so chub will be able to spawn earlier in the year and hence have a longer growing season.

4.2.2.7 Bleak (Alburnus alburnus)

Bleak can be found in the middle to lower reaches of rivers (Davies *et al*., 2004) in clean slow running waters (Wheeler, 1969). Bleak are a relatively short-lived species, taking around 2 years to reach maturity, and living a total of 3 to 4 years, rarely more than 5 to 6 years (Davies *et al*., 2004). When active, bleak will feed mostly on planktonic animals, and usually this occurs in large shoals (Davies *et al*., 2004). Once sexual maturity is reached, spawning on gravel substrate takes place from April to June, when the water temperature has reached at least 15°C (Davies *et*

al., 2004; Wheeler, 1969), and the female (of 15cm or more in length) can produce as many as 5,000 eggs (Davies *et al*., 2004).

Currently, bleak are not found in the north or west of Britain, because the water temperatures in May and June tend to be too low to permit successful spawning. Therefore, it is predicted that with climate change and the associated warmer waters, there could be a range increase for bleak (Webb & Walsh, 2004). However, this depends on whether or not bleak can access new rivers systems or whether human intervention will be needed in order for this to occur.

4.2.2.. Three-spined stickleback (Gasterosteus aculeatus)

The three-spined stickleback is ubiquitous in European and UK waters, inhabiting marine, estuarine and freshwater ecosystems (Östlund-Nilsson *et al.*, 2007). They are instantly recognisable by the presence of their three dorsal spines and by the male's red belly in courting. They are a relatively small fish, ranging from less than a gram (Sebire *et al*., 2007) to a mass of 3.4g (Maunder *et al*., 2007). They are a temperate species, usually inhabiting waters between 4 to 20°C, but having a higher upper tolerance temperature of 28°C (Moran *et al.,* 2010). They feed on worms, crustaceans, larvae and aquatic insects; they have also been known to consume their own eggs. They have been extensively studied and there is a large literature on their biology, reproduction and life history (Östlund-Nilsson *et al.*, 2007). *G. aculeatus* is generally not considered to be particularly sensitive to environmental perturbations, (Östlund-Nilsson *et al*., 2007). Given their wide temperature tolerances, varied diet and widespread distributions, it is thought that *G.aculeatus* will not be severely affected by climate change.

4.4.2.9. European Eel (Anguilla Anguilla)

European eels are instantly recognisable with long, cylindrical bodies which are covered in small scales embedded into slimy skin. They lack pelvic fins, and their dorsal and anal fins are fused together. Eels can grow to large sizes (55cm for

females and 40cm for males) weighing up to 5kg (Davies *et al*., 2004). They are found throughout Britain, and will inhabit rivers, lakes, estuaries and coastal areas throughout their life. European eels reproduce only once in their life. Adults migrate a large distance to breed, travelling to the Sargasso Sea to spawn in deep waters in the early spring. It is thought that the eggs are then slowly swept back to the coast of Britain by the warm waters of the Gulf Stream, a process thought to take at least a year (Davies *et al*., 2004). By the end of this journey, the eggs have hatched and enter British rivers as juvenile eels, known as glass eels. In spring glass eels migrate up the rivers, and spend the rest of their lives (up to 40 years) in the rivers. The cycle ends when the adult eels return to the Sargasso Sea to breed.

The European eel is fairly tolerant to a range of temperatures, pH and dissolved oxygen concentrations (Davies *et al*., 2004) and therefore may not be overly sensitive to climate change. Eels are also the only fish that are able to fish species that can move overland, and have frequently been observed moving overland in wet terrain at night. Therefore, eels have an advantage over other fish species if one river becomes too warm for them, as eels have the potential to migrate into a different river system. Whereas all other species of fish are constrained to the river in which they were born, unless moved by man. The migration of glass eels up the river is however temperature dependent, and will only occur when the water temperature reaches at least 6°C (Davies *et al*., 2004). With gradual warming and an earlier onset of spring, this may mean the glass eels enter into the river system earlier in the year.

Since the 1970's there has been a large decline in the number of glass eels reported in European waters, by as much as 99% (Vogel, 2010). As yet there is no evidence that this has affected the adult population and there is an Eel Management Plan in place presently to help increase recruitment. Given that eels, like the Atlantic salmon, inhabit marine, estuarine and freshwater habitats in their lifetime, predicting their outcome with climate change is complex and problematic (FSBI, 2007). Given their recent declines, the European Eel may face difficulties with our changing climate.

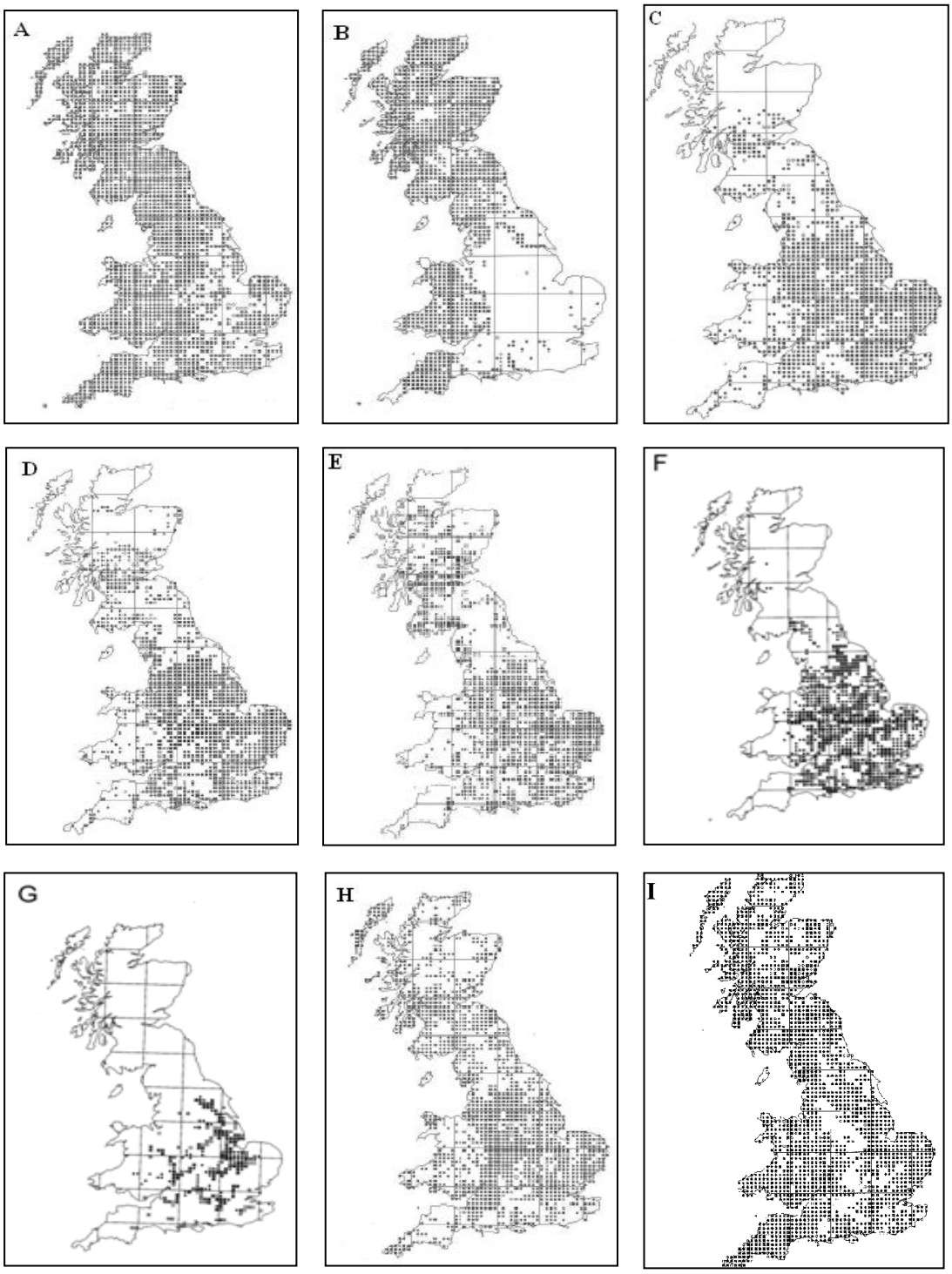


Figure 4.1. Species distribution maps in Britain. A) brown trout, B) Atlantic salmon, C) roach, D) perch, e) pike, F) chub, G) bleak, H) three-spined stickleback I) European Eel (taken from Davies *et al*., 2004). Dots represent records for each species in a 1km stretch of river based on surveys since 1972.

4.2.3. Understanding connections between the physical environment and species abundance

Whether changes in species abundance or community structure have already been seen in the non-tidal River Thames as a consequence of climate change is not known. If the fish population follows changes as seen elsewhere, it can be anticipated that climate change will benefit some species and disadvantage others. In order to predict how well the fish in UK rivers, in particular the River Thames, will respond to climate change, it is vital to first understand the complex relationship between the physical environment and the fish, but also to include and understand the interactions between fish species themselves (Milns *et al*., 2010).

Multiple linear regressions and Principal Component Analysis are the techniques commonly employed in understanding such complex datasets. The main limitations to these models is their inability to make use of incomplete data sets, bias created out of using short-time scales, and the high importance on start and end dates of any time series (Dose & Menzel, 2004). Obtaining complete, long-term data sets is often very problematic for ecological systems, due to factors such as changes in sampling methodology, effects of weather on permissibility of sampling and lost datasets as a result of changing organisations. Another big limitation of multiple linear regressions is the assumption that all explanatory factors must be mutually orthogonal, whereas most environmental variables show a degree of collinearity, such as warmer waters and lowered dissolved oxygen concentration, higher river flow and lowered temperature. This collinearity can make the results of multiple regressions misleading (Shaw, 2003).

However, a novel technique called Bayesian Networks can make use of incomplete data sets (Dose & Menzel, 2004). It is based on the principle of probabilities and the resulting graphical networks statistically represent the key links between multiple variables, providing a wealth of information about the connections in complex data sets. Using Ockham's razor, Bayesian Networks rank the probabilities and produce a model which uses the minimum number of variables necessary to explain the data (Dose & Menzel, 2004). The Bayesian Network models represent causal chains, i.e. the links in the model may be cause-effect relationships showing only relates nodes (with each variable represented as a node) that a

probabilistically and statistically related to each other by a causal dependency. Arrows connect each node, with the direction of the arrow indicating the direction of causality. Given that these networks provide information on the cause and effect relationships in a dataset, they are useful in their application of future predictions based on learnt models of past events. Therefore, by supplying evidence of past events and running a learned model, a Bayesian Network will be produced to display the most probable future outcomes.

These networks have recently been successfully applied to various ecological data sets, successfully identifying known relationships between birds and their habitats in the peak district as described in chapter 1 (Milns *et al*., 2010) and fish and the habitat and biotic conditions in the Columbia River Basin in the United States (Marcot *et al*., 2001). The study by Marcot *et al*. (2001) provided strong evidence that networks can be enhanced by taking into account expert knowledge on a system, strengthening the links between variables and the confidence in the produced network. Other multivariate analyses are not capable of computing such information. These studies demonstrate the potential for Bayesian Networks in understanding and interpreting the complex interactions in other ecosystems, such as between fish species and their changing physical environment in British Rivers.

Where rivers are concerned, alterations in water temperature, flow rate and dissolved oxygen content are all likely to change as a result of warmer air temperature and altered rainfall patterns. These three properties may react additively, synergistically or confound the effects of another variable (Brasheres, 2010). Whilst temperature is regarded as determining growth and recruitment success, it has been documented that river flow rate is the principle controlling factor influencing biomass and density of fish species, since it affects fish both directly (discharge reduced mortality) and indirectly (increased energy expenditure, reduced growth through lowered temperatures) (Nunn *et al*., 2003). However, the importance of flow may be reduced in highly regulated rivers such as the River Thames. Therefore, it is predicted that the Bayesian Network will identify temperature as being of greater importance, given that the River Thames is a highly regulated river. Consequently, as seen in the River Trent, temperature should be the principle controlling factor followed by flow rates (Nunn *et al*., 2007b). It is also anticipated that the Bayesian Network will show a relationship between roach (*Rutilus rutilus*) and perch (*Perca*

fluviatilis), as there are known and well documented interactions and competition between these two species, with roach being the stronger competitor at higher temperatures (FSBI, 2007).

Once the relationships between the physical parameters and the fish population are understood, the Bayesian Network can then be manipulated to predict the future assemblage of fish. For example, by applying general warming to the model or weather patterns such as 'wet-and-mild' winters and 'hot-and-dry' summers. Not only will Britain likely see increases in temperature and overall reduced flow rates, but it is predicted that extreme weather events, such as droughts, floods and heat waves, will increase in frequency and intensity (IPCC, 2007). Whilst little is known about how freshwater fish in the UK will respond to the general predictions of climate change, even less is known about how these 'big events' will affect the fish populations (Lake, 2003). However, even in the last 15 years, fish will have been exposed to some of these extreme events, with record-breaking weather events occurring during those years. From 1995 to 2006, 11 of the 12 years were the warmest since records began in 1850 (IPCC, 2007). In the European heat waves in the summers of 2003 and 2006, water temperatures reached 25.5°C and 27.1°C, respectively, at Oxford, with a record high air temperature of 38.5°C on the 10th August, 2003 in Kent. A dry winter of 2005/2006 followed by extreme high temperatures in the summer of 2006 led to the most severe drought in the UK for 100 years (EA). This was followed by summer flooding in 2007, with June 2007 having the highest precipitation on record for this month for Britain (until 2012). North Yorkshire received 289.9mm of rainfall in June 2007, which is nearly 500% higher than the 1961-1990 average for June (Met Office). Contrary to public perceptions of climate change and global warming, our changing climate has also brought the UK extreme cold winters; the winter of 2009/10 had a mean temperature of 1.6°C, 2°C below the 1971-2000 average, and this was accompanied by over 20cm of snowfall in southern England (Met Office). Whilst these may be extreme events now, they may well become 'normal' weather conditions in the future, and hence understanding how these events impacted fish populations can hold the key to predicting the success of future fish populations in response to anticipated climate change.

4.2.4 Aims & Objectives

There are three main aims of this study.

1) To ascertain whether there is yet any evidence of climate change in the River Thames.

    - Using a long-term (150 years) data set on air temperature and rainfall in the Thames region to determine whether there have been any general trends in climate.

    - Using a 15 year historical data set on river temperature and flow rates to establish if there is any indication that climate change is affecting the River Thames.

2) To establish whether any changes in climate conditions in the last 15 years has had an effect on the fish population of the non-tidal River Thames.

3) To determine whether Bayesian Networks can be successful applied to understanding the relationships between physical parameters and the freshwater fish population of the non-tidal River Thames.

4.3 Methodology

4.3.1. Study site

The non-tidal River Thames is 180km long, originating at Thames Head in Gloucestershire and terminating at Teddington weir, after which the river is influenced by tidal currents. The non-tidal River Thames is divided into three main sections (containing 44 contiguous reaches separated by locks and weirs) by the Environment Agency (EA) (Figure 4.2) and each section is sampled by a separate fisheries team, each using the same equipment and methods. The Upper (A) section runs from the source at Thames Head to Eynsham, the Middle (B) from Eynsham to Hurley and the Lower (C) from Hurley to Teddington.

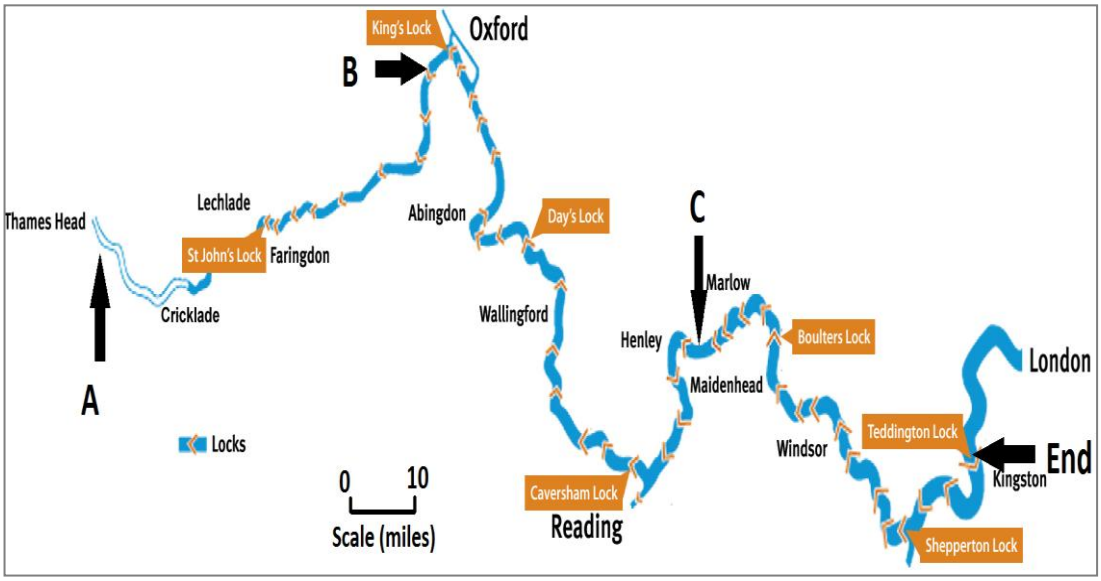


Figure 4.2. Map of the non-tidal River Thames showing the start of the three main sections as monitored by the Environment Agency. A (Upper, starting at Thames Head), B (Middle, starting at Eynsham) C (Lower, starting at Hurley) and End (Teddington Weir). (Modified from www.environment-agency.co.uk. [Date accessed 06/02.2013]).

4.3.2 Fisheries Data

All fish data were collected and supplied by the EA. Annual surveys were carried out using a purpose built boom boat for multispecies electric fishing. The following description of the methodology was provided by the EA:

Electric fishing was conducted using a purpose built 'boom boat' with two two-meter diameter fixed anodes with concentric rings of stainless steel 'droppers' (Figure 4.3). A single timed run was performed at each site in a downstream direction using Pulsed Directed Current (PDC) via an Electracatch control box, with a frequency of 50Hz generating between 10 and 15amps. Stunned fish were identified to species level and body length (as fork length, FL) was measured to the nearest mm. Fish were then released back into the river.

Figure. 4.3. Photograph of electrofishing on purpose built 'boom boat' by a fisheries team of the Environment Agency.

Sampling was carried out in daylight hours between July to early September from 1994 to 2009. Catch per Unit Effort (CPUE) values for biomass (g/min^{-1}) and density (n/min^{-1}) of each species were then calculated. The sampling technique is

biased in that it only records larger species of fish, and therefore this study does not investigate the impacts climate change or any other factor on the first year of life of any species and year class strength. In total, 26 species of fish were recorded in the EA reports, however only 11 species were included in this study: Roach (*Rutilus rutilus*), Perch (*Perca fluviatilis*), Pike (*Esox lucius*), Barbel (*Barbus barbus*), Gudgeon (*Gobio gobio*), Bleak (*Alburnus alburnus*), Tench (*Tinca tinca*), Common Bream (*Abramis brama*), Chub (*Leuciscus cephalus*), Dace (*Leuciscus leuciscus*) and the European Eel (*Anguilla Anguilla*). Based on the data obtained, these 11 species account for over 99% of the density of fish and 93% of the river's biomass. Therefore, it is considered that these species are representative of the fish community assemblage of the non-tidal River Thames. The remaining 15 species are present in such small numbers or were recorded only once in the 15 years, hence they could not provide significant information in how climate change may have affected them. Biomass and density of each species were averaged for all sites in the three reaches, providing a mean density and biomass for each species each year.

4.3.3. Physical data

Flow rate (m^3/s) and temperature (°C) data were obtained from the EA and the Centre of Ecology and Hydrology (NERC). Dissolved oxygen concentration (mg/l) and chlorophyll concentration (mg/l) data were assimilated from the Hannington and Windsor gauging stations on the non-tidal River Thames, which are operated by the EA. Table 4.1 provides a breakdown of sites along the non-tidal Thames where data were recorded by the EA. For Bayesian Network analysis, physical parameters were divided into seasons: winter (December, January, and February), spring (March, April, May), summer (June, July, August) and autumn (September, October, November).

Table 4.1 Summary of data sources of the three reaches of the River Thames.

	Fisheries Data (Density [n/min]and Biomass[g/min])	**Flow Rate (m/s)**	**Temperature (°C)**	**Dissolved Oxygen Concentration (mg/l)**	**Chlorophyll Concentration (mg/l)**
Upper (A)	Northmoor, Eynsham, Kings (2001-2009)	Eynsham 1999-2009	Hannington 2002-2009	Hannington (2001-2009, except 2007)	Hannington (2001-2009, except 2007)
Middle (B)	Sandford-Benson 1994-2009	Days 1994-2008	Sandford-Days 1994-2009	Abingdon and Caversham (2001-2009)	Abingdon and Caversham (2001-2009)
Lower (C)	Ham Loop, Penton Hook, Shepperton Weir. 1995-2009	Walton (1994-2009)	Caversham, Windsor & Teddington (2001-2009). Penton Hook (1998-2009)	Windsor (2001-2009), Teddington (2003-2009).	Windsor (2001-2009), Teddington (2003-2009).

4.3.4. Bayesian Network Methodology

In order to determine the key parameters affecting fish biomass and density, a Feature Selection was applied to the data (Saeys *et al*., 2007). A feature selection is the term commonly used in data mining, such as Bayesian Networks, to describe a technique whereby the dataset is reduced to a manageable size for processing and analysis. It is particularly important in datasets such as this where there are many features (i.e. temperature, dissolved oxygen concentration, flow rate, species biomass and density, chlorophyll concentration) but comparatively few samples (i.e. a short time series from 1994-2009). A feature selection is important as datasets usually contain more information than is necessary to build the model, and noise in the dataset makes it harder to discover meaningful patterns in the data. Using all the data available, the feature selection will rank the probability of each variable affecting another, and produces a model which uses the minimum number of variables necessary to explain the data, using the principle of Ockhams Razor (Dose & Menzel, 2004). In order to test whether the feature selection has identified the key variables and whether it is able to correctly predict a new network, a Wrapper-Based Approach was employed (Saeys *et al*., 2007). A wrapper based approach means that new networks are constantly created using different combinations of variables, and these new networks are compared to the original dataset, the 'hold-out set', and given a score as to their predictive accuracy. A Naive Bayes Classifier was used as the model in this wrapper feature selection with repeated cross-validation to the hold-out set. This technique finds the key variables in a dataset that are most robust to sampling variation and disregards the variables that are not needed, therefore reducing noise in the data (Langley & Sage, 1994).

Once the key features were identified, a Greedy search was applied to learning the Bayesian Network classifier to establish which learnt network is the best fit. The Bayes Information Criteria scoring metric was used to rank the accuracy of the learnt models, simultaneously preventing over-fitting the data by penalising any networks that are overly connected. In this way, only links between variables that have high confidence are preserved. The networks were further tested using a 'Leave One Out Cross Validation' approach in order to test the predictive power of the network. This approach performs the Bayesian analysis on one subset of the network

and validates the results against the other subsets (i.e. by comparing how changing one aspect of the network impacts other subsets of the network), and this process is performed multiple times on different subsets of the network to ensure high accuracy in the final learnt network. Bayesian Network analysis was carried out using WEKA software, software which is freely available in Java.

4.4 Results

4.4.1 Temperature

Complete long-term data sets of water temperature for the non-tidal River Thames are not available. However, the Met Office has been recording air temperature in the Thames region (at Oxford) since 1853.

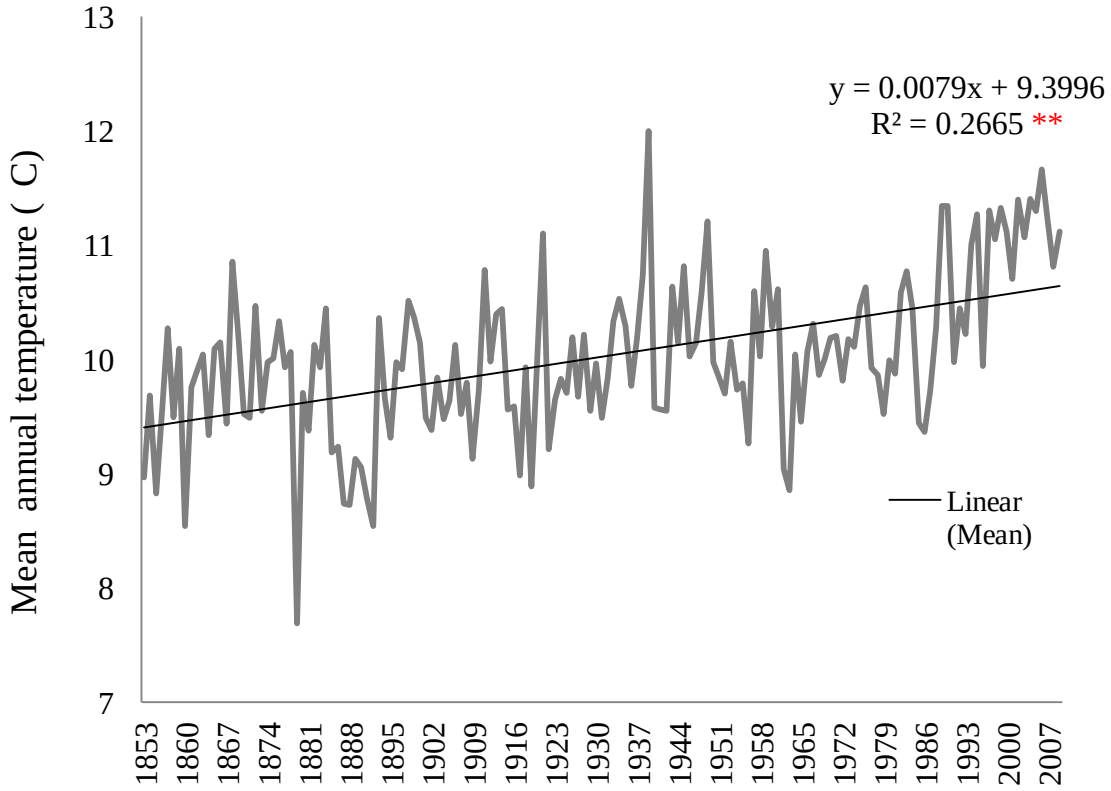


Figure 4.4. Mean annual air temperature at Oxford (data from the Met Office). There was a significant positive linear correlation between year and air temperature. Pearson's correlation coefficient: r (157) =0.516; p<0.01, r^2= 0.266 (two-tailed).

Since 1853, there has been a steady increase in the mean annual air temperature, from 9.5°C in the 1850's to 11.2°C in the 2000's. Figure 4.4 also shows that there was a steep rise in temperature from the 1990's onwards, agreeing with previous literature that the 1990's was a period of warming (Graham & Harrod,

2009). This 1.7°C increase in air temperature over the last 150 years is a significant rise and demonstrates that warming is occuring at a fast rate.

Due to the close relationship between air temperature and water temperature, it is possible to infer the impacts of this increase in air temperature to the river environment (Mohseni *et al.*, 2003). Figure 4.5 shows that there is very strong correlation between the two based on temperatures between 1990 and 2009. Therefore, one can assume that if air temperatures have risen by nearly 2°C in 150 years, then so has water temperature. However, the two temperatures will not be exactly the same, with water temperatures in the River Thames being consistenly 3°C warmer than the air.

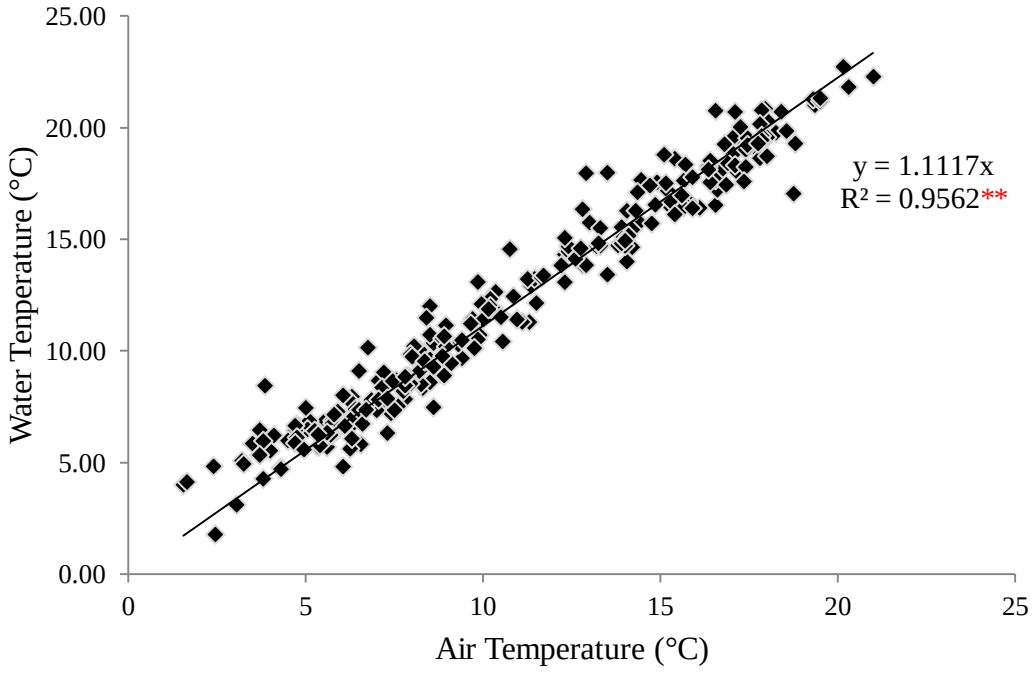


Figure 4.5 The strong positive correlation between monthly air temperature and water temperature between 1990 and 2009 on the River Thames (Sandford-Days mean). Pearson's correlation coefficient: r = 0.9778, p = 0.001, r^2= 0.9562 (2-tailed).

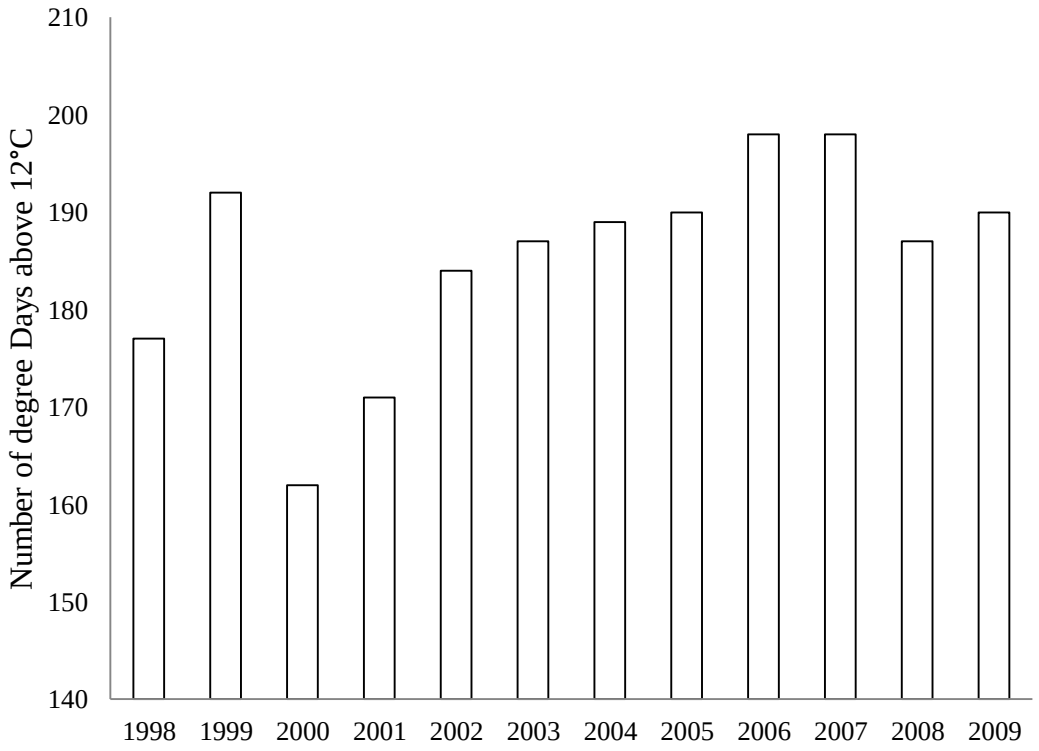


Figure.4.6. Cumulative number of degree days above 12°C from 1998 to 2009 at Penton Hook on the lower reaches of the River Thames.

The cumulative number of degree days above 12°C (taken from daily water recordings) has been shown to be a reliable indicator of recruitment (Nunn *et al*., 2010), with good recruitment in years with an above average number of cumulative degree days. The mean cumulative number of degree days above 12°C at Penton Hook over a 12 year period (1998-2009) is 185days. Figure 4.6 shows that 2000 and 2001 had low numbers of days above 12°C, with 23 and 14 days, respectively, less than the mean number of days above 12°C, and therefore may have resulted in low recruitment in cyprinids. Conversely, 2006 and 2007 both had 13 days more than the mean number of days above 12°C, thereby suggesting that these years may have resulted in higher recruitment in cyprinids.

4.4.2. Flow

Climate change models predict that there will be a decrease in rainfall in all seasons except winter, and that there will be a 20% reduction in annual rainfall by 2050 in the south-east of England (Arnell, 1998). Therefore another indication of whether or not climate change is already occurring might be a reduction in mean annual rainfall.

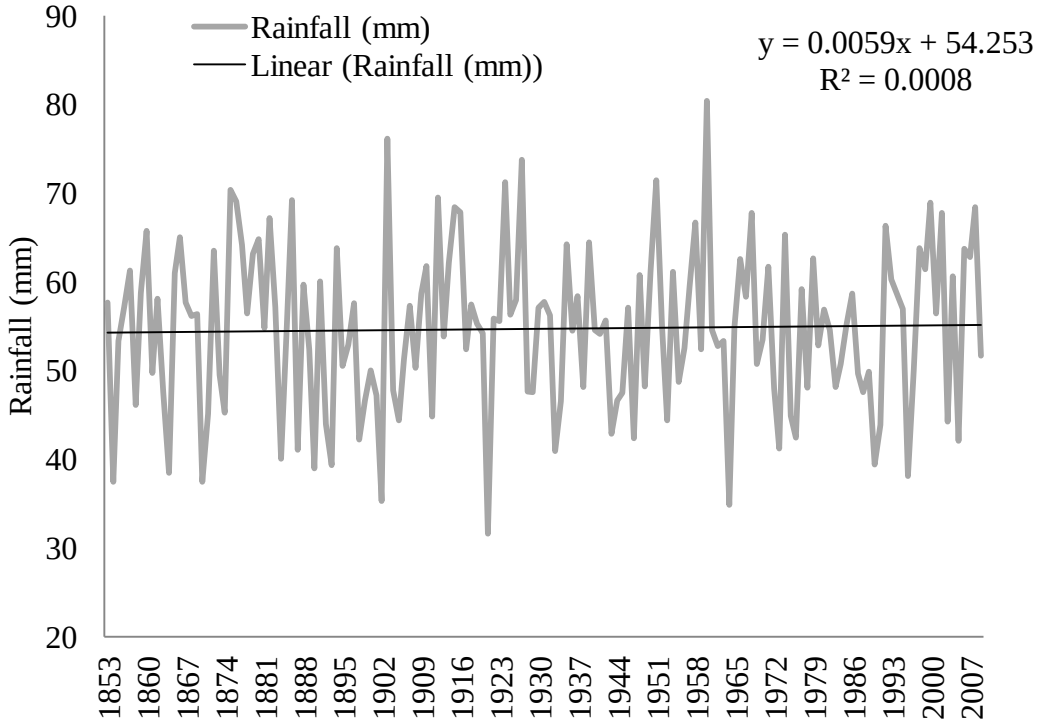


Figure 4.7 Mean annual rainfall at Oxford from 1853-2006 (data from Met Office).

Over the last 150 years there has been no significant change in the annual amount of rainfall received at Oxford. Rainfall is an important feature to rivers, since in general it governs the flow rate of a river (Nunn *et al*., 2007b). However, in the case of the River Thames, the rainfall has only a small correlation with flow rate, due to the fact that flow is highly regulated (Figure 4.8).

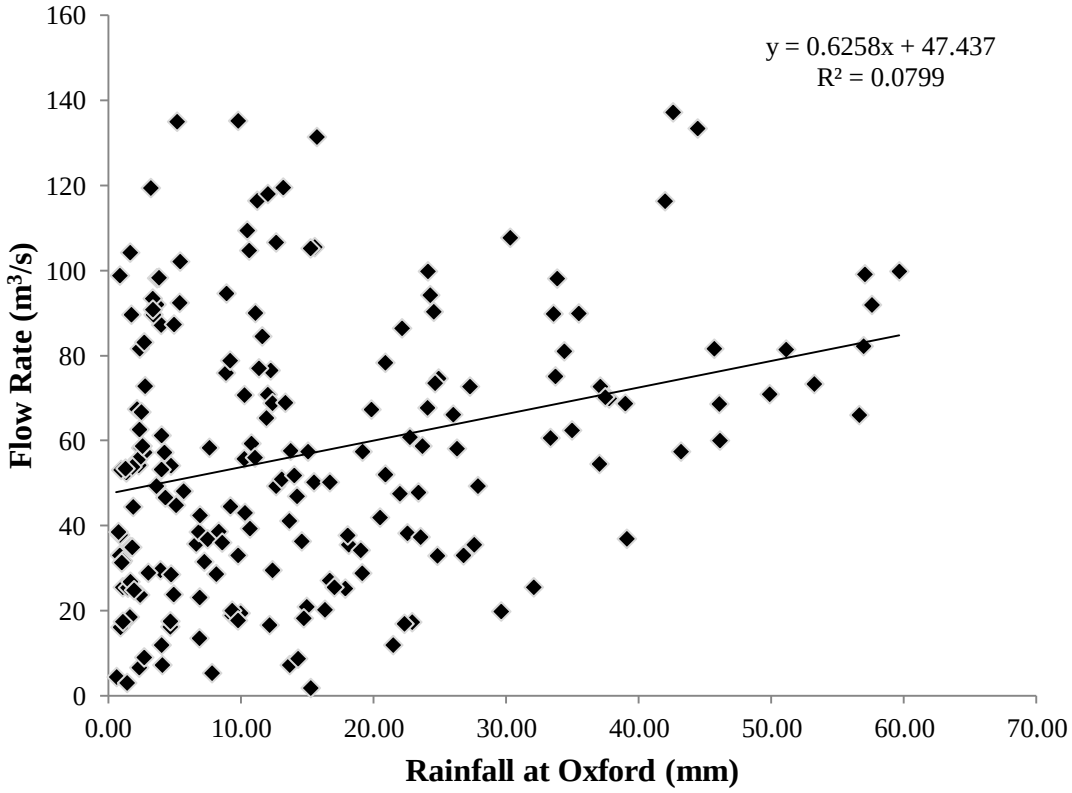


Figure 4.8. Demonstration of a small positive correlation between rainfall at Oxford and the corresponding flow rates at Farmoor in the upper reaches of the non-tidal River Thames from 1994-2010. Pearson's correlation coefficient: r=0.283, p=0.01, r^2= 0.0799 (two-tailed).

The mean winter and summer flow rates in the non-tidal River Thames are 63.6 and 13.9m^3/s, respectively. Whilst there is no clear long-term trend in flow rates in the non-tidal River Thames, there is a large degree of inter-annual variability in flow rates (Figure 4.9). In particular, there were very dry winters from 2005-2006, as depicted by low winter flow rates (24 and 40.6m^3/s, respectively), and also very wet summers in 2007 and 2008 (48.4 and 31m^3/s, respectively). In July 2007 there was widespread flooding, with the Thames region severely affected, with a mean flow rate of 73.9m^3/s, 5-fold higher than the average summer flow rate.

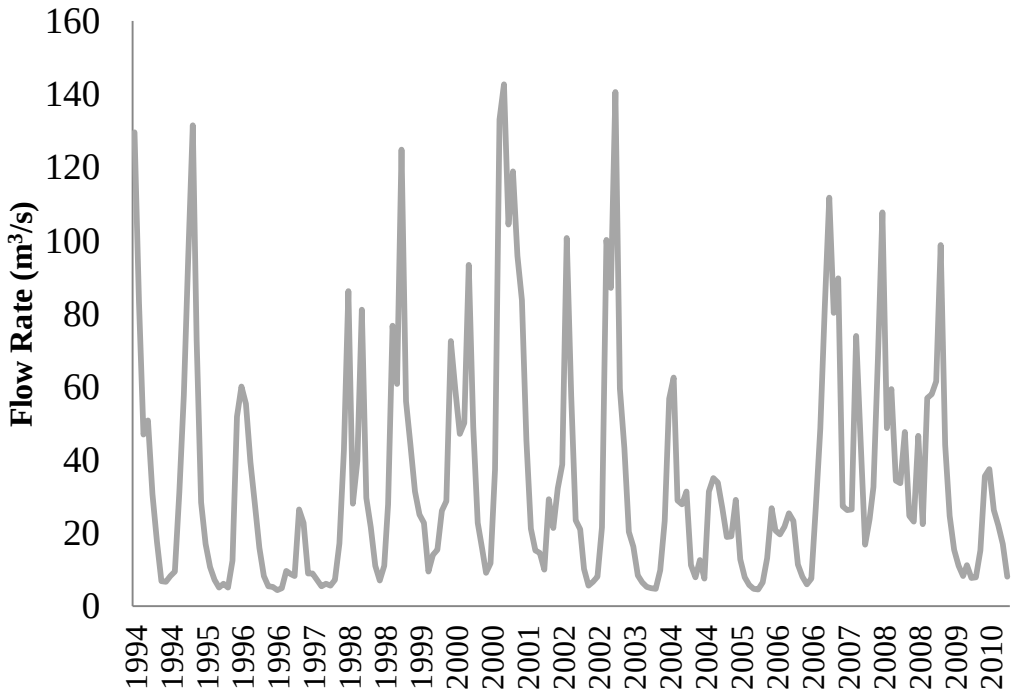


Figure 4.9. Mean monthly flow rates from all sites combined in the non-tidal River Thames from 1994 to 2010.

The changes to rainfall and hence flow rates (as shown in figure 4.8) are predicted to be different for different seasons. It is anticipated that there will be a reduction in rainfall in spring, summer and autumn, but an increase in winter. Using the monthly rainfall data from the Met Office from 1853-2009, regression analysis was applied to each month over all years to determine whether there have been changes to each month with time. Table 4.2 demonstrates that the only month which has had a significant change in rainfall is December, with an increase (+) in rainfall. There were no significant changes in rainfall for any other months; however, the general pattern appears to be an increase in winter and spring months (November-May) and a decline in summer and autumn months (June- October). These trends may become significant with time.

Table 4.2. Monthly trends in rainfall at Oxford from 1853-2009. Regression analysis was applied to the data for each month, with significances (P<0.05) denoted by *.

MONTH	$R_{2\ (RAINFALL)}$
January	0.0045 (+)
February	0.0004 (+)
March	0.0075 (+)
April	0.0016 (+)
May	0.0059 (+)
June	0.014 (-)
July	0.0241(-)
August	0.0015 (-)
September	0.0036 (-)
October	0.0079 (-)
November	0.0242 (+)
December	0.0262 (+) *

4.4.3. Responses of Fish to Physical Parameters

Data on fish density and biomass in the River Thames are only available from 1994 onwards when the Environment Agency replaced the National Rivers Authority. Whilst the archive data from the NRA would have been transferred, albeit in paper form, acquiring complete and reliable data was not possible despite many attempts. However, Williams (1967) recorded the densities of four commonly occurring fish in the non-tidal River Thames at Reading in 1959, which can be used as a comparison to data collected in recent years at the same site. These data are displayed in Table 4.3, and show that the densities seen in 1959 are not dissimilar to those seen today and also that there is a high degree of inter-annual variation in species densities. This is particularly evident for roach, where in 2003 the density was 0.7n/min^{-1} and in 2004 there was a 10-fold increase in numbers to 8.02n/min^{-1}. Given that the sampling technique catches +1year fish, it suggests that 2003 had favourable conditions, resulting in high densities of roach at Reading in 2004.

Table 4.3 Comparison of the adult population densities of four species of freshwater fish (roach, bleak, dace and perch) in the River Thames at Reading in 1959 (Williams, 1967) and between 2003 to 2008.

	Roach	Dace	Perch	Bleak	Total Density (n/min-1)
1959	0.58	0.1	0.1	1.59	2.37
2003	0.75	0.02	0.33	0.37	1.47
2004	8.02	0.24	0.6	0.91	9.77
2005	6.93	0.14	0.09	0.09	7.25
2006	2.3	0	0	0.07	2.37
2007	5.02	0.07	0.3	1.37	6.76
2008	1.32	0.01	0.17	1.12	2.62

The fish population in the River Thames is dominated by cyprinid species such as roach, dace, chub, bleak, common bream, gudgeon, tench and barbel. These species are known to have their population structure dominated by strong year

classes, which are governed by times of favourable environmental conditions. Figure 4.10 shows that there is a strong agreement between biomass and density and so in years where there are high numbers of cyprinids, there is also high biomass.

Figure 4.11 (A-C) compares the mean density of roach, chub and bleak over 15 years, and demonstrated that all three species show the same pattern. There was a high density of all three species (and all other cyprinids) in 1996 and 1997, followed by a decade of decline, followed by the start of a recovery in 2007. Electro-fishing techniques employed are size selective and so do not include the number of juveniles. Therefore the high densities in 1996 may reflect high recruitment in 1995 or earlier. The non-cyprinid species (pike, perch and the European eel) do not follow this same pattern of decline and recovery (Figure 4.12 A-C). Instead, each species has its own trend over time. Pike displays a stable density except for 2000, when there were very high densities. These high densities were seen in at least 5 separate sites and so are not thought to be due to sampling error, but rather a true representation of the pike density that year. Perch show a fluctuating pattern in density, with a downward trend with time. The European eel, which is thought to be absent from most European rivers (Feunteun, 2002), is clearly present in the non-tidal River Thames, with higher densities from 2000 to 2005.

Figure 4.10. Mean density (n/min^{-1}) and biomass (g/min^{-1}) of all cyprinids (roach, bream, chub, bleak, gudgeon, tench, dace and barbel) in the non-tidal River Thames from 1994 to 2009.

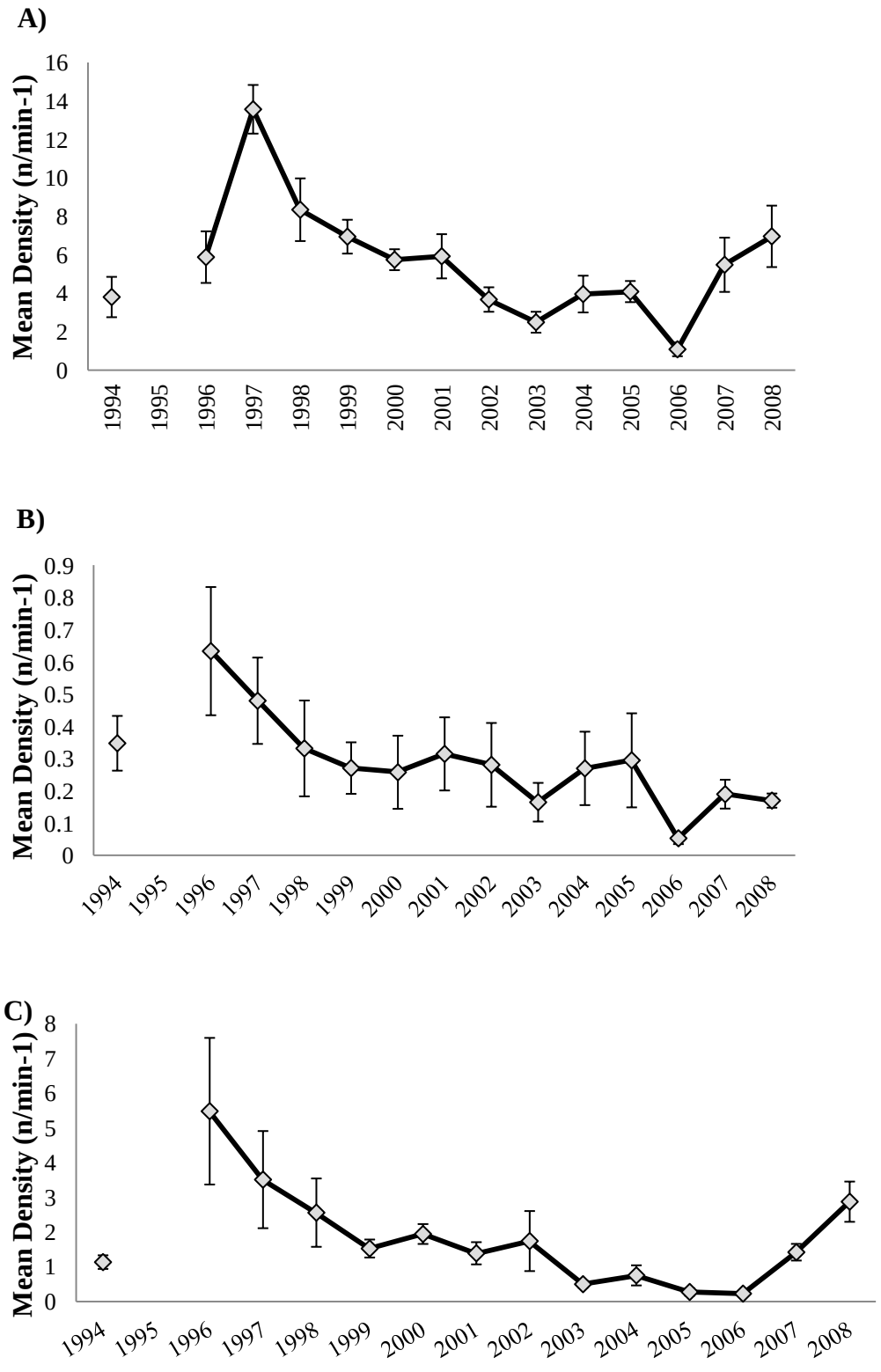


Figure 4.11. Mean densities of cyprinid species in the three reaches of the non-tidal River Thames from 1994 to 2008: A) Roach, B) Chub and C) Bleak. No data were available for 1995. Data reported as means ± SEMs.

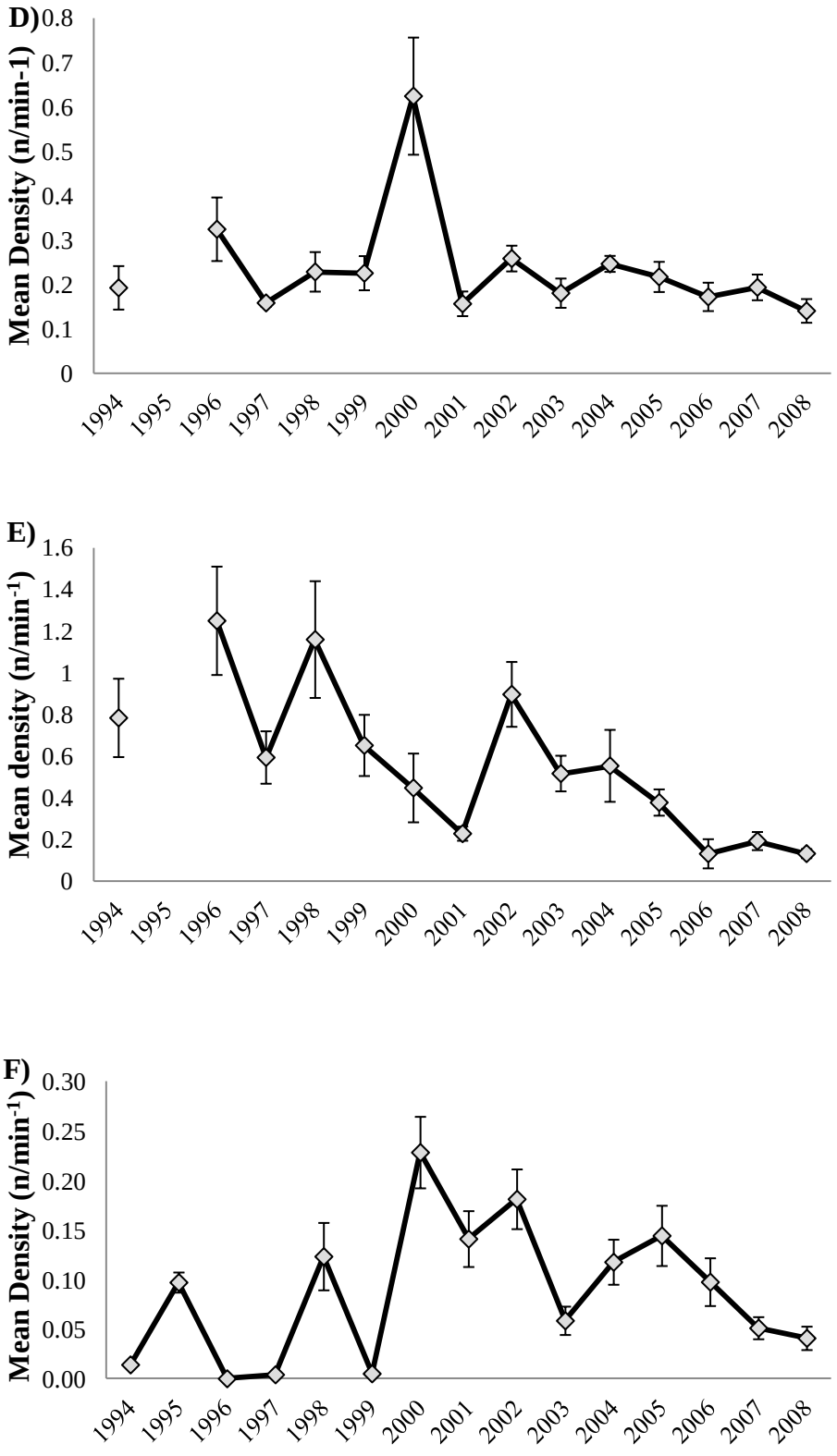


Figure 4.12. Mean densities of non-cyprinid species in the three reaches of the non-tidal River Thames from 1994 to 2008: D) Pike, E) Perch and F) European Eel. No data were available for perch and pike in 1995. Data reported as means ± SEMs.

Figure. 4.13 Shannon-Weiner diversity index, H' (solid line) and species richness, S (dotted line) for all fish recorded from all sites in the non-tidal River Thames from 1994 to 2009.

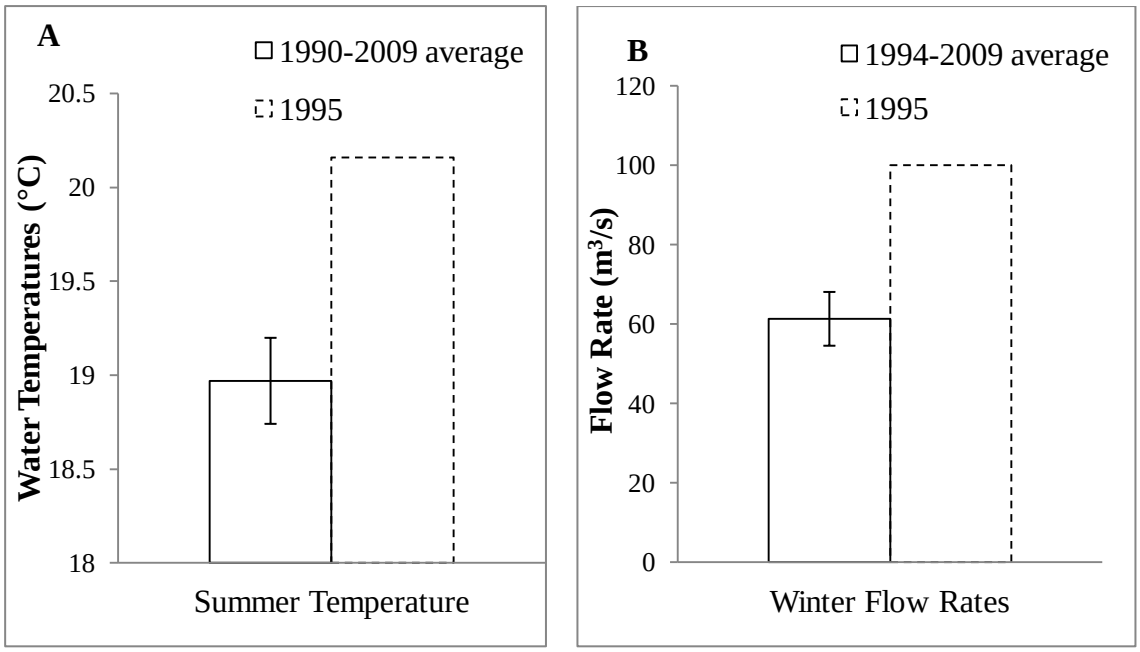
Species richness, S, refers to the number of species in a given area, or in this case the number of species recorded in EA surveys in any particular year. The Shannon-Weiner (H') index is a measure of the diversity of an ecosystem, it takes into account both richness and evenness. Typically values range from 0-4, with 0 indicating that all the species in a sample are the same and 4 indicating a highly diverse ecosystem. Figure 4.13 shows that the H' values for the non-tidal River Thames are characteristically low, with a mean of 1.01, indicating that the River Thames fish population is dominated by a small number of species. The H' values in Figure 4.13 included not only the 11 selected species (see section 4.2.2), but also any species that were recorded each year, even if in low numbers and therefore were excluded from the main analysis. In 1996 there was a peak in the H' to 1.57, suggesting that there was an increase in species diversity that year, but it was not sustained beyond 1996. This peak in H' coincides with the peak in cyprinid numbers (as seen in figure 4.11), suggesting that the conditions that were favourable for other

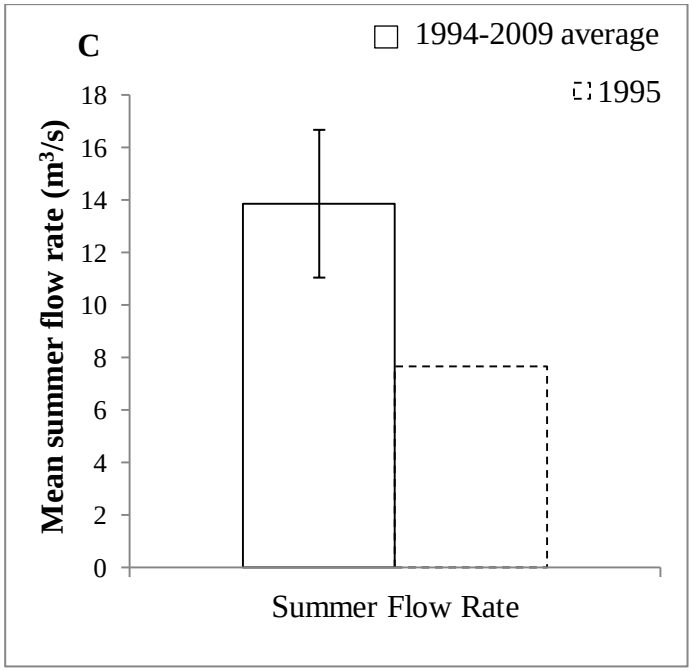
species, namely the cyprinid species Rudd (*Scardinius erythrophthalmus*) and Silver Bream (*Blicca bjoerkna*).

4.4.4 Causes of high cyprinid numbers in 1996-1997

Figure 4.10 shows that there was a peak in densities of the warm water cyprinids (chub, bleak and roach) in either 1996 or 1997, after which there was a rapid decline. Since the sampling technique only catches adult fish, it is important to consider the weather patterns from 1995, as it is the conditions in 1995 and 1996 which would have had the greatest impact on adult numbers caught in the subsequent years.

1995 can be generalised as a warm and dry year (there was a wet start to the year, with high flow rates, followed by a summer with temperatures higher than average) (Figure 4.14, A&B). However, 1995 experienced a dry summer with lower than average flow rates (Figure 4.14.C), which extended into the winter months and into 1996, which again had lower flows for the rest of the year (Figure 4.15). 1996 can be generalised as a cool and dry year (Figure 4.15A&B). There was markedly less rainfall in 1996, with flow rates only 58% of the 15 year mean (Figure 4.15B). The densities of all cyprinids were much lower in 1998, which could possibly be due to the extremely cold January in 1997 (Figure 4.16), with temperatures as low as -0.2°C. Given that this very cold spell followed on from a year with below average temperatures, these conditions could have meant high over-winter mortality due to reduced growth in the previous year.





Figures 4.14. A) Mean summer temperatures (June-August) of 1995 compared to the 1990-2009 average (±SEM bars). B) Mean winter flow rate (Jan-Mar) for 1995 compared to the 1994-2009 average (±SEM bars). C) Mean summer flow rate (June-August) for 1995 compared to the 1994-2009 average (±SEM bars).

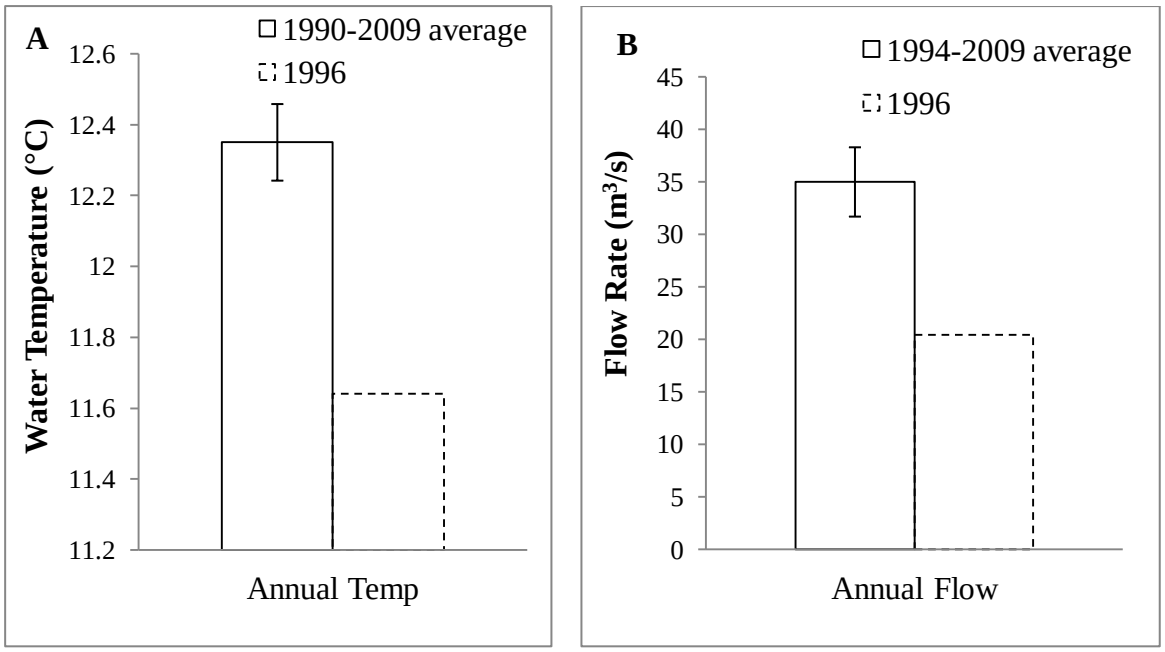


Figure 4.15. A) Mean annual temperature for 1996 compared to the 1990-2009 average (±SEM bars). B) Mean annual flow rate for 1996 compared to the 1994-2009 average (±SEM bars).

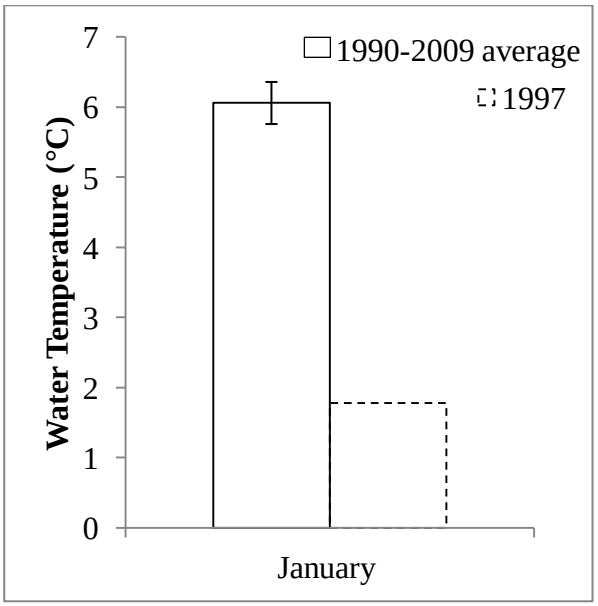


Figure 4.16. Mean water temperature for 1997 compared to the 1990-2009 average (±SEM bars).

4.4.5. Application of PCA and Bayesian Networks

A Principal Component Analysis (PCA) can be conducted on large datasets with multiple variables as a tool of data reduction to identify the key parameters or factors that are responsible for the majority of variation. A PCA was applied to the fisheries data from the non-tidal River Thames to determine the main factors controlling the density of fish. For this, physical data were grouped into seasons (winter: December to February, spring: March to May, summer: June to August, autumn: September to November).

Figure 4.17 is the Scree Plot obtained in SPSS (v15) for a PCA. Based on eigenvalues above 1, the scree plot identifies that there are 5 principle factors (Figure 4.17). These five factors account for over 96% of the variation in the data, and all variation is accounted for by 6 factors (Table 4.4).

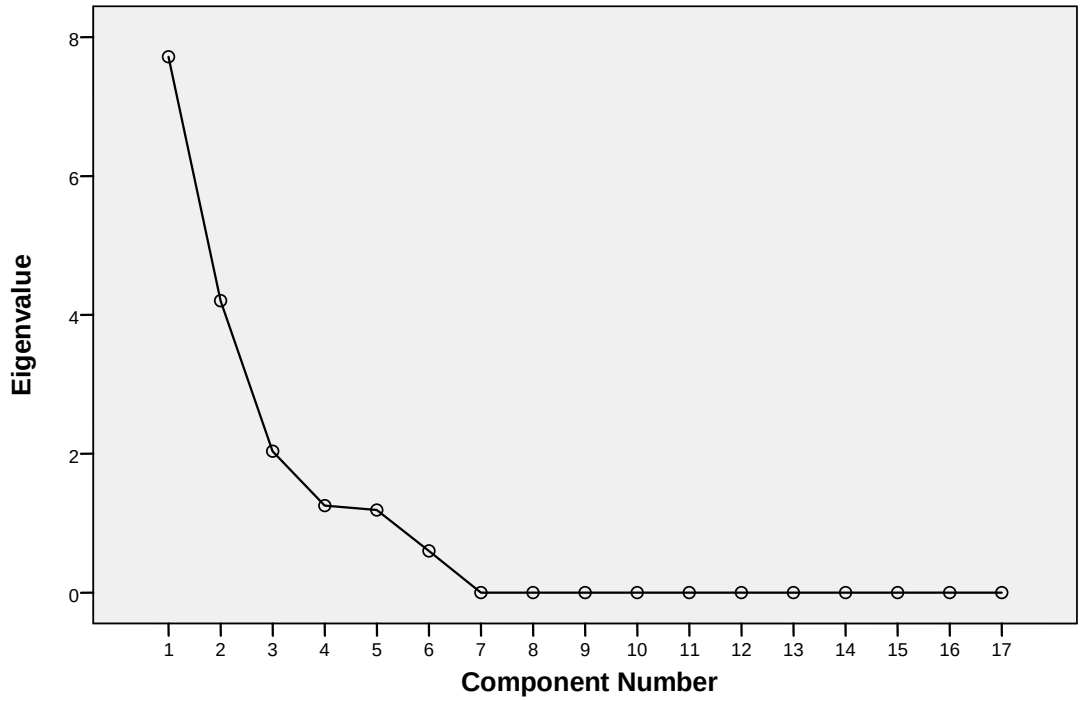


Figure 4.17. Scree Plot obtained in SPSS for a PCA based on 17 factors affecting density of fish in the Non-Tidal River Thames (seasonal temperature, flow, dissolved oxygen concentration, chlorophyll concentration). Based on Eigenvalues above 1, five principle components were identified (1-spring temperature, 2-autumn flow, 3-summer temperature, 4-autumn temperature, 5- spring flow).

Table 4.4. A Total Variance Explained table produced by SPSS for Principle Component Analysis. 100% of variance in the dataset is controlled by 6 components (1-spring temperature, 2-autumn flow, 3-summer temperature, 4-autumn temperature, 5-spring flow, 6- winter flow).

	Initial Eigenvalues		
Component	Total	% of Variance	Cumulative %
1	7.716	45.389	45.389
2	4.204	24.727	70.116
3	2.036	11.975	82.091
4	1.253	7.371	89.463
5	1.190	7.002	96.464
6	.601	3.536	100.000
7	1.61E-015	9.46E-015	100.000
8	8.86E-016	5.21E-015	100.000
9	4.06E-016	2.39E-015	100.000
10	2.37E-016	1.39E-015	100.000
11	1.18E-016	6.92E-016	100.000
12	5.59E-017	3.29E-016	100.000
13	-4.0E-017	-2.35E-016	100.000
14	-1.9E-016	-1.10E-015	100.000
15	-3.5E-016	-2.08E-015	100.000
16	-4.5E-016	-2.62E-015	100.000
17	-7.4E-016	-4.38E-015	100.000

The Kaiser-Meyer-Olkin Measure of Sampling Accuracy should be applied to each test in PCA in order to determine the appropriateness of factor analysis (i.e. whether the dataset lends itself to PCA). Values are always between 0-1, and it is considered that a KMO value of 0.6 is the minimum value at which the data are acceptable for PCA. Table 4.5 shows the output from SPSS for the KMO and Bartlett's test and given that the KMO value is 0.3, it is deemed that further statistical analysis in PCA is not appropriate. Therefore based on the dataset we have for the non-tidal River Thames, due to the large amount of missing data and noise within the dataset, a PCA cannot be accurately carried out with any confidence.

Table. 4.5 Output from SPSS for a Kaiser-Meyer-Olkin and Bartlett's Test of Sphericity. Rejection of PCA is recommended due to the KMO value being less than 0.6.

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.309
Bartlett's Test of Sphericity	Approx. Chi-Square	75.922
	df	36
	Sig.	.000

Similar to Principal Component Analysis, Bayesian Networks can apply a Feature Selection to identify the main controlling variables and discount variables which have little significance, in order to simplify a model. A Feature Selection is necessary when carrying out data mining analysis such as Bayesian Networks, as often data sets contain information that is not central to building a model. The Feature Selection therefore helps to identify the principal factors that are involved in the model, which can then be applied into building a Bayesian Network. However, unlike PCA, Feature Selections are able to make use of incomplete datasets and therefore are more accurate at identifying key factors within a dataset. The PCA identified 6 factors that accounted for all the variability in the data, whereas the Feature Selection identified 7 (split between 3 main principle factors). Further to this, it provided much more in-depth information about which fish species were affected by which parameters, giving much more ecologically significant results.

Table.4.6 Results from a Naive Bayes feature selection showing the three highest ranking variables affecting the density of fish species in the non-tidal River Thames.

Principle Features	**1**	**2**			**3**		
Physical Parameters	**Spring Temp**	**Summer Temp**	**Autumn Temp**	**Autumn flow**	**Winter flow**	**Spring flow**	**Summer flow**
Species	Roach	Roach	Roach	Pike	Bream	Pike	Pike
	Perch	Pike	Pike	Bleak	Tench	Barbel	Bream
	Pike	Barbel	Bleak	Barbel	Eel	Eel	Tench
	Barbel	Eel	Eel	Eel			
	Gudgeon						

The Naive Bayes Feature Selection (Table 4.6) revealed that spring temperature is the primary controlling factor influencing the density of five fish species in the River Thames. Indeed, temperature in all seasons except winter was most important, followed by flow, in all seasons, particularly in autumn. It also identified that dissolved oxygen and chlorophyll concentrations were not important factors in predicting fish density, therefore they were omitted from the subsequent analysis.

The Feature Selection also revealed that the relative importance of each of these factors was species-specific, with roach most affected by temperatures and bream most affected by flow. Therefore, whilst overall, temperature may appear to be the most important factor, the impacts of the physical environment appear to affect different species in different ways, and so there is perhaps no one physical parameter that is of primary importance.

For some species, such as perch and gudgeon, the only factor that seems to be important is spring temperature. The ways in which high or low, or early or late, spring temperatures affect perch and gudgeon can be explored in models produced by Bayesian Networks. Each variable is represented as a circular node in the model, and the relationships between nodes are shown by arrows, with the direction of the arrow indicating the direction of causality. Therefore, within the model, the circular nodes represent variables that are probabilistically and statistically related by a causal dependency. Within the model, each circular node is accompanied by a table showing a figure [-inf...] and a percentage. The number in the bracket represents the mean value for that node, i.e. for warm water cyprinid [-inf-2.5725] explains the mean density of warm water cyprinids was 2.57n/min^{-1}, and the mean flow rate was 86.475m^3/s in winter (1994-2009). The top line of the table represents values below the average, and the bottom line of the table representing higher than average values.

By applying the conditions seen in 1995 into the Bayesian Network, it correctly predicted that the following year there was a high density of warm water cyprinids (i.e. roach, chub and bleak) as displayed by a high percentage (87.5%) in the [2.89-inf]. Starting at step 1 (Figure 4.18), the model was adjusted to show 100% in [-inf-2.5725] for density of WarmWater Cyprinids. By placing the 100% in this top bracket sets the model to accept that in that year there was a lower than average density of warm water cyprinid species. The next step (2) is to manipulate the model to replicate the high winter flow rates that were experienced in Jan-Mar of 1995 (100% in [86.475-inf]), followed by a higher than average summer temperature (3) (100% in [18.872667-inf]. These manipulations of the network resulted in higher densities of warm water cyprinids the following year (T+1), with 87.5% probability of an above mean density (4).

What is also interesting to note is that perch is directly affected by the density of warm water cyprinids and the summer temperatures, and again the network correctly identified that the conditions seen in 1995 resulted in higher densities in 1996 (71% above the mean value for perch densities) (5), as also seen in Figure 4.12E.

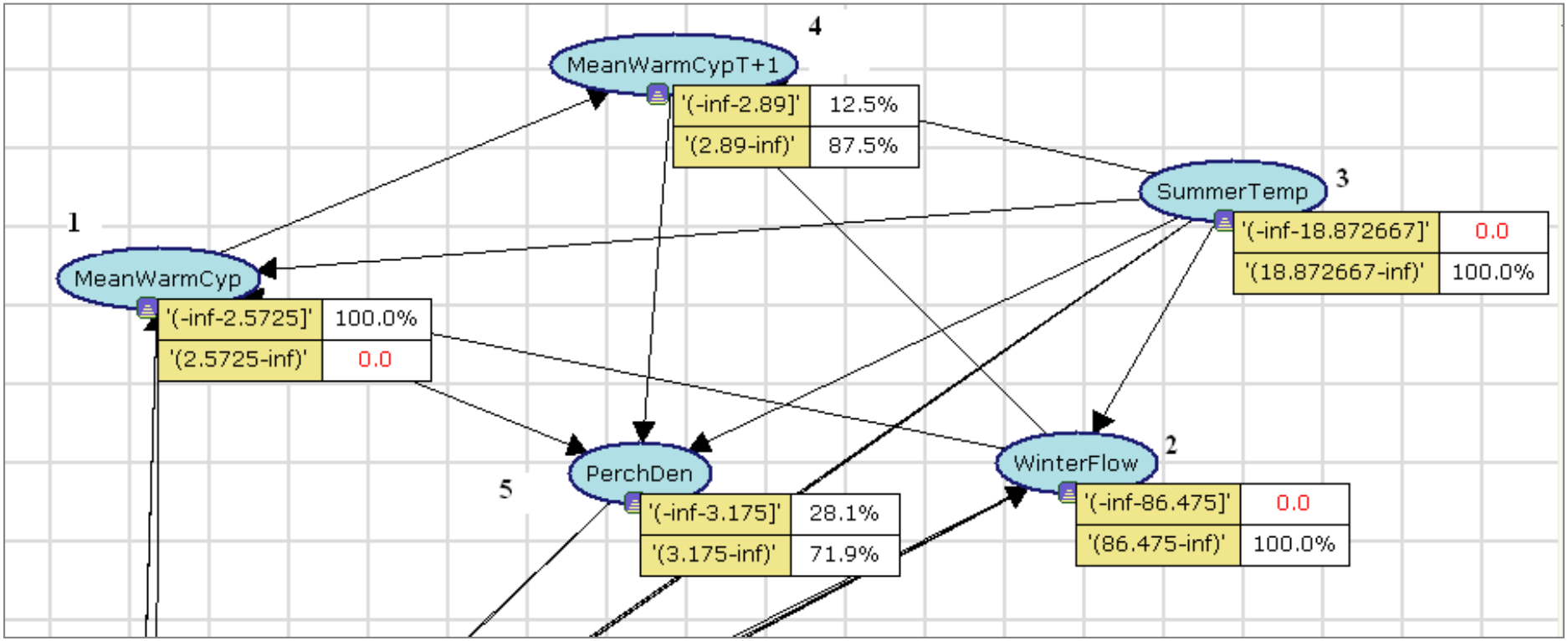


Figure 4.18. A screen capture from part of a created learnt Bayesian Network, whereby the parameters summer temperature and winter flow have been manipulated to represent the conditions experienced in 1995 to predict their effects on the density of warm water cyprinid fish. Each variable is represented by a node and the arrows connecting nodes represent the direction of causality and dependency. Other variables included in this dataset (e.g. spring temperature and flow etc) were not manipulated and therefore not included in screen capture.

4.5 Discussion

There is evidence for gradual warming in the Thames region, which over the last 150 years has seen a rise of 1.7°C in annual mean air temperature. If this rate continues, this could result in a mean annual temperature of 14°C by 2050, which is in line with current predictions (IPCC, 2007). Given that the mean global temperature has remained stable for over 10,000years (IPCC, 2007), this recent rise in temperature of over a degree is considerable.

Whilst this increase in temperature strongly suggests that fish in the River Thames have already been affected by climate change, there is no evidence that rainfall or flow rates have changed significantly over the last 150years, except in the month of December. There is still a lot of disagreement in the literature regarding what may happen to flow rates in the future, and this depends largely on what model is used to make the predictions. For the UK, Sefton & Boorman (1997) quote an overall increase in rainfall, whereas Arnell & Reynard (1996) predict no annual change in runoff; even though frequency of rainfall is reduced, they predicted an increase in intensity of rainfall events. Mulholland *et al.* (1997) quote that in Britain we can expect to experience a summer wet season and a winter dry season. However, in warm summer months, evapotranspiration may prevent the rainfall events increasing runoff. More recently, using improved modelling techniques, the predictions are for a reduced flow in all seasons and scenarios (Johnson *et al.*, 2009). The results from this study suggest that there is, in fact, higher rainfall in the winter months and a reduction in summer months. This study has also highlighted that the flow rates in the River Thames vary considerably from year-on-year, which agrees with the results of a study by Johnson *et al.* (2009). Furthermore, there is not a strong correlation between rainfall and flow rates in the River Thames, unlike most rivers, due to the fact that the River Thames is highly regulated. This agrees with previous research on the River Trent, which is also highly regulated (Nunn *et al.*, 2010). Therefore it is not likely that the long-term projections of changes in rainfall will have dramatic effects on the already variable nature of the flow rates of the River Thames.

The fish population has survived through decades of high flow variability. This may be an indication that any change in flow rates will not have a significant impact on the fish population of the River Thames. Elsewhere, declining river flows have been a major cause of loss of species in rivers (Xenopoulos *et al*., 2005). The Naïve Bayes Feature Selection showed that mean flow rates are of lesser importance than water temperatures in influencing fish populations in the River Thames. Since short flood events are thought to be less important in determining recruitment than the mean flow rates over a given period (Nunn *et al.*, 2010), it is likely that temperature will be the primary physical parameter controlling fish populations in the River Thames in the future. Whilst it appears that temperature is the primary controlling factor, it is prudent to be mindful of other variables that may not have been included in the dataset that may affect fish biomass and density, such as food availability, extreme weather events, invasive species, pollution spills and disease outbreaks. However, the Naïve Bayes Feature Selection highlighted that although temperature may be more important in general, different species may be affected by temperature in different seasons. The situation is therefore far more complex than merely stating that temperature or flow is the primary controlling factor, as has been done to date.

The biomass and density of the fish species investigated in this study showed some interesting trends. All the 8 species from the cyprinid family showed a high density in either 1996 or 1997, followed by a decade of decline to a low in 2006, and then they began to increase in 2007. Furthermore, this pattern was not reflected in any of the non-cyprinid species studied. The density of pike has stayed relatively stable, except for a high density in 2000. Perch density fluctuated year-on-year, with a steady overall decline. Whilst the European Eel, thought to be largely absent from European rivers now, showed its highest densities in the years when cyprinid densities were low.

Since the 1970's there has been a 99% reduction in the number of glass eels reaching the UK and the European eel is now a managed species (there is an Eel Management Plan in place) in an attempt to improve recruitment (Vogel, 2010). The reason for the large decline in the Eel population across Europe is not fully understood. However, it appears that there is a population of eels in the River Thames, although they are perhaps not present in the same numbers as prior to the

1980s. Furthermore, it appears that in years when weather conditions are not favourable for all the other species of the River Thames, the eel seems to thrive.

Cyprinid populations are known to be dominated by strong cohorts from years where conditions are favourable in the first year of life. Therefore the high density in 1996 may represent a particularly strong cohort, but it took a further 10 years for another strong cohort to dominate the population. Since the sampling technique only captures adults, it can be assumed that the strong cohort of 1996 was caused by environmental conditions that were favourable in 1995 or earlier. Favourable conditions for cyprinids are when the water temperatures are higher than average, i.e. years where the cumulative number of days above 12°C is above average (Nunn *et al.*, 2010) or when there is a warm-dry summer (Grenouillet *et al*.. 2001). This warm-dry summer was seen in 1995 (Figure 4.14), indicating that as climate change progresses and temperatures increase and summer rainfall decreases, there may well be an increase in the number of strong year classes of cyprinid species. Therefore, it is likely that climate change will benefit cyprinid species, particularly the warm water species such as roach, chub and bleak.

There is strong confidence that there was in fact a peak in biomass and density of the cyprinid species in 1996 and 1997, given that this same peak was seen across all the cyprinid species. However, given the limited data on variables other than temperature, flow and dissolved oxygen concentration, the reason for this apparent peak cannot be deduced. What cannot also be said with great confidence is whether the values for biomass and density in 1996/97 were in fact the peak, or that they were the norm, followed by a decade of decline. This is therefore a limitation to this study, in that a data-set of 15 years is not long enough to establish long-term trends. Similar studies on land have access to much longer data-sets, for example the National Butterfly Monitoring Scheme has been recording the appearance of butterflies across Britain since 1976 (Roy & Sparks, 2000). Even in these substantially longer data sets it has still been questioned whether they are of a significant amount of time to determine long-term trends and the effects of climate on them. The importance of continuing sampling and recording in rivers is therefore of paramount importance if we are to continue to investigate how fish respond to continuing changes in climate.

The Bayesian Network also correctly identified that perch densities were affected by the summer temperature and also by the density of warm water species. In the case of this study, as seen in Figures 4.11 and 4.12E, perch numbers were highest in 1996, when the numbers of warm water cyprinids were also high as a response to higher water temperatures. However, as roach numbers continued to increase into 1997, the numbers of perch rapidly declined, therefore agreeing with literature that roach is the stronger competitor and can depress the population of perch.

The literature emphasizes the crucial role of summer conditions, as these dictate the amount of growth that is likely and therefore how much overwinter mortality there may be. Whilst this is true, it is also prudent to take into account the winter conditions. A long hot summer followed by adverse winter conditions may still result in high overwinter mortality (Grenouillet *et al*., 2001), as was perhaps seen as a consequence of the particularly cold winter of 1997. This indicates that the extreme weather conditions, whether it is a particularly hot summer or indeed an unusually cold winter, may indeed have a greater impact than a general trend and the importance of the weather during the winter months should not be overlooked.

In all species there was a low in density in 2003, coinciding with the widespread heat wave across Europe in the summer of 2003. Whilst the cumulative number of days above 12°C was not particularly high (Figure 4.6), the days that were hot well surpassed mean summer temperatures. In that year the River Thames experienced high temperatures coupled with drought (seen in the low flows in figure 4.9). Surveys of fish density and biomass are carried out in the summer months. It is possible that fish had moved from the main river channel to cool water refugia (either deeper waters or under shady areas at the sides of the river) and so were not sampled by the electro-fishing technique, which only samples the middle of the river. Another possibility is that these unfavourable conditions did result in fish deaths in the summer months and so fewer were caught in sampling. Whilst warm-dry summers are usually good for recruitment, it may have been too severe and not only lowered recruitment but was also negative for the adult population of fish. Very high temperatures have been shown to reduce fish community biomass (Yvon-Durocher *et al*., 2010). While most British fish are well within their thermal tolerance limits (Arnell, 1998), the heat wave of 2003, when water temperatures reached 25.5°C,

produced water temperature near the upper physiological tolerance limits of many fish (e.g. pike [25°C], roach [27°C], tench [26°C] and perch [28°C]) (Webb & Walsh, 2004).

In the Rhone river, it was found that over a period of 20 years, chub and barbel (warm water species) replaced bleak and dace (cool water species) (Daufresne *et al*., 2003) (NB. bleak is considered a warm water species in Britain [Webb & Walsh, 2004]). The same pattern was not seen in the River Thames, with all species of cyprinids following the same pattern. This suggests that in the River Thames at least, there is not the inter-specific competition, where as one species is disadvantaged, another benefits. What negatively affects one species of cyprinid appears to negatively affect them all. And since this study suggests that the wet winters followed by a warm-dry summer (as predicted to occur with climate change) are beneficial for cyprinids, the River Thames may see an increase in all cyprinid species, therefore increasing total fish biomass. Since cyprinids are the main coarse fish species targeted by recreational anglers, climate change could prove to be beneficial, not just from an ecological standpoint but also economically, given the large income that anglers bring to the economy in the River Thames region.

The results from this data set only begin to elucidate how climatic conditions might affect the fish population of the River Thames. We still know relatively little about how conditions in relatively large rivers such as the River Thames affect the biomass and community structure of fish populations (Hughes, 1998). It has been stated that the general trend of higher recruitment in warmer, drier years no longer seems to apply, due to a southward shift in the Gulf Stream since the 1990s (Nunn *et al*., 2010). However, in this study, Nunn's recent findings did not hold true for the River Thames. Rather the established trend was evident suggesting that indeed warm-dry summers are important for recruitment. There has been a large variation in the density of all fish species in the River Thames over the last 15 years, but whether this is as a result of responses to climatic conditions or just normal fluctuations is not clear. Previous research has shown that many other important and dominant species in other rivers also display substantial variation with time (Araujo *et al*., 2000). Moreover, confidence in the accuracy and reliability of the dataset is questionable. In some species there is a 10-fold variation in density from year-to-year, and whilst this might be true, it cannot be ruled out that this is not an accurate representation. As

already stated, the fishing methodology is biased in that it only targets larger fish and, furthermore, only those swimming in the middle of the river. Therefore, fish that are either too small, located in weeds and plants at the sides of the river or indeed too deep will not be represented in the data set. To add to this, fish are mobile and not stationary at one reach from year-to-year, which may affect biomass and density values for each reach of the river.

Understanding the ways in which physical parameters affect the fish population is a complex task indeed, further clouded by the fact that in the River Thames, in particular, there is heavy re-stocking of most coarse fish species (Johnson *et al*., 2009). On top of a plethora of other variables that may affect the fish population, the interactions between the species themselves may also be extremely important. Therefore to fully grasp the array of interactions taking place, it is sensible to turn to modelling techniques such as Bayesian Networks that can mathematically quantify multiple variables and their interactions. In this way, the impacts of climate change can be more fully understood and therefore predicted. The Bayesian Networks produced in this study demonstrated that they can be successfully applied to fish populations and physical parameters, albeit only in initial trials. Therefore they may prove to be a powerful predictive tool for establishing how climate change may alter the community structure of fish in the non-tidal River Thames and beyond.

4.6 Conclusion

Whilst climate change is predicted to have its greatest effects in the northerly latitudes, such as Britain, very little is known about how British freshwater fish may respond to any changes. This study has provided evidence that there is already gradual warming in Thames region with a 1.7°C increase in mean annual air temperature. There has also been a statistical increase in mean precipitation in December over the last 150 years. However, due to the River Thames being highly regulated, there is not a strong correlation between precipitation and river flow rates. The results from a 15 year dataset conclude that the flow rates in the River Thames are highly variable over time and show no clear trends or patterns that might be expected with climate change. Therefore, it is unlikely that the long-term projections of changes in rainfall will have dramatic effects on the already variable nature of the flow rates of the River Thames.

Over the more recent past, there have been a number of extreme weather events, such as record high summer temperatures (2003) and high flood events (summer 2007). The fish inhabiting the non-tidal River Thames have displayed different responses to the changes in their physical environment. All cyprinid species, such as roach, dace and bream displayed a similar pattern in density and biomass over a 15 year time period. All the 8 species from the cyprinid family showed a high density in either 1996 or 1997, followed by a decade of decline to a low in 2006, and then they began to increase in 2007. However, this pattern was not followed by non-cyprinid species, such as perch, pike and the European Eel. The density of pike has stayed relatively stable, except for a high density in 2000. Perch density fluctuated year-on-year, with a steady overall decline. Whilst the European Eel, thought to be largely absent from European rivers now, showed its highest densities in the years when cyprinid densities were low. These results demonstrate, that there will likely be interfamilial differences in the responses to climate change, and that conditions that are favourable for one cyprinid species, may well be suitable for all cyprinid species.

This study also investigated the potential for Bayesian Networks to be applied to complex ecological datasets relating to aquatic habitats. The Bayesian Networks were able to correctly identify key relationships in the data, both between fish species and their environment and also key interactions between different species. Therefore, Bayesian Networks may be a useful tool in predicting the impacts of climate change on freshwater ecosystems. The networks also indicated that cyprinid species may benefit from the warm-and-dry summers that are predicted to become typical with climate change. Since it appears that all cyprinid species have similar trends in biomass, these results provide hope that cyprinid species, at least, may cope with the predicted changes.

Chapter 5. Assessing the effects of chronic thermal stress on the stickleback *Gasterosteus aculeatus*.

5.1 Abstract

The effects of warming waters as a result of climate change on the freshwater fish population of Britain remains largely unknown. Models predict that air temperature could rise between 2 and 6°C by the end of the century. However, the responses of freshwater fish in Britain to these increases and the mechanisms behind responses are little understood. This study provides evidence that small chronic increases in temperature of only 2-6°C can elicit a stress response at the biochemical, cellular and whole organism level in a species of fish native to Britain. This study examined the effects of chronically elevated water temperature (realistic of anticipated climate change) on the stress response system of the three-spined stickleback, *Gasterosteus aculeatus*, using a Biological Indicator Approach. A small increase of 2°C (above current summer mean water temperature) resulted in a stress response at the cellular (higher neutrophil: lymphocyte ratio) and whole organism level (lowered condition factor and growth rates). A 6°C rise in temperature resulted in a stress response at the biochemical level (higher cortisol and glucose concentrations), as well as the cellular and whole organism level. These stress responses will ultimately lead to impacts at the population and community level. *G. aculeatus* is considered to be temperature tolerant and resilient species, and therefore these results indicate that climate change may indeed prove to be stressful for these and less hardy species.

5.2. Introduction

Very little is known about whether increases in temperature predicted by climate change models may prove to be stressful for British freshwater fish. Most studies to date have examined the effects of warming at the species and population level (Shuter & Meisner, 1992; Webb & Walsh, 2004). However there is a distinct

lack of information of the molecular and physiological mechanisms that may be affected by climate change. Of the few studies carried out at the individual level, the stress has either been acute or higher than anticipated with climate change (Brian *et al*., 2008; Currie *et al*., 2008; Perez-Casanova *et al*., 2008), thereby offering limited ecologically significant information. The Inter-governmental Panel for Climate Change (IPCC) published a Special Report on Emissions Scenarios (SRES) which provides estimates for temperature in the coming century (IPCC, 2007). The highest projections are for a 6.4°C increase in temperature above the current mean (Table 5.1). Since the Thames is dominated by cyprinid and percid species, increases of up to 6.4°C may well not reach the lethal limits for most species. However, even if lethal limits are not reached, that is not to say that chronically elevated temperature may not act as a stressor, and have adverse effects.

Table 5.1 Projected global average surface air temperature increases by the end of the 21st century (as compared to the 1980-1999 average) (taken from IPCC, 2007)

Case	Temperature change (°C at 2090-2099 relative to 1980-1999)	
	Best estimate	*Likely* range
Constant year 2000 concentrations	0.6	0.3 – 0.9
B1 scenario	1.8	1.1 – 2.9
A1T scenario	2.4	1.4 – 3.8
B2 scenario	2.4	1.4 – 3.8
A1B scenario	2.8	1.7 – 4.4
A2 scenario	3.4	2.0 – 5.4
A1FI scenario	4.0	2.4 – 6.4

The mean summer temperature (June-Aug) for the non-tidal section of the River Thames is 18.9°C (1990-2009 average, data collected from EA). The mean summer temperature over this time period has ranged from an average of 16.9°C in 1993 to a high of 20.6°C in the summer of 2003. SRES B1 is based on a greenhouse gas concentration of CO_2 of 600ppm by the end of the century (currently it is at 379ppm [IPCC, 2007]). This is the lowest emissions scenario with a convergent

world based on a service and information economy, whereby carbon dioxide emissions will increase slightly in the next few decades. This is by far the most optimistic scenario; however it will possibly result in global temperatures increasing by up to 3°C and by a mean of 1.8°C by 2100 (Table 5.1). Working on a basis of a 2°C increase above the current day summer mean on 19°C brings predicted summer water temperatures to 21°C. SRES A1F1 scenario is based on a fossil fuel intensive economy, with greenhouse gas emissions at 1550ppm by 2100. Therefore this scenario predicts a nearly four-fold increase in emissions and could lead to global temperatures increasing by 6.4°C. Using a 6°C increase, this would bring the average summer temperature to 25°C.

Air temperature and water temperature follow an S- shaped function. A linear relationship exists between air and water between 5°C and 25°C. Above 25°C evaporating cooling means water temperature increases at a slower rate than air temperature (Mohseni, *et al.*, 2003). However, the mean range of water temperatures likely to be experienced with climate change does not go beyond this 25°C, and so it can be assumed that the increase will be linear. That is to say, a 1°C increase in air temperature will lead to roughly a 1°C increase in water temperature.

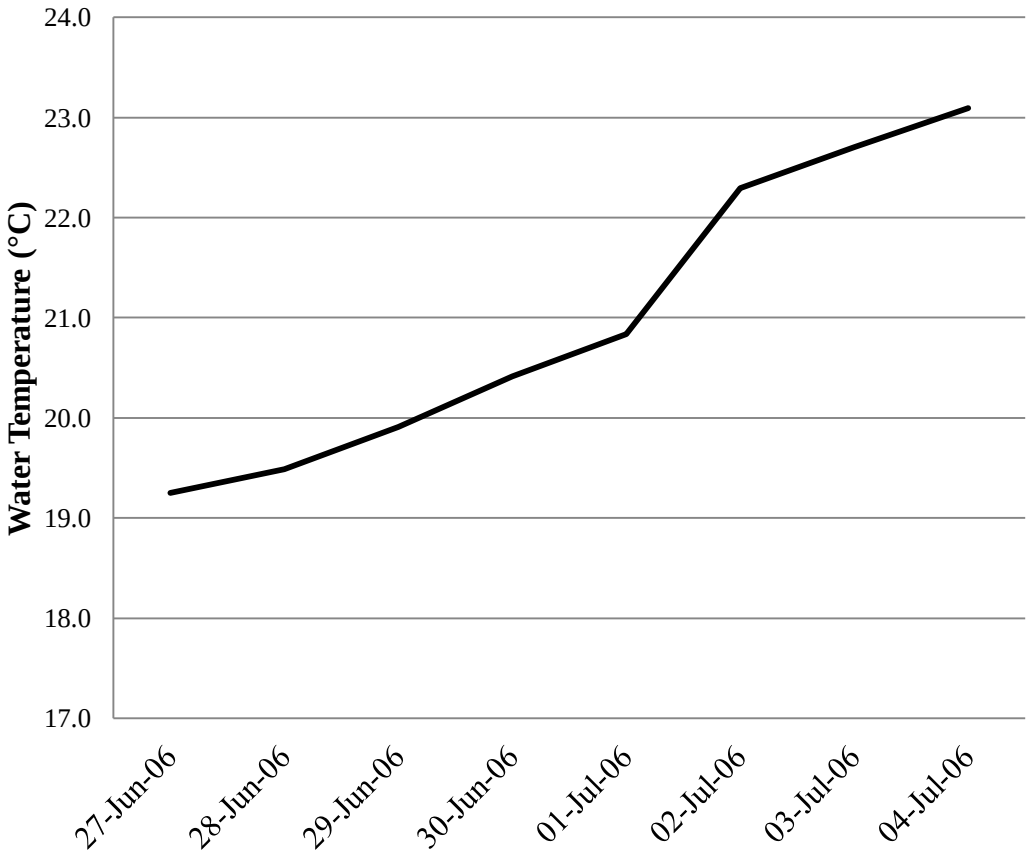


Figure 5.1. Water temperature at Penton Hook on the River Thames showing a 4°C rise in temperature over an 8-day period (June- July) in 2006 (Data obtained from the Environment Agency).

Although the temperature regimes of 19°C, 21°C and 25°C are based on what is expected to become typical for summer temperatures based on best and worst case scenarios, these temperatures already occur in the River Thames. These high temperatures may not persist for a sustained period of time, which is what is predicted with climate change. Nevertheless, fish in the River Thames can be exposed to temperatures as high as 23°C and also experience rapid increases in temperatures. Figure 5.1 shows that over a relatively short time scale (8 days), the water temperature at Penton Hook in the non-tidal River Thames increased from 19.3°C to 23.1°C. Therefore, regardless of climate change predictions, these extreme high temperatures that already occur could pose a stress to the fish of the non-tidal River Thames.

Few studies have yet investigated whether these smaller increases in temperature will have any significant effects on fish. A study by Moran *et al*. (2010) tried to replicate the potential climate change scenarios by exposing the three-spined stickleback (*Gasterosteus aculeatus*) to a 4°C temperature increase above ambient temperature. This study showed that even a robust species like the three-spined stickleback was greatly affected by this increase in temperature, with a 60% reduction in biomass. Whether this reduction in stickleback biomass was a result of increased temperature, decreased dissolved oxygen concentration, or a combination of both, was not clear. However, the study did highlight how even small increases in temperature, well within the realms of climate change, may cause significant sub-lethal effects on British fish.

The three-spined stickleback (Figure 5.2) has been used extensively in research over the years, firstly as a model species for studies on evolution and speciation and more recently as a sentinel for assessing the effects of endocrine disrupting chemicals. It also lends itself to studies on climate change; being small, robust and ubiquitous in European waters, including the River Thames. It is generally not considered to be overly sensitive to environmental perturbations, thus allowing measurement of a range of sub-lethal endpoints. It is also one of the few fish species whose whole genome has been sequenced, allowing endpoints at the genetic level to be assessed. The biology of *G. aculeatus* is well understood and has been shown to be suitable for laboratory studies. Other commonly occurring species in the River Thames, such as the Roach (*Rutilus rutilus*) and Dace (*Leuciscus leuciscus*), have been shown to become agitated and stressed by laboratory experimental conditions, such as the presence of humans feeding the fish and cleaning tanks (Brunel University experience). *G.aculeatus* quickly adapts to new situations and after a few days of confinement can become accustomed to the presence of people.

Figure 5.2. Image of a three-spined stickleback, *Gasterosteus aculeatus.* (Picture obtained from http://fishbase.us/photos/PicturesSummary [date accessed: 31st Jan 2013]).

It is important to understand the ways in which chronically elevated temperatures may elicit a stress response, in this instance, in the three-spined stickleback, *G.aculeatus*. Stress can lead to reduced growth, reduced reproduction and increased susceptibility to disease (Adams, 1990). Therefore even though an increase in temperature may not surpass the zone of tolerance for most fish, it can still be detrimental to the health of the individual, leading ultimately to changes at the population level. In order to investigate the impact of sub-lethal stress, a Biological Indicator Approach (Adams, 1990) was adopted, whereby a variety of stress indices were used: biochemical (cortisol and glucose concentrations), cellular (white blood cell counts) and whole organism responses (hepatosomatic index, condition factor, ventilation rate and growth rate). The Biological Indicator Approach uses indicators of stress at each level of biological organisation to assess the overall health of the organism. No single indicator can provide a complete insight into the extent of stress, but the probability of detecting stress is increased if several indicators from primary cellular responses to whole organism responses are used in conjunction with one another (Adams, 1990). The results from these biological endpoints will indicate the overall health status of the individuals at each temperature regime and allow predictions of the effects of the stress-induced changes.

5.2.2 Biochemical Responses

5.2.2.1 Cortisol

The hormone, cortisol, has been used in many studies as an indicator of stress. Cortisol is produced by the interrenal tissue in the head kidney in response to Adrenocorticotrophic Hormone (ACTH), and is one of the first responses an organism will have to stress. Cortisol has several purposes. Firstly, it helps shunt energy away from non-essential activities, i.e. digestion, excretion and growth. Secondly, the presence of an elevated cortisol concentration in the blood initiates glycogenolysis and gluconeogenesis to release stored glucose in liver and muscle, thereby providing more energy to resist the stressor and regain homeostasis (Martinez- Porchas *et al*., 2009). Whilst in the short-term cortisol has a positive effect, prolonged cortisol production can have serious implications for the health of the fish. Since it diverts energy away from non-essential activities, it can result in reduced growth rates, lowered reproductive ability and a suppressed immune response (Thomas, 1990).

Measuring the levels of circulating cortisol is one of the earliest indicators of stress (Thomas 1990) and the traditional method for obtaining the cortisol concentration is through sampling the plasma. Plasma cortisol concentrations have been shown to be reliable and highly temperature sensitive (Perez- Casanova *et al*., 2008). However, elevated cortisol concentration is a generalised stress response, and so it is also occurs in times of experimental and handling stress (Adams, 1990). Obtaining the cortisol concentration in plasma requires netting the fish, exposure to air and blood collection; all of which would result in a stress response, not just of the fish being sampled, but also any other fish in the tank (Scott *et al*., 2008). Therefore acquiring reliable measures of plasma concentrations in fish is problematic. For small species, and therefore small blood volumes, this means only one measurement can be made at the end of the experiment, upon termination of the individual. Recent studies have shown that fish release steroids into the water via their gills (Ruane & Komen, 2003; Scott & Ellis, 2007), allowing for cortisol concentrations to be determined from water samples. These water concentrations are not only directly

proportional to that of the plasma concentrations, but they are also detectable by analysis by using radioimmunoassays (RIA) (Ellis *et al*., 2007) and enzyme-linked immunosorbant assays (ELISA's) (Fanouraki *et al*., 2008). Measuring cortisol concentrations in the water rather than the blood has many advantages. It permits the same individuals or populations of fish to be monitored over time, thus allowing an assessment of the effects of chronic stress (Scott & Ellis, 2007). Although cortisol is thought to be a better indicator of acute stress, chronic stress can also result in prolonged elevation of the plasma cortisol concentration. Given that it is a non-invasive technique, water cortisol concentrations do not reflect any cortisol released due to handling stress, hence better representing the magnitude of stress caused by temperature alone (Sebire *et al.,* 2007).

The movement of cortisol from blood to water is thought to be via passive leakage, and studies have validated the premise that the concentration of free steroid present in the water is closely correlated to the concentration of physiologically active steroid in the plasma (Scott & Ellis, 2007). However, the release rate will never be exactly the same as the plasma concentrations, as it can vary depending on factors such as gill surface area, changes to gill permeability, steroid lipophilicity, and conversion of free steroids from one steroid to another (Scott *et al*., 2008). The body mass of the fish, the volume of water and the flow rate may also affect release rate (Scott & Ellis, 2007), therefore it is essential to have the same mass of fish in each tank and also the same flow rates. There can also be differences in steroid release rates depending on the maturation of the individual, with mature three-spined sticklebacks releasing higher amounts of cortisol than immature individuals (Sebire *et al*., 2007). However, this species did not show a difference in cortisol concentrations between the sexes (Sebire *et al*., 2007). For that reason, it may not be crucial to know the sex of the individuals, but the life stage may be important.

In assessment of chronic stress using cortisol, it is important to sample the water at the same time of day each time, since cortisol concentration follows an endogenous diurnal rhythm, being higher at night than the day (Lorenzi *et al*., 2008). The pattern of cortisol release in the day depends on the species and has been shown in several studies to peak after the morning feed (Lorenzi *et al*., 2008).

Despite these variations in cortisol release rates between species, life stages and daily cycle, cortisol concentration is still considered to be a key biological indicator (Martinez-Porchas *et al*. 2009). Water cortisol concentrations have been calculated for many fish species and validated against plasma cortisol concentrations, including the three-spined stickleback (Sebire *et al*., 2007), Atlantic salmon and rainbow trout (Ellis *et al.*, 2007), ensuring that this non-invasive technique for assessment of cortisol status is a reliable indicator of stress.

5.2.2.2 Glucose

Glucose is a simple monosaccharide and is the most important carbohydrate in the body, as it is the primary source of energy. Glucose levels are tightly controlled, since large deviations from the basal rate can result in conditions such as hyperglycemia (persistently high levels) or hypoglycaemia (persistently low levels). Normal human glucose levels range between 3.6-5.8 mmol/L (or 64.8-104.4mg/dL). A study by Martinez-Porchas *et al*. (2009) compared literature on the basal and post-stress glucose concentrations of 14 species of fish. It reported basal concentrations of between 0.17mmol/l in Atlantic Cod (*Gadus morhua*) and 6.1mmol/L in the Sunshine Bass (*Morone chrysops x saxatis*).The highest post-stress glucose level recorded for these species was for sunshine bass at 10.5mmol/L, a 1.7-fold increase above the basal level. The largest fold-increase was for the Emerald Rockcod (*Trematomus bernachhii*), being a 5-fold increase (1.5mmol/L pre-stress to 7.5mmol/L post-stress). The amount of glucose circulating in the blood is elevated in times of cortisol production due to glycogenesis and glycogenolysis by the liver and muscle (Martinez-Porchas *et al*., 2009). This additional glucose in the blood ensures that the fish has adequate circulating energy substrate to restore and maintain internal homeostasis (Martinez- Porchas *et al*., 2009; Perez-Casanova *et al*., 2008). The use of blood glucose concentrations as a biological indicator has received contradictory reviews. Glucose levels have been shown to provide variable results for studies on Atlantic Cod (*Gadus morhua*) (Perez- Casanova *et al*., 2008), and it has been suggested that glucose concentration responds more to acute than chronic stress (Adams, 1990). However in some studies with chronically stressed fish, there were higher levels of basal plasma glucose (Barton *et al*., 1987). Experimental

stresses such as over-crowding and handling stress (Adams, 1990) can also increase glucose concentrations. However, blood glucose has been reported to be a sensitive, reliable indicator of environmental stress (Silbergeld, 1974) and it is still one of the most commonly measured changes when investigating stress responses in fish (Iwama *et al.*, 1998) and generally shows little variation in the basal levels (Silbergeld, 1974). Although not suitable as an isolated indicator, as is true for most indices of stress, when used in conjunction with other biological indicators, it may provide further insights into the early responses to stress and support data from other biological indicators employed (Martinez-Porchas *et al.*, 2009).

5.2.3 Cellular Stress Responses: White Blood Cell Counts

Fish have well developed immune systems, an important characteristic given that fish are continuously exposed to pathogens in the aquatic environment (Schrek, 1990). The white blood cells, or leukocytes, provide the first line of attack and defence. The proportions of each white blood cell subtype are affected by stress. Fish blood contains 5 types of white blood cells: neutrophils, lymphocytes, eosinophils, monocytes and basophils, with the first two comprising over 80% of white blood cells (Figure 5.3). Neutrophils are primary phagocytic white blood cells and proliferate in times of infection, inflammation and stress. Lymphocytes, of which there are several sub-types (T-cells and B-cells), are involved in several immune functions, such as immunoglobin function (Davis *et al.*, 2008).

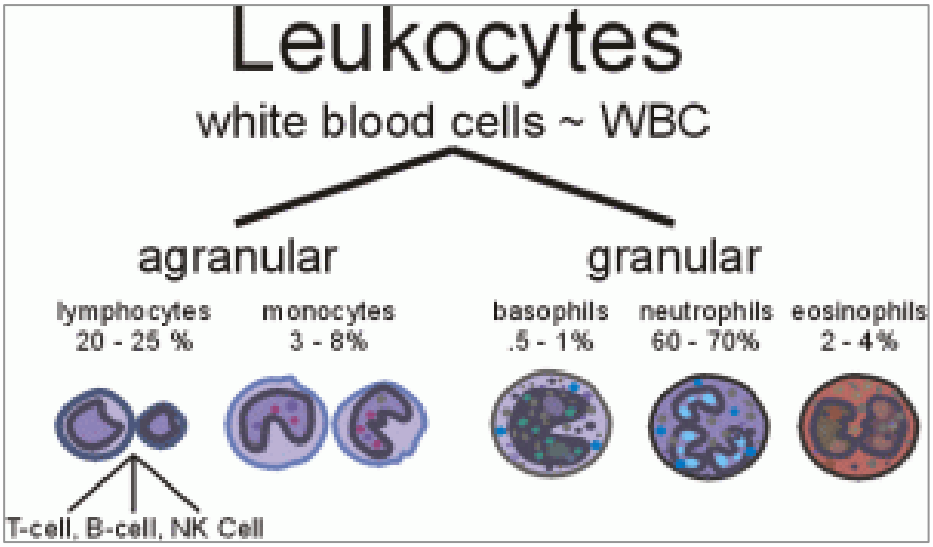


Figure 5.3. Generalised model of white blood cell types and proportions. (Image obtained from http://whiteblood-cells.net/types-of-white-blood-cells/ [date accessed 31st Jan 2012]).

Stress is known to affect the immune system and in some cases can be immunoenhancing in the short run. However, chronic stress, through prolonged elevated cortisol levels, can be immunosuppressive (Dhabher 2002) by altering the composition and proportions of leukocytes (Anderson, 1990; Dhabhar, 2002). Assessment of the immune system can be used as a powerful stress indicator (Schrek, 1990), particularly using the Neutrophil: Lymphocyte ratio (N: L ratio).

In times of stress and production of cortisol, the number of neutrophils circulating in the blood increases, a response known as Neutrophilia. This is due to an influx of neutrophils from the bone marrow into the blood. At the same time, there is a decrease in the number of lymphocytes in the blood, a response known as Lymphopenia. This is not due to destruction of lymphocyctes, but rather to redistribution or 'trafficking' of lymphocytes from the blood into other components such as the skin, lymph nodes and bone marrow (Davis *et al*., 2008; Dhabhar, 2002). This is thought to occur so that there is an increase in the number of lymphocytes in the organs that are likely to be susceptible to an infection. The blood N: L ratio can therefore provide a good indication of the levels of stress of an individual compared to another individual, with a higher N: L ratio indicating higher stress. Whilst these N: L ratios are becoming increasingly recognised as powerful indictors of stress, care must be taken in interpretation of this indicator. Unless they are used in conjunction

with a disease test, the immune-competence or how well an individual may be able to fight an infection cannot be confidently predicted (Davis *et al.*, 2008).

The leukocyte profile (the N: L ratio) is a longer lasting indicator of stress than that of cortisol, with the response lasting hours to days rather than minutes to hours. If the cortisol concentration is chronically elevated, it can lead to long-term elevation of the N: L ratio, such as is seen in humans suffering from Cushing's disease (Davis *et al.*, 2008).

It is thought that higher environmental temperatures enhance specific immune responses, whereas lower temperatures are thought to be immunosuppressive (Le Morvan-Rocher *et al.*, 1995). If climate change-induced warming of water itself acts as a stressor, one can assume that higher temperatures will result in a higher N: L ratio. A study by McFarlane & Curtis (1989) showed that when chickens were exposed to chronically elevated temperature, the cortisol levels returned to basal rates after 7 days (suggesting acclimation) but the leukocyte response was more enduring. This study demonstrates that leukocyte profiles are potentially a more reliable indicator of chronic stress than the cortisol concentration, and that higher temperature can be immunosuppressive rather than immuno-enhancing.

Leukocyte profiles can therefore provide a wealth of information as to whether fish will find warmer waters, representative of climate change, a stressor itself or a benefit to aid the immune system. Moreover, leukocyte profiles have also been shown to be reliable indicators of future performance and viability, as summarised by Davis *et al*. (2008), therefore providing potentially telling information as to the likely success of certain species currently found in Britain as water temperature rises.

5.2.4. Whole Organism Responses

5.2.4.1 Ventilation Rate

As waters warm, the metabolic rate of a fish increases and so oxygen requirements are greater. In order to meet the higher oxygen demands, respiration

rates also increases. Warmer water also holds less oxygen, and this under-saturation coupled with increased demand leads to the 'Temperature Oxygen Squeeze' (Pörtner & Knust, 2007). Therefore higher temperatures can act as a stressor by limiting the amount of available oxygen. Ventilation rate can be used as a very sensitive indicator of stress in fish; however, it cannot be used to accurately quantify the degree of stress (Martinez-Porchas *et al.*, 2009). In chapter two it was demonstrated that even small increases in temperature decreased the aerobic capacity of five species of coral reef fish, although there was variation in the degree of change between families. That study also showed that fish were unable to increase their maximal oxygen uptake, and so all decreases in aerobic scope were caused by increases in their resting oxygen consumption. It has been shown that even small chronic increases in resting respiration rate can have large effects on growth (Rice, 1990), due to 'oxygen dependent size limits' (Pörtner & Knust, 2007). Oxygen limitation models predict that temperature-dependent aerobic limits are experienced earlier by larger fish than by smaller fish, and therefore oxygen availability determines the size to which fish will grow (Pörtner & Knust, 2007). Consequently, reductions in aerobic capacity could result in smaller individuals, which may translate into future years of reduced fecundity (Pörtner & Knust, 2007). Although freshwater fish in the River Thames are currently not living close to their lethal thermal limits, as some coral reef fish are, rises in temperature could have significant effects on their aerobic capacity, which will then have implications for the long-term survival of the population. Non-invasive techniques, such as quantification of gill movements of fish at rest, can provide useful information as to whether a fish is experiencing stress which may ultimately affect its growth and development. Whilst not as accurate and quantifiable as measuring the amount of oxygen consumed in a given time using a respirometer (as was used in the studies reported in chapter two and three), counting gill movements does not require any interaction with the individuals and is therefore less stressful. Hence, counting gill movements is a more suitable technique for this study, given that several other end points will also be measured that would be affected by the stress involved in holding fish in a respiromter.

5.2.4.2 Hepatosomatic Index and Condition Factor

Both hepatosomatic index and condition factor relate to an individual's potential for growth by indirectly assessing the energy status of a fish. The production of cortisol is known to cause mobilisation of glucose stored in the liver, to provide energy to resist the stressor. Therefore, in times of chronic stress, the size of the liver can decrease. Liver size is affected by many factors such as: season, life stage, nutrition and non-allometric growth (Owen *et al*., 2010). The glycogen stores in the liver can also become depleted with high metabolic rates (Barton *et al*., 1987). Since fish are pokilothermic, warmer waters, such as may be associated with climate change, will cause fish to have a higher metabolic rate. It is widely recorded that water temperature and food availability are the two most important factors determining growth rate in fish (Pottinger *et al*., 2011). If food availability is high enough to compensate for the increased metabolic rate, then glycogen stores in the liver may not be compromised. However, if temperatures are high and food availability is low, then the hepatosomatic index may decline, suggesting a stress response.

There have been many reports that cortisol is linked to reduced growth and lowered condition factor in fish (Barton *et al*., 1987; Davis *et al*., 2008, Robertson *et al*., 1963). It is thought that cortisol production as a result of a stress response causes metabolism to shift to protein catabolism and thereby reduces the growth of the individual (Barton *et al*., 1987). Condition factor and hepatosomatic index can provide a complimentary set of data indicating whether or not a stress that might be expressed at the biochemical level is severe enough to cause effects at the whole organism level and reduce growth. These indicators are extremely important, since the growth of an individual is closely linked to its ability to survive the first year of life and also the potential to reproduce.

By acquiring simple measurements of length and body mass, Condition Factor (Fulton's K) can be calculated.

$$K = (\text{mass}/\text{fork length})^{3} \times 100 \qquad \text{(Ricker, 1975)}$$

Condition factor, due mostly to the simplicity of determining it, is one of the most widely used indicators of general health in fish. A decline in condition factor is

generally ascribed to a decline in environmental conditions and stress, such as high stocking densities (Ellis *et al*., 2002).

The Hepatosomatic Index (HSI) is based on the principle of the liver weight (a glycogen store) as a percentage of the whole body weight:

HSI= (liver weight/ body weight) x100

This ratio gives information regarding the amount of energy that is available to an individual, with lower glycogen stores in stressed individuals. Since these energy stores deplete over a period of time, decreases in HSI can indicate that a chronic stress is present. The HSI can vary naturally with season and developmental stage and so care must be taken when comparing ratios from different studies or within a study over a prolonged period of time (Chellappa *et al*., 1995). Furthermore, gender is also known to affect the HSI, with female medaka having higher HSI values than males at all ages (Teh & Hinton, 1998). Therefore it is important to know the sex of the individuals in order to make accurate comparisons.

5.2.5. Aims of study

This *in-vivo* study aims to expose a commonly occurring UK freshwater fish, the three-spined stickleback *Gasterosteus aculeatus*, to realistic temperature increases predicted to occur by the end of the century as a consequence of climate change. Based on the IPCC predictions, fish will be exposed and acclimated to the temperature regimes of 19°C, 21°C and 25°C for a period of 3 weeks, to investigate whether there are any stress responses to a prolonged elevated temperature. A suite of biological indicators, from biochemical to whole animal, will be measured to examine which physiological stress responses are activated and the degree of stress each temperature increment causes. These stress responses may well be subtle, but they may still provide a wealth of ecologically significant information on the impacts of thermal stress on fish in a warming climate.

5.3 Preliminary Investigation 1. Accurate control and monitoring of experimental water temperature

5.3.1. Introduction

Given the numerous studies on the effects of climate change, many experiments now require manipulation of temperature in laboratory testing. However, keeping temperature constant over a period of time may not be easy, and is open to scrutiny, as many studies involve recording temperature only once a day, and hence may fail to record any fluctuations over a 24hour period. Since manipulating the temperature forms the basis of these experiments, it is essential that methodologies are developed to ensure minimal fluctuations and accurate recording.

This preliminary study was conducted prior to final confirmation of the methodology to be employed, and so the following temperature regimes were trialled: 20°C, 22°C and 24°C. These are all within the range to be used in my planned *in-vivo* study investigating the effects of chronic thermal stress on the three-spined stickleback.

It is usual for studies to record the temperature during the day. However, in order to be able to state that fish have been exposed to a constant temperature, temperatures need to be measured over a 24-hour period, to determine whether or not there are daily fluctuations. Fluctuations in water temperature within the experimental room will also need to be investigated and controlled, as distance from the header tanks may affect warming or cooling in pipes and hence influence the ability to control water temperatures in the fish tanks. Two new pieces of equipment were also tested in this trial. The first was a submersible temperature logger, Tinitag (Tinitag TG-4100, Gemini Data Loggers), which was be programmed to measure and record temperature every minute over a 3 week period to a reported accuracy of 0.01°C.The second piece of equipment was the Rena Smart Heater, which is a submersible heating element with thermostat that allows temperature to be controlled to the 0.5°C level. The efficiency of this heating element was compared to that of the normal tank heaters currently used at Brunel University (Visi-Therm Heaters).

5.3.2. Aims

The main aims of this preliminary study were to determine the best method of maintaining a constant water temperature within a range of no more than ±0.5°C. This would ensure that there was no overlap in regimes (for either 20, 22, and 24°C or 19, 21 and 25°C regimes). Secondly, this study aimed to determine how best to accurately monitor and record water temperature over the course of the study. To achieve this, the following steps were carried out:

1. To investigate whether there are diurnal patterns in temperature of the water supplied from the header tank.
2. To check the accuracy of the Tinitags
3. To investigate whether there is any effect on water temperature of distance from the header tank.
4. To determine whether individual heaters in tanks and appropriate insulation provide greater temperature stability.
5. To investigate whether dissolved oxygen concentration (DOC) decreases with increasing temperature

5.3.3. Methodology

Experimentation was carried out between May-June 2010. Daytime air temperature recordings within the laboratory ranged from 21°C to a maximum of 28°C. 3 x 20L tanks were supplied with water from the same header tank. The flow rate for each tank was set to 20L/h; therefore the entire water in the tank was replaced every hour. Oxygen was supplied to each tank via an air stone.

5.3.4. Results

5.3.4.1 Daily fluctuations in water temperature supplied by the header tank

It is standard procedure in many such temperature manipulation studies that temperature is recorded daily but usually only in daylight hours (Nilsson *et al*., 2009). However, these recordings will be in day time and therefore will not detect what fluctuations, if any, occur throughout a 24hour period. Tinitags can provide recordings of temperature continuously over an entire experimental period.

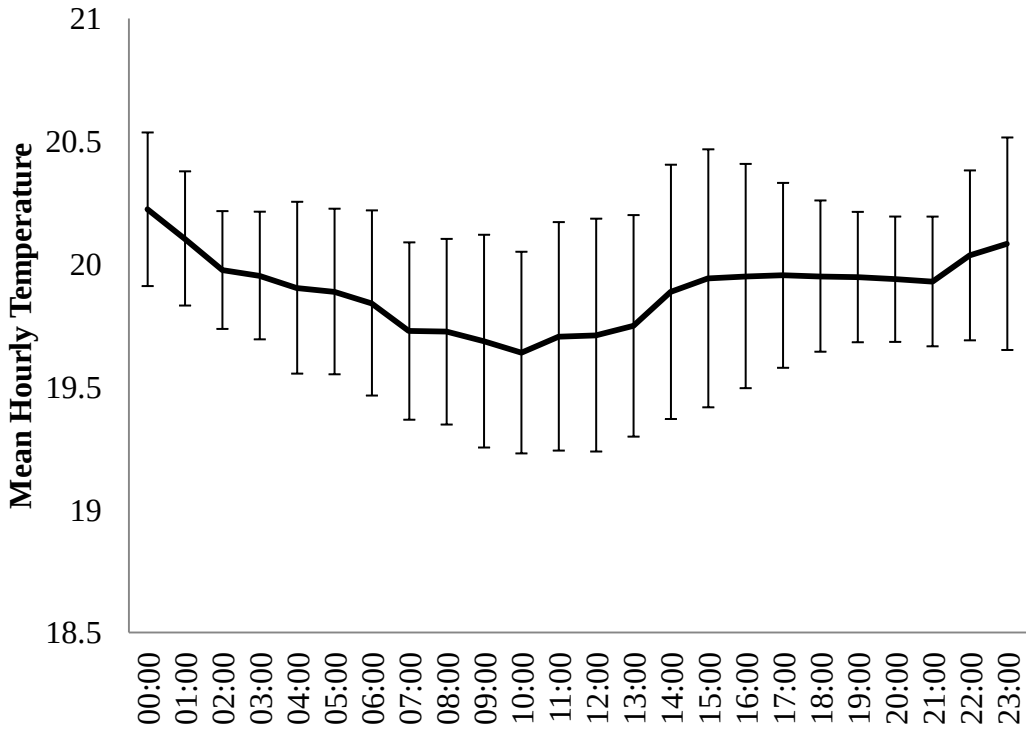


Figure 5.4. Hourly mean temperatures (±SD) taken from Tinitag recordings set to record every minute over 5 days. Tank heated solely by header tank set to 18°C.

Figure 5.4 shows that there is a small diel variation in water temperature supplied by the header tank. Whether this is as a result of header tank temperature fluctuating or whether it is room temperature creating a fluctuation in individual tank temperature is not clear. The mean temperature over this 5 day period was 19.9°C. Whilst this is extremely close to that desired 20°C, there were fluctuations over this time period that follow a daily pattern, with lower temperatures in the day.

Consequently, only recording the temperature in the day can give a false understanding of the true temperatures that fish are exposed to over an experimental period.

5.3.4.2 Accuracy of the Tinitags

Three tanks were set up, all being supplied by the header tank alone, which was set to 18°C and which subsequently achieved a mean water temperature of 20°C in the fish tanks (due to the heating effect of the room). Six Tinitags were programmed with the date and time and programmed to start recording at exactly the same time. Two Tinitags were placed at the bottom of each of the tanks. Tinitags are reportedly accurate to the 0.01°C level. The standard deviations for tanks 1-3 were 0.04, 0.08 and 0.07°C, respectively; therefore a mean accuracy of 0.06°C was achieved. This is less accurate than the manufacturer's guidelines; however this is still sufficiently accurate to provide confidence in the data on the water temperatures in the tanks.

5.3.4.3 Affect of distance from header tank on individual tank temperatures

One end of the room is close to the door, which is periodically opened throughout the day for fish feeding and access to other experiments being carried out in the room. This, and the fact that heat may be lost in the pipes on its way to the furthest tank, warranted an investigation to see if the tank furthest away from the header tank has the poorest control of water temperature. Tank 1 represents the tank closest to the header tank and tank 3 represents the tank furthest from the header tank (and therefore closest to the door).

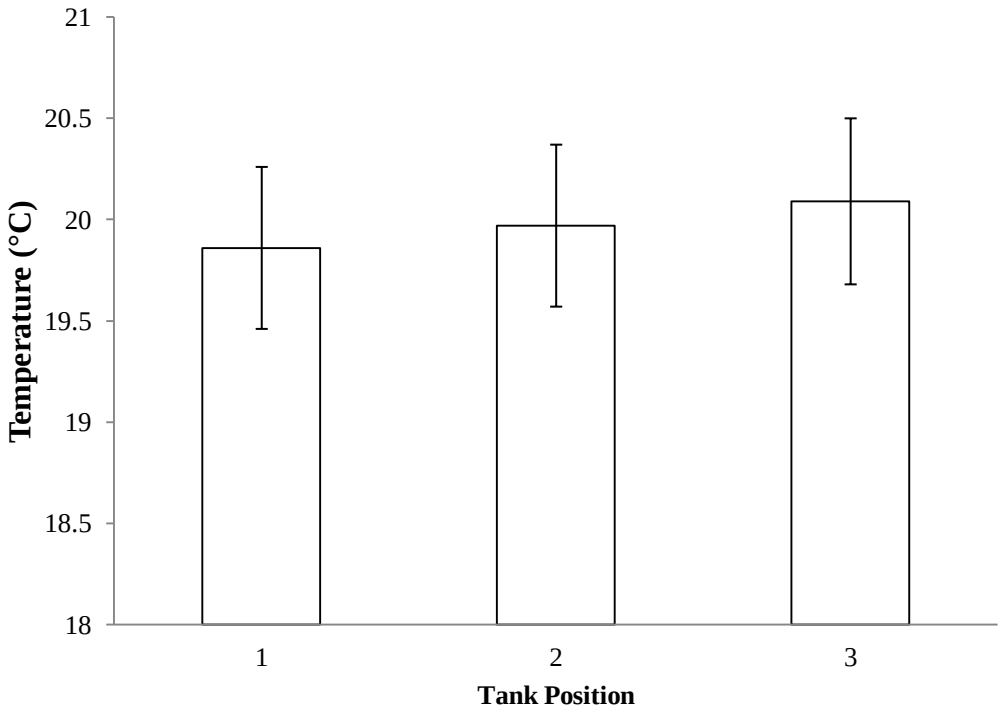


Figure 5.5. The effect of distance from the header tank on mean water temperatures in the fish tanks. All tanks received water from header tank set to 18°C: 1 being closest to the header tank and 3 being furthest away from header tank and closest to the door. Tank means (±SD): 1 = 19.8°C ±0.4, 2 = 19.97°C ±0.4, 3= 20.09°C ± 0.41. One-way ANOVA f[2, 32.4], p<0.05. Tukey's post hoc test showed the water temperature in tank 1 to be different from that in tanks 2 & 3, but tank 2 was not significantly different to tank 3.

The difference between the mean water temperature of tank 1 and 3 was 0.29°C. The air temperature in the room was warmer than that of the water in the header tanks, and therefore additional contact in pipes could have warmed the water entering tanks 2 and 3 more than those positioned closer to the header tank (i.e. tank 1). However, the main study will be carried out in the winter months in a temperature controlled room, and so air temperature will not be above experimental temperatures and will be kept relatively constant. This should prevent air temperature having a negative impact on water temperatures in the tanks.

5.3.4.4. Effects of insulation and individual heaters in tanks

Fish tanks were insulated by polystyrene tiles that were fixed by tape to 3 sides of each tank. A plastic sheet, cut to the size of the tank, was placed on top of the tank to prevent heat leaving through the surface of the water. The front of the tank was left clear for inspection of fish. Fish tanks were supplied with water from the header tanks and comparisons made to determine whether insulating the fish tanks reduced the fluctuations in the water temperature over a 27 day period.

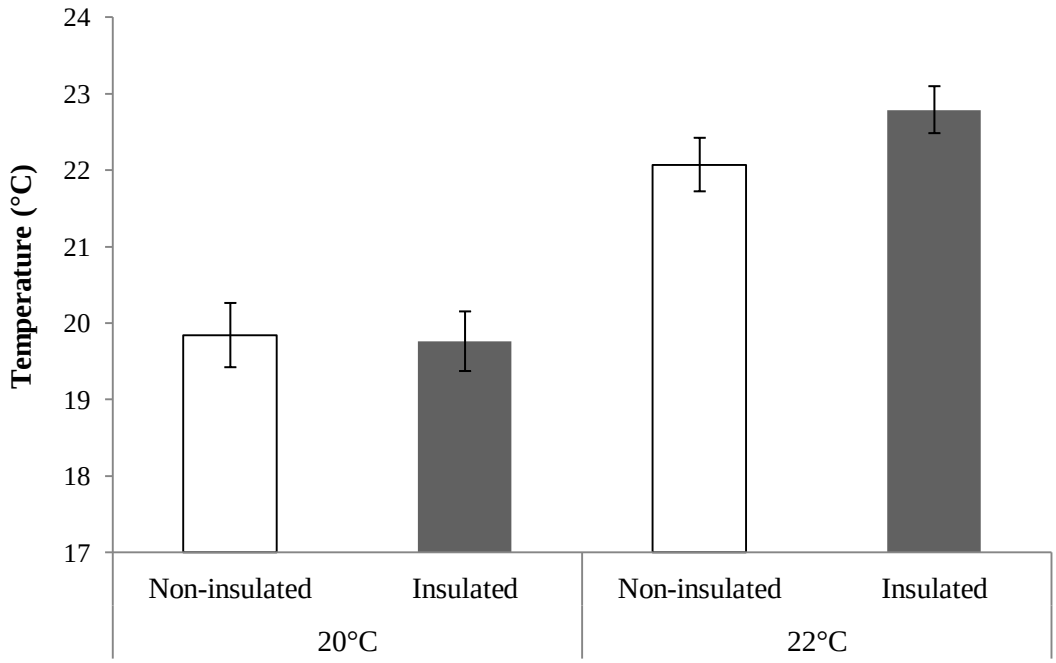


Figure 5.6. Effectiveness of polystyrene insulation on fish tank temperature fluctuations. Mean temperatures (±SD) for each tank over a period of 27 days, recorded by Tinitags. 20°C [header tank set to 18°C]: non-insulated (mean 19.83°C±0.42), insulated (mean 19.76°C ±0.39); 22°C [header tank set to 20°C]: non-insulated (mean 22.07°C ±0.35), Insulated (mean 22.78 °C ± 0.31).

Insulating the tank in the 20°C and 22°C trial did reduce the standard deviation slightly. In the case of the 22°C trial, the mean water temperature in the fish tanks was higher than the desired 22°C when insulated, but this can be overcome

by reducing the temperature of the water in the header tank. What is important here is that the fluctuation in temperature (i.e. the standard deviation) is reduced. Whilst the decrease in fluctuation is not significant, the polystyrene tiles provide a second function by preventing fish in one tank from seeing the fish in another tank, which may be stressful. Therefore, polystyrene tiles around the outside of the tank will be included.

Two different brands of heating elements were trialled. The Reno Heater is a thermostatically-controlled heating element that claims to control temperature to within 0.5°C. The Visi-Therm heaters were also trialled because these are the standard heating element used at Brunel University.

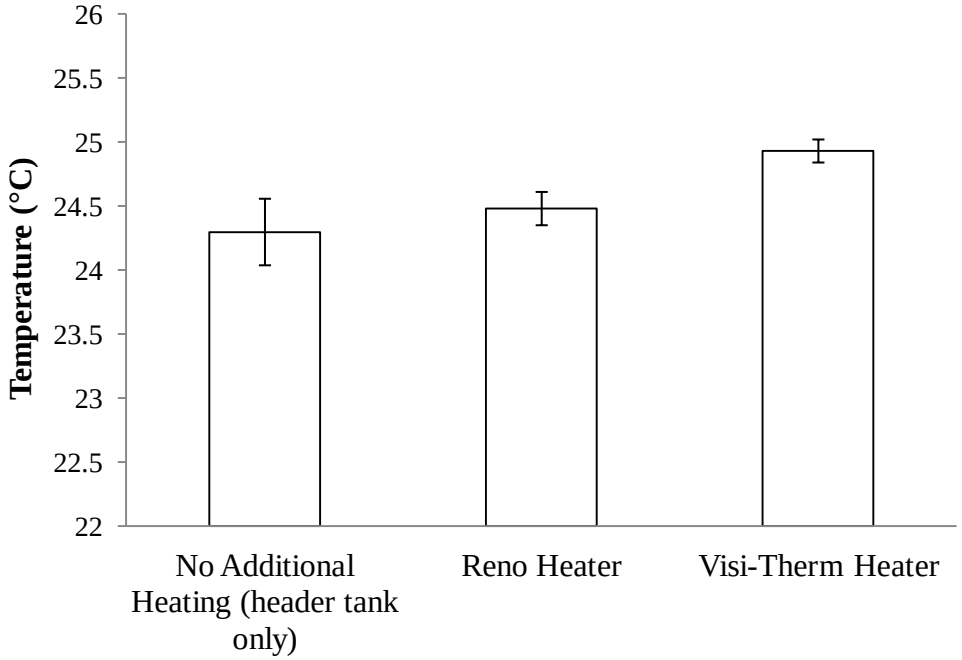


Figure 5.7 Comparison of header tank, Reno and Visi-therm heaters on tank temperature. Mean temperatures and SD for each tank over a period of 27days, recorded by Tinitags. Header tank water set to 24°C: Header tank only (mean 24.29 °C +/-0.26), Reno Heater (mean 24.48 °C +/- 0.13), Visi-Therm Heater (mean 24.93°C +/- 0.09).

A Visi-Therm heater was better than a Reno Heater at reducing the fluctuations in temperature, and both greatly improved temperature stability when compared to water from the header tank that received no further temperature control. Whether relying solely on header tank temperatures, or using individual heaters or

insulation, there was no overlap in temperature regimes, providing strong confidence that fish will be able to be kept at distinctly different temperature conditions throughout the experiment. However, by using individual heaters and insulating the tanks, fluctuations in temperature in each tank can be further minimised.

5.3.4.5. Effect of increasing water temperature on the dissolved oxygen concentration

The dissolved oxygen concentration (mg/L) was recorded hourly between 9am-5pm for each temperature regime for 3 days. Tanks were not supplied with an air stone for this trial.

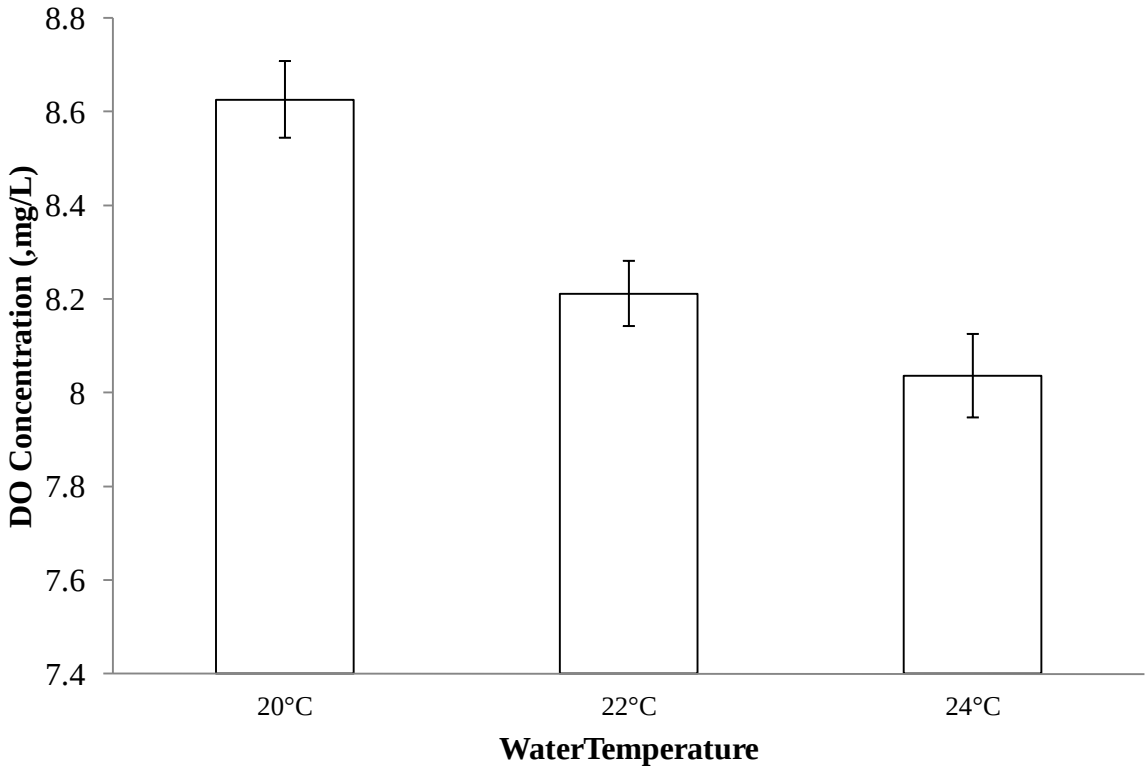


Figure 5.8. The effect of water temperature on the dissolved oxygen concentration (mg/L) in tanks containing water held at 20°C, 22°C and 24°C over a 3-day period. Higher temperatures result in lower oxygen availability (One-way ANOVA, $p<0.01$ level)

It is usual for oxygen concentration in water to either be measured in mg/L (Brian *et al.*, 2009; Crampton *et al*., 2003; Moran *et al*., 2010) or as a percentage of oxygen saturation in air (DO saturation %) (Nilsson *et al*., 2004 & 2009; Pörtner *et al*., 2001). If water had a DO saturation of 100%, this is the maximal amount of oxygen that can be dissolved in water at a given water temperature, pressure and salinity (Crampton *et al*., 2003). Based on this study, the mean DO saturation % for each temperature regime was 95.8 ±0.72% (20°C), 95.4± 0.47% (22°C) and 95.2 ±0.68% (24°C). At higher temperatures, the percentage of oxygen saturation can still be fairly high (as in this experiment); however, the actual amount of oxygen present in water, i.e. the concentration in mg/L can be lower (as shown in Figure 5.8). Therefore, it is often more useful to measure the amount of oxygen present in water in mg/L (Crampton *et al.*, 2003). The amount of available oxygen in the water did significantly decline with increasing water temperature. This result was expected, since it is known that warmer water holds less oxygen. However, even at 24°C, the dissolved oxygen concentration was still above the concentration considered low for most fish species, and therefore should not be a limiting factor.

It has been considered that the partial pressure of oxygen (PO_2) in water also has an effect on the amount of oxygen that is available (Verberk *et al*., 2011). Partial pressure refers to pressure exerted by an individual gas, such as oxygen, in a mixture of gases and is affected by the pressure and the temperature. Verbeck *et al.* (2011) state that it is not a case that either PO_2 or mg/L is more important than the other, but that both play a role, along with the rate of diffusion of the gas, in the bioavailability of oxygen in water. It may be that if the oxygen concentrations in Figure 5.8 were converted to PO_2, the apparent difference in oxygen levels might be slightly less significant. However, not all of the information is available (since pressure of the water or air was not accurately measured) to confidently convert the concentration of O_2 to PO_2.

It is generally considered that an oxygen concentration in water above 7mg/L is more than sufficient for healthy fish development (Crampton *et al.*, 2003). In fact, hypoxic conditions as low as 2.8mg/L have been shown to have no effect on fish, such as the fathead minnows (Brian *et al.*, 2009). *G.aculeatus* is a hardy species and can cope with dissolved oxygen concentrations as low as 2.0mg/L (Moran *et al.*,

2010), and therefore it is extremely unlikely that a concentration of 8mg/L will be stressful for *G. aculeatus.*

Despite this, it is important to keep all variables other than temperature constant, and so tanks will be supplied with an oxygen stone whose supply will be adjusted accordingly to ensure that DO concentrations do not fall below 7mg/L in the tanks. Dissolved oxygen concentrations will be kept below 9.0mg/L, however, as above this level water can become so saturated in oxygen. Water that is supersaturated in oxygen can cause 'Gas Bubble Disease' in fish (air pockets forming under the skin of fish, particularly the face, tail and eyes) (Bouck, 1980), which can be lethal in extreme cases.

5.3.5. Conclusions and suggested experimental set-up

A final trial was carried out using a smaller tank size (10L) immediately prior to conducting the experiment, due to a limited number of available 20L tanks. 2x 10L tanks were set up with insulation as described above and the header tank was set to 18°C. The mean water temperature achieved was 19.77°C ±0.07 (SD) compared to 19.76°C ±0.39 (SD) previously in 20L tanks. Therefore, the smaller volume tank had reduced fluctuation in temperature and was deemed to be suitable for the experiment.

Based upon the results of these trials, the following steps were used to ensure accurate control and monitoring of water temperature:

- Control
    - Volume of tanks: 10L
    - Individual Visi-Therm heaters were placed in all tanks to raise water temperatures to the desired values.
    - One layer of polystyrene tiles was added to the sides and backs of each tank and a plastic lid placed onto each tank.
    - Dissolved oxygen concentrations were kept constant and uniform across tanks by use of an air stone in each tank.

- A temperature controlled room was used to ensure that air temperature does not fluctuate and affect the water temperatures in the fish tanks.

- Monitoring
  - TinyTags were placed in each tank to record water temperature every 30 minutes over the entire experimental period, in order to provide an accurate account of the water temperatures in tanks. Data was downloaded at the end of each week of experiment for constant monitoring.
  - Temperature readings were complemented with twice daily temperature and dissolved oxygen concentration readings.
  - A thermometer strip was placed at the front of each tank to allow quick assessment of the water temperature.

5.4. Preliminary Investigation 2. Cortisol Validation Study

5.4.1 Introduction

Cortisol is passively released via the gills into the water at concentrations that have been shown to be detectable by techniques such as Enzyme-Linked Immunosorbent Assay (ELISA) and Radioimmuno Assays (RIA's). The underlying principle of this approach is that the release rate of cortisol into the water is directly related to that in the plasma. Measuring cortisol in the water rather than plasma has many advantages. The main benefit is that it is non-invasive and so the concentration measured reflects stress caused by the thermal conditions and not due to handling stress, as is the case when obtaining plasma samples. Also, it allows repeated cortisol measurements over a period of time. This allows investigations into the effect of chronic stress on cortisol concentrations, whereas plasma concentrations can only give you a value for that moment in time. The principle of measuring cortisol in the water rather than plasma is that the concentrations should be roughly equivalent. However, the release rate into water will never be exactly the same as the plasma concentrations. Factors such as gill surface area, gill permeability, steroid lipophilicity and conversion of free steroids from one steroid to another (Scott *et al*., 2008) can all alter the concentrations of cortisol detected in water samples as opposed to being detected in the plasma.

Prior to carrying out a study on the effects of temperature increases on cortisol concentrations, a preliminary investigation was carried out to establish whether cortisol can be detected and measured in water samples. Similar validation studies have previously been carried out by Scott *et al*. (2008) for the three-spined stickleback, but it is prudent to re-apply these validation steps for each experiment.

5.4.2. Aims

1. To establish the volume of water needed for extraction.
2. To establish extraction efficiency using solid phase extraction (SPE) cartridges (Sep-Pak ® Plus C_{18}, Waters Ltd, UK).
3. To quantify background cortisol concentrations in header tank water.
4. To ascertain a suitable reconstitution solvent (ELISA buffer or ethanol).
5. To compare basal and stressed cortisol rates.
6. To establish whether water samples will need to be diluted prior to ELISA.

5.4.3.1 Volume of water needed for extraction

Within the literature, either 1litre (Ellis *et al*., 2007) or 500ml (Ruane & Koman, 2003) of water is typically collected for extraction prior to an ELISA or RIA. Here, the concentrations of cortisol in both a 1L and 500ml sample of water from the same tank were compared to determine whether cortisol was detectable at the same concentration in both. The smaller the volume of water that needs to be collected the more advantageous, due to the time taken in extraction.

1L and 500ml of water was collected from the outflow pipe of a tank containing unstressed fish (n=10). 5ml and 2.5ml of methanol were added, respectively, and samples placed in a fridge for an hour to prevent biological degradation.

Cortisol was detectable in both the 1L sample (0.17ng) and in 500mL sample (0.08ng). Given that the concentration in both samples was the same (0.08ng/500ml is equivalent to 0.16ng/L), 500ml is deemed an adequate volume of water to collect and process.

5.4.3.2 Extraction efficiency of C_{18} cartridges

Samples of water were spiked with a low and high concentration of cortisol that could be expected in unstressed and stressed fish, to test the extraction efficiency of the cartridges. Non-reproducing *Gasterosteus aculeatus* have a basal cortisol

release rate of approximately 0.2ng/g/h, and with most release rates falling between 0.2 and 1.0ng/g/h (Sebire *et al*., 2007). It has also been reported that acutely stressed individuals (handling and confinement stress) of the same species having cortisol release rates of 3.5ng/g/h (Scott & Ellis. 2007).

With a mean fish mass per tank of 18g, tank capacity of 10L and a flow rate of 10L/h (therefore 1L and hour), this equates to:

LOW SPIKE= 0.2 x 18 /10= 0.36ng of cortisol in 1 litre of water
HIGH SPIKE= 3.5 x 18/10= 6.3ng of cortisol in 1 litre of water

A 30ng/ml stock solution of cortisol in methanol was made. For the Low Spike, 10µl (0.3ng/L) was added to 1litre of header tank water, and 200µl (6ng/L) was added to 1Litre of header tank water for the High Spike, representing the expected basal and stressed cortisol release rates. A total of 8 samples for each spike were tested.

The concentration of cortisol detected in the high spike was 4.9±1.39 (SD) ng/l (1:20 dilution) and the concentration for the low spike was 0.25±0.007 (SD) ng/l (1:10 dilution). Therefore, based on the high spike concentration and low spike concentration, the extraction efficiency of the C_{18} cartridges was 77% and 70%, respectively. However, the readings for the high spike fell closer to the IC_{50} (50% B/B_o) and therefore an extraction efficiency of 77% will be used in future calculations.

5.4.3.3 Background concentrations of cortisol in header tank water

Eight x 1L samples of water were collected directly from the header tank (which supplies the fish tanks with water) was extracted and analysed by ELISA. A 1L purite water sample was also analysed by the same process. The results showed that the concentration of cortisol in the purite water sample was too low to detect. However, there was a detectable amount of cortisol in the header tank water, at a concentration of 0.29±0.14 (SD) ng/L. Therefore all tanks will be receiving a low

concentration of cortisol and this amount must be deducted from the final cortisol concentration recorded from the outflow pipes of the tanks containing fish.

5.4.3.4 Reconstitution using solvent

The high spike which was reconstituted in ethanol had an extraction efficiency of 36%, compared to 77% when reconstituted in ELISA buffer when using C_{18} cartridges (Sep- Pak). Given the higher extraction efficiency with buffer, it was therefore used thereafter in preference to ethanol, since solvents like ethanol can interfere with the ELISA technique and potentially affect readings. In accordance with the ELISA Kit manufacturer's guidelines (Cayman Chemical), the buffer provided should be used to reconstitute cortisol from plasma, urine and fecal samples. The justification for trialling ethanol was that cortisol has a logP (octanol-water) of 1.43. The logP value acts as an indicator of the compound's lipophilicity and solubility, and a value of 1.43 for cortisol could indicate that it is somewhat hydrophobic and so needs to be dissolved in an organic solvent. However, given that cortisol was successfully reconstituted in buffer and subsequently detected by ELISA, and that this procedure follows the manufacturers guidelines, all samples were reconstituted in ELISA buffer prior to analysis.

5.4.3.5 Basal and stressed cortisol release rates

The basal or unstressed cortisol concentrations were determined from a 500ml water sample from a tank of fish which were undisturbed. Two water samples from stressed fish were obtained by placing 3 fish into each of two 500ml beakers of water for 3 minutes. The process of netting and confinement in a small volume of water was considered to be adequately stressful for the purpose of this study.

The cortisol release rates were calculated as follows:

$$\textit{Release rate (ng/g/h)} = (C \times F) / M \qquad \text{(Ellis } \textit{et al.}\text{, 2007)}$$

Where C= Concentration of cortisol (ng/l), F= Flow rate (L/h) and M= total mass of fish in tank. The resting, or unstressed, cortisol concentration (0.17ng/ml) therefore equates to a release of 0.12ng/g/h (corrected for 77% extraction efficiency and based on a flow rate of 8.85L/h and mass in tank of 17.1g). This release rate is similar to that recorded by Sebire *et al*. (2007) of 0.2ng/g/h for unstressed fish.

The concentration of cortisol in the water of fish stressed for 3 minutes was 0.3ng/ml and 0.56ng/ml (corrected for 77% extraction efficiency). This equates to release rates 3.22 ng/g/h and 3.8ng/g/h (based on 500ml water sample, with a total mass of 3.37g [n=3] and 5.86g [n=3]). These acutely stressed fish therefore released cortisol at a rate comparable to that reported by Scott and Ellis (2007) of 3.5ng/g/h.

In previous validation studies for the three-spined stickleback, it has been found that there is strong positive curvilinear correlation between water and plasma concentrations of cortisol (r_s=0.82, n=24, p<0.001) (Sebire *et al*., 2007). These authors also reported that a cortisol release rate of 3ng/g/h approximately equates to plasma concentrations of 75ng/ml.

Table. 5.2. Comparison of water cortisol concentrations and release rates for unstressed and stressed fish.

Samples	*Actual Concentration (ng/l)*	*Release Rate (ng/g/h)*
Basal (Unstressed)	0.17	0.12
3 minutes stressed (3.37g)	0.3	3.22
3 minutes stressed (5.86g)	0.56	3.8

Basal rates of cortisol release from sticklebacks used in this study are slightly lower than the range that is cited in the literature for *G.aculeatus* of 0.2-0.3ng/g/h (Sebire *et al*., 2007). Some mature sticklebacks have been recorded to release higher concentrations of cortisol at around 0.7ng/g/h, but no differences have been seen between the sexes (Sebire *et al.*, 2007). The fish in the tanks were mixed sex but were not yet reproducing, and there was no evidence of nest building. Therefore it can be assumed that sexual maturity had not yet been reached, further supporting the fact that the basal rates obtained from the water samples were realistic, if on the low

side of that cited in literature. In acutely stressed fish, a 25-fold increase in cortisol levels in the water has been reported (Ellis *et al.*, 2007). In this study, there was a 27-fold and a 32-fold increase between the basal concentration and the concentrations in the water samples from stressed fish. However, it must be noted that different fish were used for each sample and so the differences in release rate are a guideline only. It should also be noted that although these rates are similar to those cited by Ellis *et al*. (2007), it is anticipated that the release rates from the main experiment will be less as the stress will be chronic and not acute.

5.4.3.6 Final dilutions of water samples

The detection range of the cortisol ELISA was between 0.016 and 1.6ng/ml. Therefore, it was necessary in some cases to dilute samples so that the concentrations fell within the detection range. Where the expected concentration already falls in this range, no dilution is necessary. However, ideally any readings should fall between 20-80% on the standard curve, with no more than a 20% disparity between the replicates, to provide accurate concentrations.

The ELISA could detect the concentration of cortisol in the water from either diluted samples (1:6, 1:10) or non-diluted samples. However, in some cases a 1:20 dilution was too low to provide reliable readings. Therefore, each water sample was assayed both with no dilution and a 1:6 dilution.

5.4.4 Summary

- 500ml of water will be collected from the fish tanks for extraction, which should be enough to detect both basal and stressed release rates of cortisol.
- All water samples will be assayed at no dilution and a 1:6 dilution.
- Blank water samples will be taken on each sampling day and recorded blank concentrations will be subtracted from final cortisol concentrations for each fish tank.

5.5. Preliminary Investigation 3. Flow Cytometry for Leukocyte Profiles

5.5.1. Introduction to Flow Cytometry

Flow cytometry for cell counting is becoming increasingly popular. The traditional method of using microscopes to count cells is both extremely labour intensive and subjective. A flow cytometer can accurately count up to 50,000 cells in 3 minutes, allowing quick processing of a large number of samples, which is essential when dealing with fresh blood.

Flow cytometry works on the principles of Forward and Side Scatter using a laser beam. The Side Scatter provides information about the granularity of the cells, with higher side scatter (SSC^{high}) indicating greater granularity of the cell. The Forward Scatter informs you of the relative size of the cells, with a higher forward scatter (FSC^{high}) indicating a larger cell. Therefore based on what is known in the literature regarding the sizes and granularity of blood components, it is possible to determine from FSC/SSC profiles different blood constituents. 'Gates' are set up in defined regions to represent each cell type, e.g. a gate for lymphocytes with the software stating the percentage of cells represented in each gate. By processing blood from fish in each temperature regime, it can be investigated whether there is an increase or decrease in the percentage of cells in any particular gate. This will be used as the basis for the N: L ratio, since neutrophils are Granular (FSC/ SSC^{high}) and lymphocytes are smaller and non-granular (FSC/SSC^{low}) (Scharsack *et al.*, 2004).

In order to determine the parameters of the gates, a set of preliminary trials was carried out to determine the best methodology of processing blood and also where gate parameters should be placed.

5.5.2. Preparation of Blood

5.5.2.1 Whole Blood

Blood was collected from a three-spined stickleback after a lethal overdose in buffered MS222. 900µl of HBSS (Hanks Balanced Salt Solution) was added to the whole blood to prevent degeneration of cells. A stock solution of 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6$) dye (Sigma) was prepared in ethanol at 500µg/ml and a 10x diluted stock solution in HBSS was prepared just prior to staining. 50µl of the dye solution was added to each blood sample10 minutes prior to flow cytometry, as the dye can only be exposed to light for a short period due to its photodynamic toxicity. $DiOC_6$ is a fluorescent dye which binds to membranes, mitochondria and endoplasmic reticulum. It has been shown in studies to be an effective dye for blood cells when using flow cytometry (Inoue *et al.*, 2002). A minimum of 20,000 cells were acquired in a linear mode by the flow cytometer.

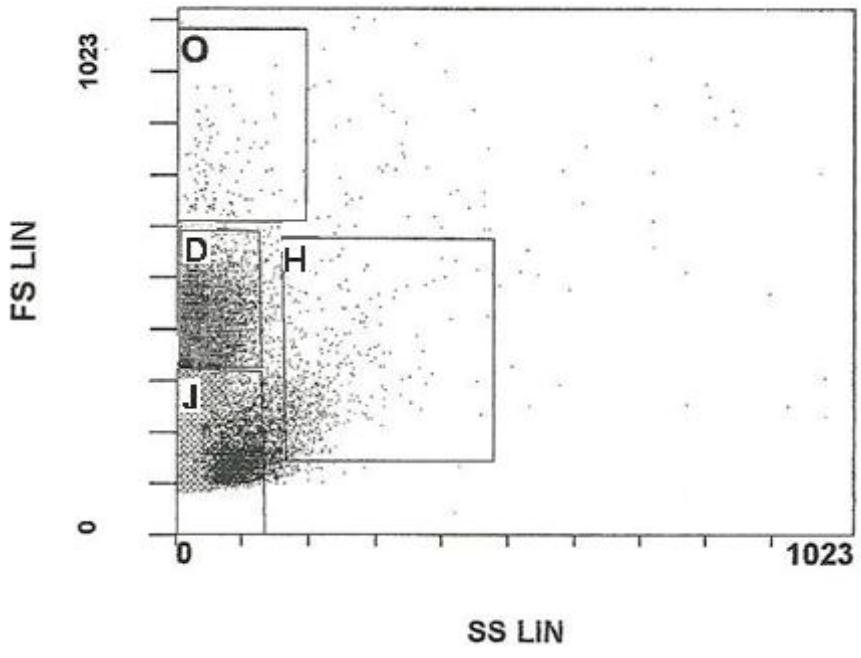


Figure 5.9. Display of a FS/SS profile from a flow cytometer for a whole blood sample. Standard 'Gates' J,D,H and O refer to different components of blood with [J] and [D] representing erythrocytes, [H] leucocytes and [O] presumed clumped cells.

Table 5.3. Statistical analysis of the results produced by the flow cytometer for each FSC/SSC profile. The percentage of cells detected in each gate is provided (% gated).

Statistical Analysis

PROGRAM INFORMATION

File:- 00008872 2191.LMD								
Gate:- Ungated								
Compensation:- Advanced								
Filename:- 00008872 2191.LMD								
Mean Calculation Method:- LOG-LOG								
Region	Number	%Total	%Gated	X-Median	X-Mean	X-Mode	X-CV	X-HPCV
D	21919	21.92	21.92	24.0	34.2	10.0	75.8	94.9
G2/M tel	41837	41.84	41.84	6.2	7.4	5.6	0.0	5.5
H	1323	1.32	1.32	205.0	224.1	165.0	26.8	3.5
J	73802	73.80	73.80	26.0	34.9	21.0	70.2	52.2
O	292	0.29	0.29	57.0	71.0	41.0	63.0	2.6
S tel	44172	44.17	44.17	6.1	7.4	5.6	0.0	5.5
T	75722	75.72	75.72	2.9	3.2	5.6	0.0	4.9

When using whole blood samples, it is not possible to determine the different subsets of leukocytes. Gate O indicated that cells in this gate are very large (Figure 5.9). It is assumed that cells in this gate are multiple cells that have clumped together, and so data from this gate will be removed in the final analysis. Gates D and J accounted for a total of 95.72% of the cells (Table 5.3), which is assumed to largely represent the erythrocytes along with white blood cells. Erythrocytes usually account for anywhere up to 50 % of all cells in the blood. Within gates D & J there may well have also been non-granulocytes (lymphocytes) that could not be distinguished from other cell types also present. Given that erythrocytes account for such a large percentage of blood cells and that gating prevents distinction of leukocyte sub-populations, the next step was to remove erythrocytes from whole blood and isolate the white blood cells.

5.5.2.2 Isolation of White blood cells

Blood was collected from the caudal peduncle using a heparised capillary tube and decanted into an epindorf tube (0.5µl). Blood samples were centrifuged for 4 minutes at 12,200rpm (8050x g). The top layer of plasma was pipetted off and 200µl of HBSS added. This was carefully overlaid onto 200µl of Leukocyte

Separation Medium (LSM) in a separate microcentrifuge tube. Without disturbing the mixture, it was centrifuged for 30minutes at 750x g. This caused the erythrocytes and basophils to form a pellet at the bottom of the tube, leaving the neutrophils, lymphocytes, thrombocytes and monocytes at the saline- LSM interface (known as a buffy coat). 100µl of the buffy coat was collected by Pasteur pipette and reconstituted in 850µl of HBSS. 50µl of prepared $DiOC_6$ dye stock solution was added 10minutes prior to flow cytometry.

5.5.3.3. Gate Setting

Figure 5.10 shows a diagrammatic representation of where it was expected that each cell constituent would fall and therefore where gates can be placed (Scharsack *et al*., 2004).

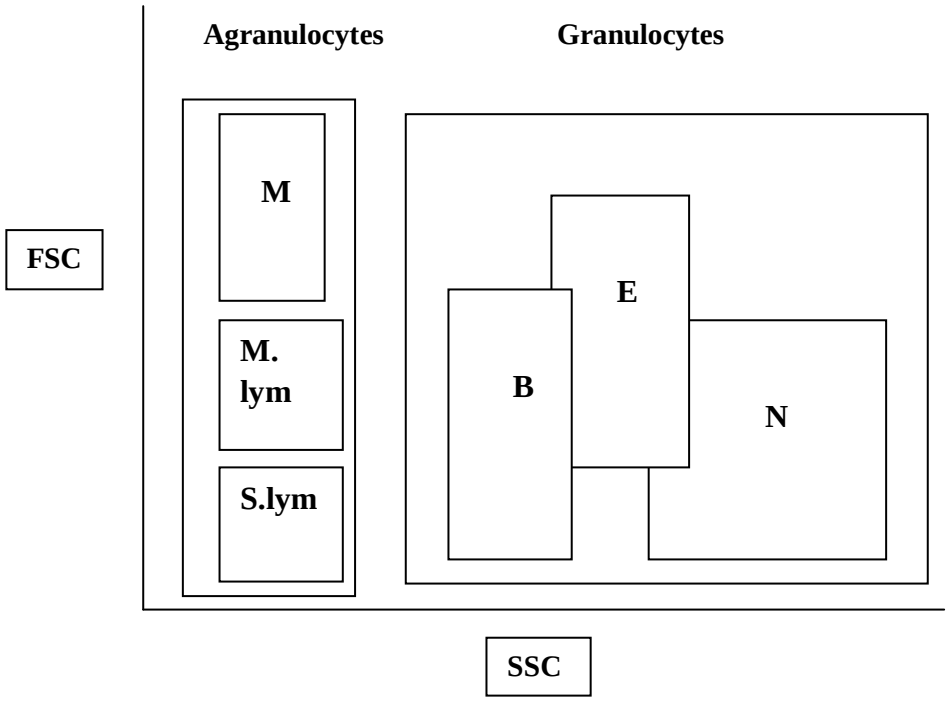


Figure 5.10. Diagrammatic representation of a typical flow cytometry result of fish blood when red blood cells have been removed. It shows the positions of the agranulocytes (comprised of M= monocytes, M.lym=medium lymphocytes and S.lym= small lymphocytes) and granulocytes (B= basophils, E= esinophils and N= neutrophils).

Once red blood cells have been removed, it was possible to see that the remaining cells broadly divided into two groups, agranulocytes (lymphocytes and monocytes) and granulocytes (Esoinophils, neutrophils and basophils). However, basophils were removed in the LSM separation procedure. Two gates were used, one representing the agranulocytes (FSC/SSC^{low}) and the other representing the granulocytes (FSC/SSC^{high}). Given that the percentage contribution of monocytes to agranulocytes is small, as is the contribution of esoinophils to granulocytes, the percentages in each gate were used to represent the ratio of neutrophils to lymphocytes (i.e. the N: L ratio).

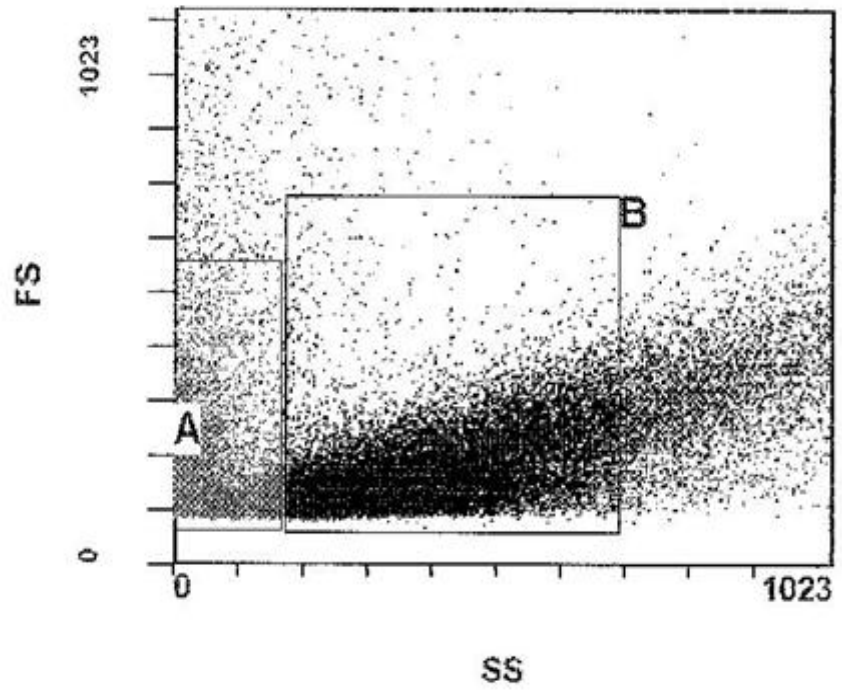


Figure 5.11. Profile of flow cytometry of isolated white blood cells from an unstressed fish. Gate A refers to agranulocytes and gate B to granulocytes.

Table 5.4. Statistical analysis from a flow cytometer for the blood sample used in the figure above showing 18.03% of cells were in gate A (agranulocytes) and 63% of cells in gate B (granulocytes).

Statistical Analysis

PROGRAM INFORMATION

File:- T3.1.LMD

Gate:- Ungated

Compensation:- Advanced

Filename:- T3.1.LMD

Mean Calculation Method:- LOG-LOG

Region	Number	%Gated	X-Mean	X-HPCV
A	9013	18.03	81.7	###
B	31647	63.29	365.8	25.0

Once the erythrocytes have been removed, it was clearer to see an even spread of cells (Figure 5.11) with 18% in gate A and 63% in gate B (Table 5.4). When these gating parameters for gate A and B were subsequently trialled on the blood from three additional unstressed fish a mean percentage of 21 ± 3% cells fell in gate A (representing agraunlocytes) and 62± 2% in gate B (representing graunulocytes). Therefore these gate parameters will be used on isolated white blood cells (as described in 5.8.2.2) to determine the granulocyte: agranulocyte ratio (effectively the N: L ratio).

5.6. Methodology

5.6.1 Fish Collection and Husbandry

Fish were obtained from CEFAS laboratories (Weymouth, UK), where they were bred to be free from parasites and disease. Fish were held in large stock tanks in ambient temperatures (mean 14°C ± 2°C) for 4 weeks (prior to being moved into experimental tanks). Fish were in mixed sex groups (sex unknown) but were prevented from breeding by using a winter photoperiod of 8L: 16D (light 8am-4pm, with a 15minute dawn and dusk phase) and by the absence of any nesting material in the tanks. The use of a winter photoperiod to prevent breeding has been well documented (Maunder *et al*., 2007; Sebire *et al*., 2007). Fish were fed 4 times daily on frozen blood worm. *G.aculeatus* has a relatively high thermal tolerance for a British fish (critical temperature 28°C) and therefore the temperatures used in this study (19°C, 21°C and 25°C) will not pose a lethal threat to the fish (Moran *et al*., 2010)

5.6.2 Potential experimental stresses and mechanisms to minimize stress

1) Tank Confinement: Fish moved into experimental tanks and left to acclimate for 3 weeks to their tanks and co-habitants prior to sampling. This time period, based on previous observation of stickleback behaviour, is thought to be sufficient time for the fish to settle into a new environment.
2) Competition for food: Fish fed 4 times daily until satiation and therefore food is not thought to be a limiting factor.
3) Competition for mates/territorial aggression: Fish were prevented from entering breeding behaviour by a winter photoperiod and lack of nesting material. Therefore, fish should not initiate breeding or be competing for mates.
4) Social Hierarchy: Sex of fish not known at beginning of experiment, therefore assumed that the groups are of mixed sex. Hierarchies usually established in times of courtship and mating and therefore not considered to

be significant in this study, as breeding should be prevented by use of a winter photoperiod.

5) Predators: No predators present
6) Handling: Handling kept to an absolute minimum. Fish handled at the very beginning of the experiment, in order to be weighed. All endpoints were non-invasive.
7) Disturbance from daily temperature and DO readings: Readings of tank temperature and DO were made twice daily, usually to coincide with feeding, in order to minimise entry into the room. Noise was kept to a minimum at all times.

5.6.3 Temperature Control and Regulation

The experiment was carried out between January and April 2011 in a temperature-controlled room with air temperature set to 21°C. Using the current-day summer mean river temperature and the IPCC predictions B1 and A1F1 for temperature increases, the following temperature regimes were selected:

- 19°C (±0.5°C) (representing typical current summertime water temperatures)
- 21°C (±0.5°C) (representing current average plus B1 of +2°C)
- 25°C (±0.5°C) (representing A1F1 of +6°C)

Ninety fish in total were used in the study. Sample size was estimated using power analysis calculations based on the size of the effect expected, the variability in sampling and a desired statistical significance ($p<0.05$). Information gained from pilot studies and literature where used to inform the size of the effect expected and variability in samples from studies in similar species. It was established that 30 fish would be needed per temperature treatment in order for statistical significance to be seen. Ten fish were wet weighed (mean weight 1.8g) and placed in each of the 9 x10L tanks (3 at each temperature regime) (18th January 2011). Fish were left to acclimate to their tanks for 3 weeks at ambient water temperature (16°C). After this, the header tank water was heated to 17°C, which subsequently heated the tanks to 19°C. Fish were maintained at the control water temperature of 19°C for a period of

4 weeks, as this length of time has been cited as a suitable acclimation period for chronic studies (Martinez-Porchas *et al*., 2009). Temperatures were subsequently gradually increased in tanks by individual VisiTherm Heaters over a period of 23 days (for those tanks at 25°C) and 18 days (for those tanks at 21°C), until target temperatures were reached. This strategy was employed to avoid an acute shock due to any rapid temperature increases. Visi-therm heaters were placed in the control tanks (tanks that remained at 19°C) but not turned as a stable 19°C was achieved by header tank alone, but the presence of the heaters ensured uniformity in tanks. Flow rate was set to 10L/h, allowing complete renewal of water every hour. Water in each tank was aerated with an air stone so that the oxygen concentration was maintained between 8.0-9.0mg/l. Temperature was controlled and monitored as discussed in section 5.3.5.

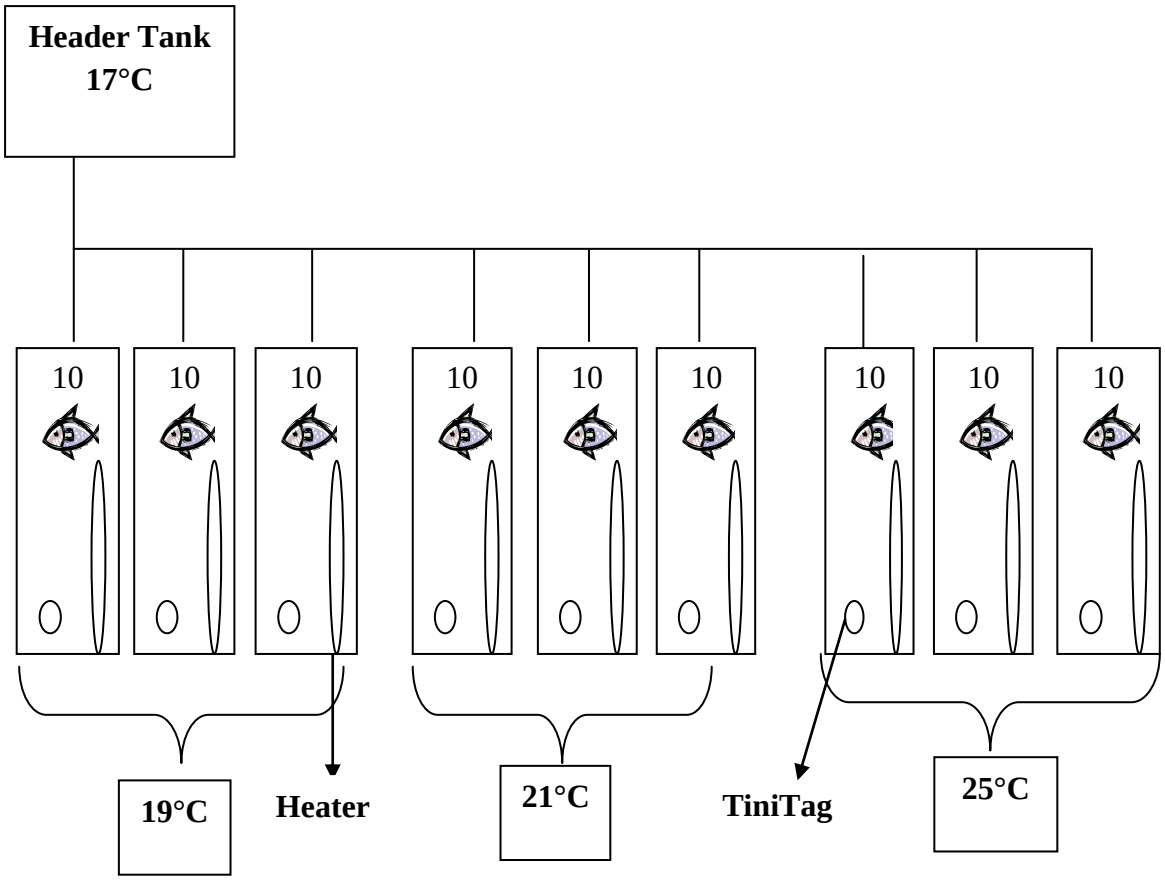


Figure 5.12. Diagrammatic representation of the experimental set-up. There were three tanks for each water temperature: 19°C ±0.5°C; 21°C ±0.5°C; 25°C ±0.5°C. The header tank feeding all tanks was set to 17°C. Tanks at 21°C and 25°C were also heated with individual Visi-Therm heaters with water temperature recorded continuously by Tinitags. Tanks were insulated with polystyrene tiles on sides and back, and a plastic lid placed on top of each.

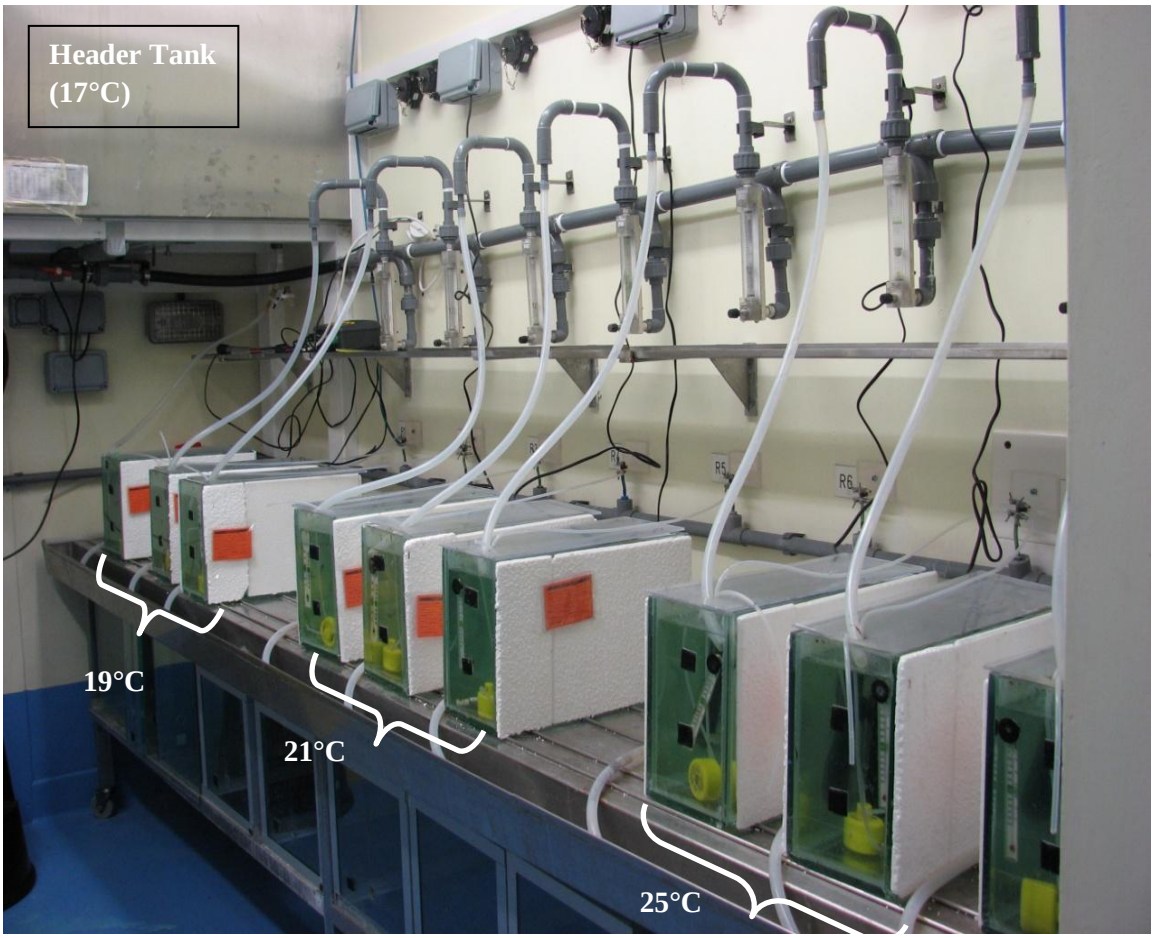


Figure 5.13. Photograph of the actual experimental set-up, showing the position of the header tank in relation to the 9 individual fish tanks.

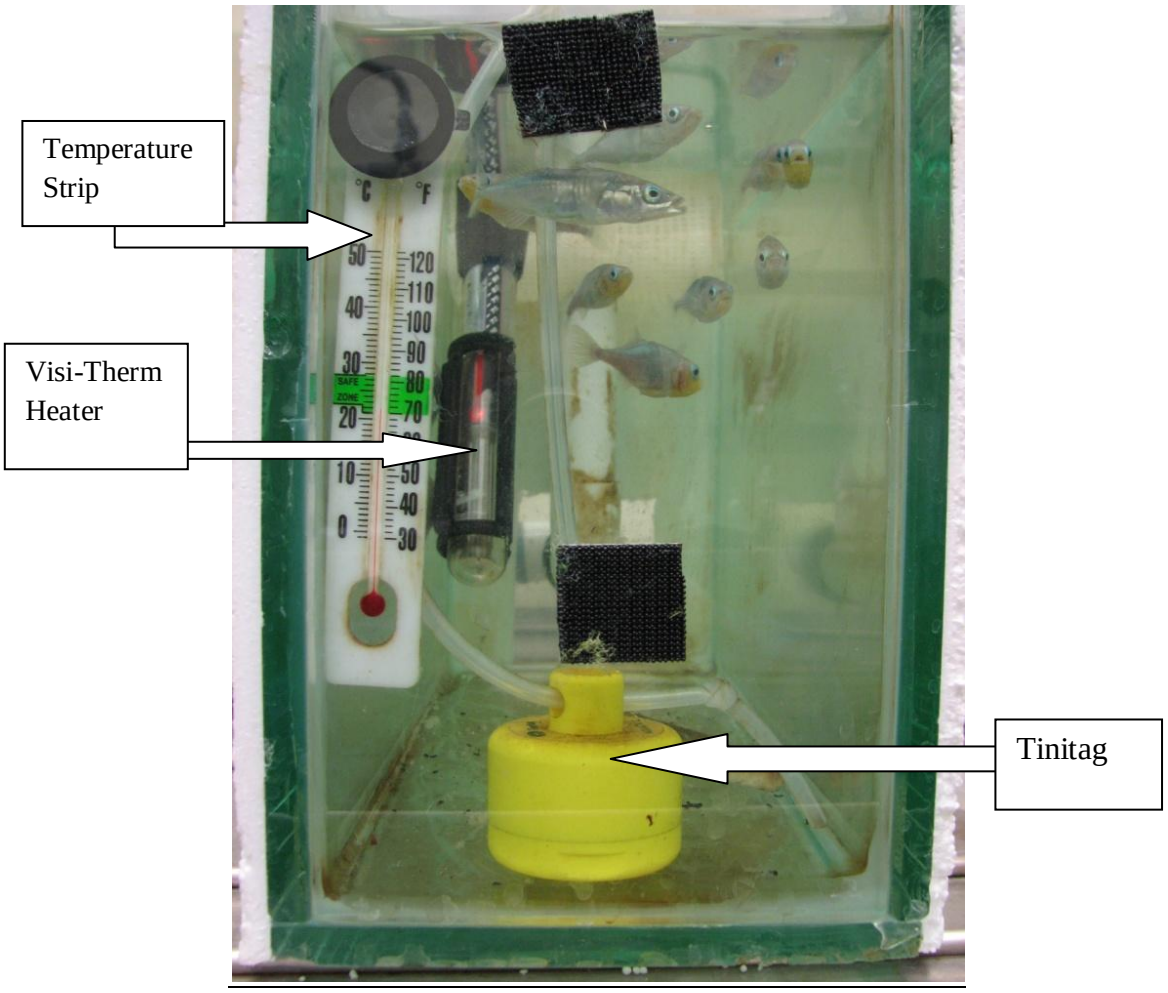


Figure 5.14. Photograph of the inside of a tank, showing the Visi-therm heater, thermometer, Tinitag and 10 three-spined sticklebacks.

5.6.4 Sampling Methodology

5.6.4.1 Ventilation Rate

Breathing rate was estimated by counting the opercula movements covering the gills, with each opercula movement representing a breath. For each observation, the time taken for 30 opercula movements was determined. With fish remaining in their experimental tanks, they were acclimated to the presence of an observer for 10 minutes prior to the official observation period. Ten minutes was deemed to be sufficient time for fish to become acclimated to my presence. This was determined in a pilot study where frozen blood worm was added to the front of the tank and I timed how long it took for fish to feed in my presence. Within 9 minutes, all fish in the tank were feeding and had resumed 'normal' behaviour. Whilst efforts were made to ensure each fish in the tank was observed, by taking note of particular markings or defining features of individuals, it cannot be guaranteed that all individuals were observed. In order to increase the chances of random sampling, thirty observations were made for each tank both prior to the temperature increase and at the end of the experiment. Thus a total of 90 observations for each temperature treatment were recorded at the start and the end. Fish were observed each time between 8-9am, prior to their morning feed, as there can be increased breathing post-feeding, due to digestion (Bry, 1982). Ventilation Rate was calculated as:

$$\text{Ventilation Rate} = (30 \text{ gill movements} / \text{ time taken}) \times 60$$

5.6.4.2 Termination of Experiment

Fish were sacrificed by lethal overdose in MS222 and destruction of the brain. Each fish was weighed (nearest 0.01g) and its length measured (nearest 0.1mm) using vernier callipers. The caudal peduncle was severed and blood collected using a heparised capillary tube. Blood was decanted into microcentrifuge tubes kept on ice. Whole blood was centrifuged for 4minutes at 1400rpm (126 g). Plasma was collected and stored at -20°C for determination of glucose concentration. The remaining blood cells were processed for blood cell counts by flow cytometry,

as described in section 5.5.2.2. The body cavity was opened and the liver removed and weighed, for calculation of the Hepatosomatic Index.

5.6.4.3 Hepatosomatic Index and Condition Factor

The length of each fish was measured with venier callipers to the nearest 0.1mm, and wet weighed to the nearest 0.01g. The liver was excised from each fish and weighed to the nearest 0.01g. Fulton's condition factor (K) and Hepatosomatic Index (HSI) were calculated as described in 5.2.4.2.

5.6.4.4 Water Cortisol Concentration

500mL of water was collected from the outflow pipes between 8-9am on sampling days. This time was prior to the morning feed, to prevent post-feeding cortisol spikes (Sebire *et al*., 2007). Samples were collected: once at 19°C before rising temperatures, +1hr after experimental temperatures were achieved, +1 day, +2 days, +5 days, then once every week for the duration of exposure. 2.5ml of methanol was added to the sample and each was then placed in a freezer for 45mins to prevent biological degradation. The water sample was pumped through a Solid Phase Extraction (SPE) cartridge (C_{18}, Cayman Chemicals). Cortisol is then eluted from the SPE Cartridge with 5ml of methanol, which was then evaporated under a steady stream of nitrogen. The remaining residue was then re-constituted in ELISA buffer and stored frozen at -20°C until subsequent analysis using an ELISA. Water cortisol concentrations are calculated as described in 5.4.3.5.

5.6.4.5 Blood Glucose Concentration

Blood samples were collected from each individual by severing the caudal peduncle. Blood samples were centrifuged at 14 000g for 10 minutes at 4°C. The plasma was pipette off without disturbing the white buffy layer. Due to the small volumes of plasma collected, samples were pooled, with plasma from three

individuals resulting in one sample. Therefore plasma was not utilised for glucose analysis for the remaining individual in each tank due to insufficient volume for analysis. This provided three samples per tank and therefore nine samples per temperature treatment. Plasma samples were then stored in the freezer at -80°C until later analysis. 15µl of plasma per sample was used for analysis, which allowed for three repeat readings (at 5µl each). Analysis was carried out using a Glucose Assay Kit (Cayman Chemicals).

5.6.4.6. Neutrophil: Lymphocyte Ratio

Isolated white blood cells were prepared as described in section 5.5.2.2 and analysed using a flow cytometer; 20,000 cells were analysed. Two previously set gates (A & B) calculated the relative percentages of agranulocytes and granulocytes (N: L ratio).

5.6.4.7. Statistical Analysis

Statistical analysis was conducted using SPSS v15. For non-normally distributed data (as for ventilation rates), non-parametric testing was conducted using Mann-Whitney U-test and Kruskal-Wallis. Intra-thermal regime tank differences were analysed by conducting a one-way ANOVA for each biological endpoint to determine whether there were differences between tanks for each temperature treatment. Inter-thermal regime differences were subsequently analysed by one-way ANOVA followed by a Tukey's post-hoc test to determine the significance of differences between temperature regimes. For hepatosomatic index and condition factor, a two-way ANOVA was used to factor in gender as well as temperature effects. For hepatosomatic index and condition factor, an LSD pair-wise comparison was used to compare the means within factors instead of Bonferroni as there were less than four levels in the factors (three for temperature and 2 for gender), and this type of post-hoc test is better at reducing chances of making a Type 1 Error in these cases. The Sum of Squares Type 111 approach was used as it is an un-weighted

means method which was needed in order to deal with the unequal sample sizes, as there were different numbers of male and female fish in each temperature regime.

5.7. Results

5.7.1 Temperature

After an initial three week settling in period, tanks were slowly raised from ambient temperature of 16°C to the control temperature of 19°C over a period of two weeks (day 1 to 13). All tanks were then maintained at a temperature of 19°C for a period of four weeks (day 13 to 40). The temperatures were then raised by individual Visi-therm heaters (except in the three control tanks that remained at 19°C). Temperatures were increased in three tanks to 21°C over a period of 18 days and another three tanks to 25°C over a period of 23 days (day 41 to 64). This slow increase in temperature was used to prevent acute thermal stresses to the fish and allow them to gradually acclimate to their new thermal environment.

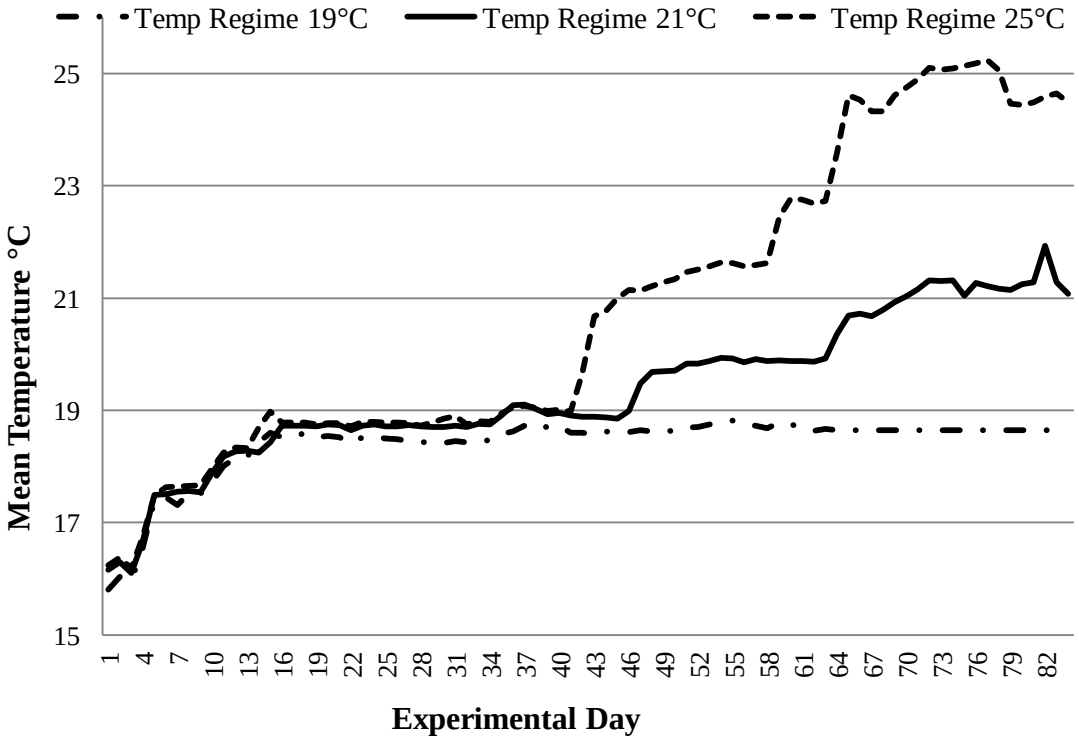


Figure 5.15. Mean water temperature for each experimental regime (n=3) over the entire experimental period. Raising water temperature from acclimation of fish to tanks at 19°C occurred between days 13 and 40, raising temperature occurred between days 41 and 64, and exposure to the desired raised temperatures occurred between days 65 and 85.

The temperatures over the 3 weeks (day 65 to 85) for each regime were 18.7°C±0.23 (target 19°C), 21.1°C± 0.26 (target 21°C) and 24.7°C± 0.3 (target 25°C). There was no overlap in temperature regimes and the fluctuations were smaller than reported in similar studies.

5.7.2 Dissolved Oxygen Concentration

Dissolved oxygen concentrations were recorded twice daily throughout the experimental period.

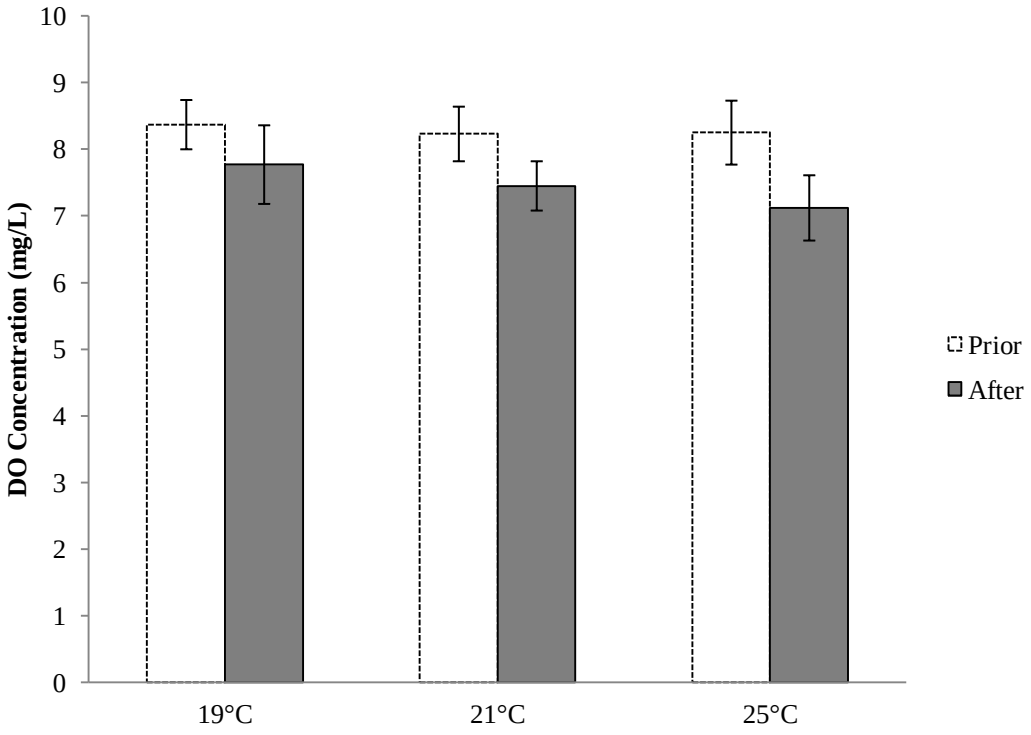


Figure 5.16. Concentrations of dissolved oxygen in the fish tanks for each temperature regime, both prior to increasing the temperature (i.e. all tanks at 19°C) and after raising temperatures to 21°C (n= 3 tanks) and 25°C (n=3 tanks). There was no significant difference in DO prior to raising the temperature. There were significant decreases in DO after water temperature had been raised for both 21°C and 25°C (one-way ANOVA, F [2,11], $p<0.05$).

At the start of the experiment, when all tanks were set to 19°C, there were no differences among the tanks. Once temperatures had been raised in 6 of the tanks, the oxygen concentration fell as the water temperature increased. Therefore, the three

tanks at 25°C had a lower dissolved oxygen concentration than the tanks at 21°C and 19°C. This is to be expected given that warmer water holds less oxygen. However, in all tanks the mean dissolved oxygen concentrations were higher than 7mg/l, and hence were the above the concentrations known to be stressful for *G.aculeatus* (Pottinger *et al*., 2011).

5.7.3 Gender allocation

Fish were assigned randomly to tanks without prior knowledge of gender. Although fin clips were taken from each fish for genetic sex determination, it was possible to determine the sex by the appearance of their gonads. Some of the males were also easily identified by the development of an orange/red throat, which they acquire in times of sexual maturity and reproduction (Aoki, 2010).

Table 5.5. Numbers of male and female fish in each temperature regime (3 tanks per regime).

	Female	*Male*	*Ratio (F:M)*
19°C	10	19	0.5
21°C	8	22	0.36
25°C	18	12	1.5

Despite the random nature in which fish were allocated to tanks, there were a higher proportion of females in the tanks at 25°C than at the lower temperatures, where males dominated.

5.7.4 Molecular Stress Response

5.7.4.1 Cortisol Concentrations

Cortisol release rates were determined for each group of fish prior to raising water temperatures, and then at intervals over the experimental period. However, results for the third week were not valid due to error in the ELISA analysis. The mean cortisol release rate at each sampling time for each temperature regime was calculated and plotted.

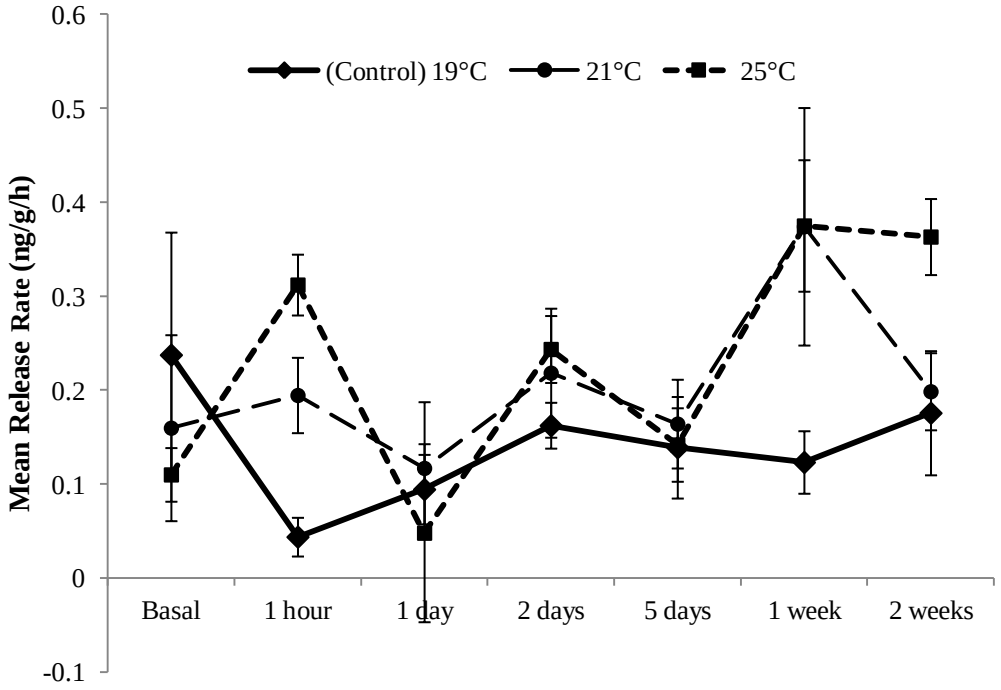


Figure 5.17. Mean (±SEM) cortisol release rates for each temperature regime over a two week period.

Figure 5.17 shows that basal cortisol release rates for all three temperature regimes prior to the experimental period ranged between 0.10 and 0.23ng/g/h. These results agree with the findings from the validation study (where the basal cortisol release rate was 0.12ng/g/h) and also with literature, which states that basal rates for the *G.aculeatus* are approximately 0.2ng/g/h (Sebire *et al.*, 2007). The highest

cortisol release rates occurred in the highest temperature regime of 25°C, with 0.37ng/g/h after 1 week at this temperature. Cortisol concentrations at the water temperature of 19°C fluctuated the least, with a maximal cortisol release rate of 0.175ng/g/h. The concentrations of cortisol appeared to fluctuate over the course of the study for each temperature regime (Figure 5.17). Whilst no intra-thermal regime differences were detected (i.e. within each temperature regime), there were inter-thermal regime differences (i.e. between temperature regimes). The mean concentrations of cortisol were statistically higher in the tanks held at warmer water temperatures of 25°C (Figure 5.18). Water samples from fish held at 21°C did not have significantly elevated cortisol concentrations ($p=0.072$). However, with a larger sample size, the apparent increase may have become significant.

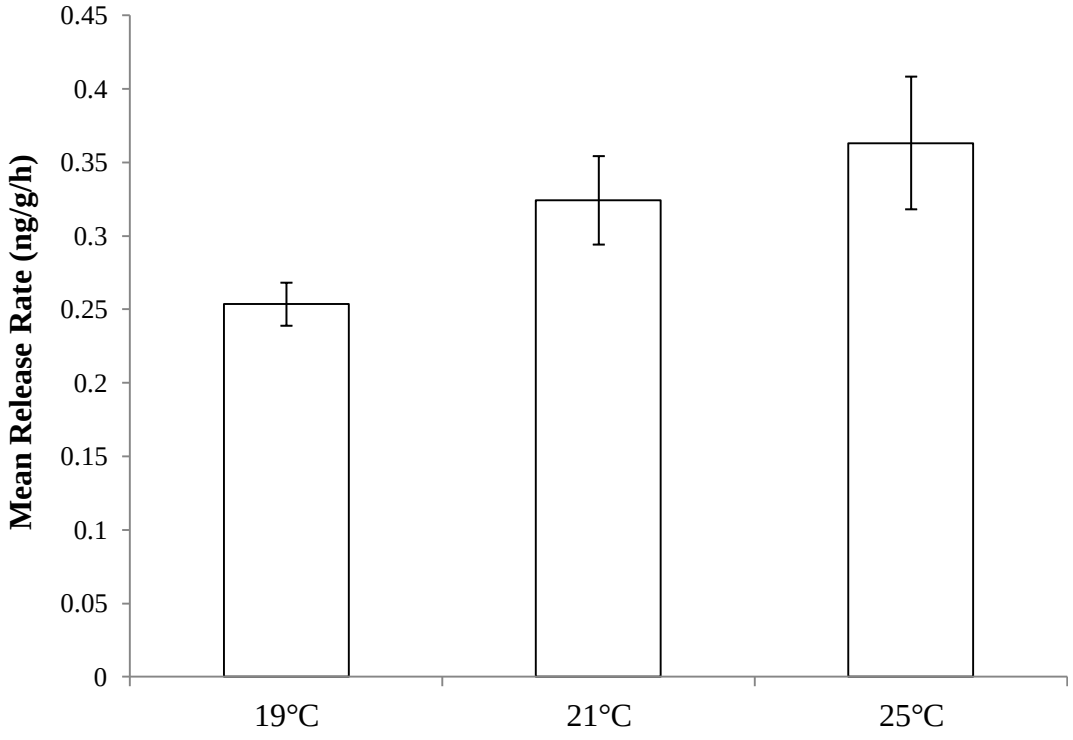


Figure 5.18. Mean (±SEM) cortisol release rates for each temperature regime over the two week experimental period. There was a significant increase in water cortisol concentration with temperature (Log10 transformed for normality, one-way ANOVA, $F [2, 6.30]$ $p<0.05$). Tukey's post-hoc test showed that water cortisol concentrations for fish held at 25°C were significantly higher ($p<0.05$) than those at 19°C.

Mean cortisol release rates fell in the range previously recorded in a similar study (Sebire *et al.*, 2007), however the mean release rate was much lower than the values reported for this species when acutely stressed (3.5ng/g/h) (Scott & Ellis, 2007). Given that temperature was slowly increased and the fish were not handled in anyway, it would have been very surprising if the release rates in this experiment were as high as Scott & Ellis reported. Therefore, the results for cortisol suggest that a higher temperature of 25°C was indeed stressful but the stress was chronic rather than acute.

5.7.4.2 Glucose Concentrations

Pooled plasma samples were analysed for glucose concentration at the end of the experiment. Plasma samples were pooled for reasons described in 5.6.4.5.

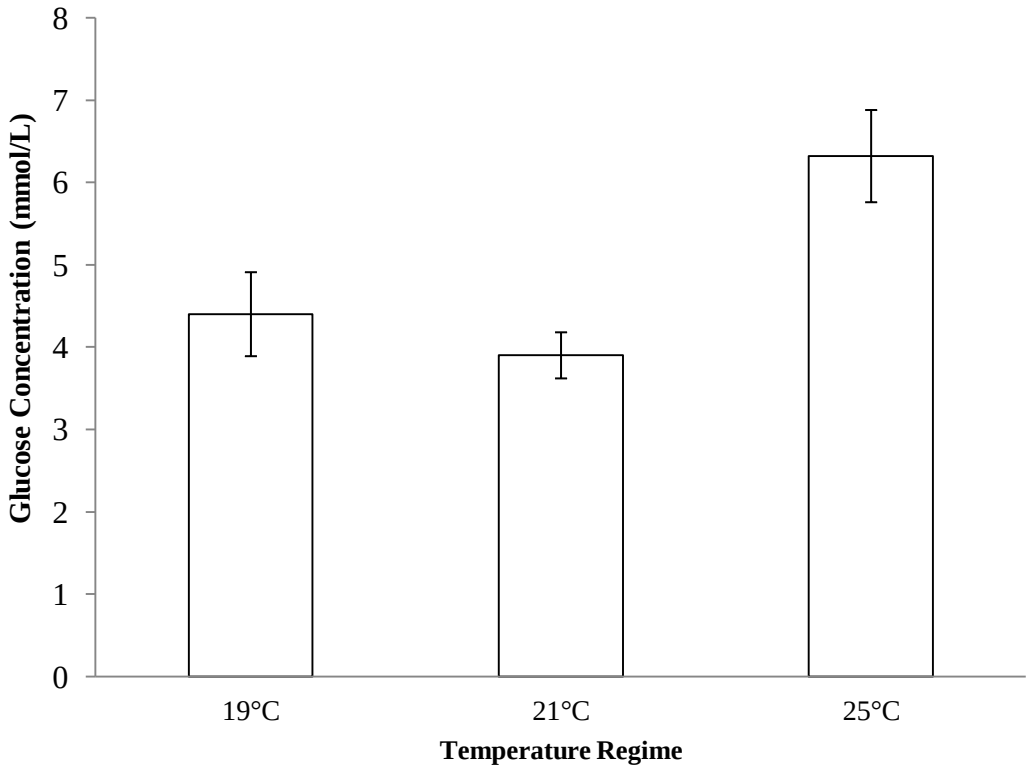


Figure 5.19. Mean glucose concentrations from pooled plasma samples of fish held in each temperature regime for 3 weeks (±SEM bars) (n=9 per temperature regime). There was a significant increase in plasma glucose concentrations with temperature (one-way ANOVA, F [2, 7.47] p<0.005). Tukey's post-hoc test showed that glucose concentrations of fish held at 25°C were significantly higher (p<0.05) than those held at 19°C or 21°C (21°C not different from 19°C).

No statistical differences were detected in plasma glucose concentrations between tanks within the 19°C and 21°C temperature regimes. However fish held in the middle tank at 25°C had significantly higher plasma glucose concentrations than those in the other two tanks held at 25°C ($p<0.05$). Whilst there was no difference in the mean glucose concentrations between fish held at 19°C and 21°C (4.40mmol/L and 3.90mmol/L, respectively), there was a significant (1.6-fold) increase in blood glucose concentration in fish held at 25°C (6.32mmol/L). These values for plasma glucose concentrations are within the range occurring in other species of fish exposed to a stress (Martinez-Porchas *et al.*, 2009).

5.7.5 Cellular Stress Response: The N : L Ratio

Based on gate settings determined in preliminary flow cytometry trials (see section 5.5.2.3), the relative percentages of lymphocytes and neutrophils were estimated. Cells captured in Gate A represented agranulocytes (lymphocytes) and those in Gate B represented granulocytes (neutrophils). Although this method did not actually visualise the individual cells for identification, it allows a measure of the relative proportions of cells with similar properties to be recorded for each temperature regime.

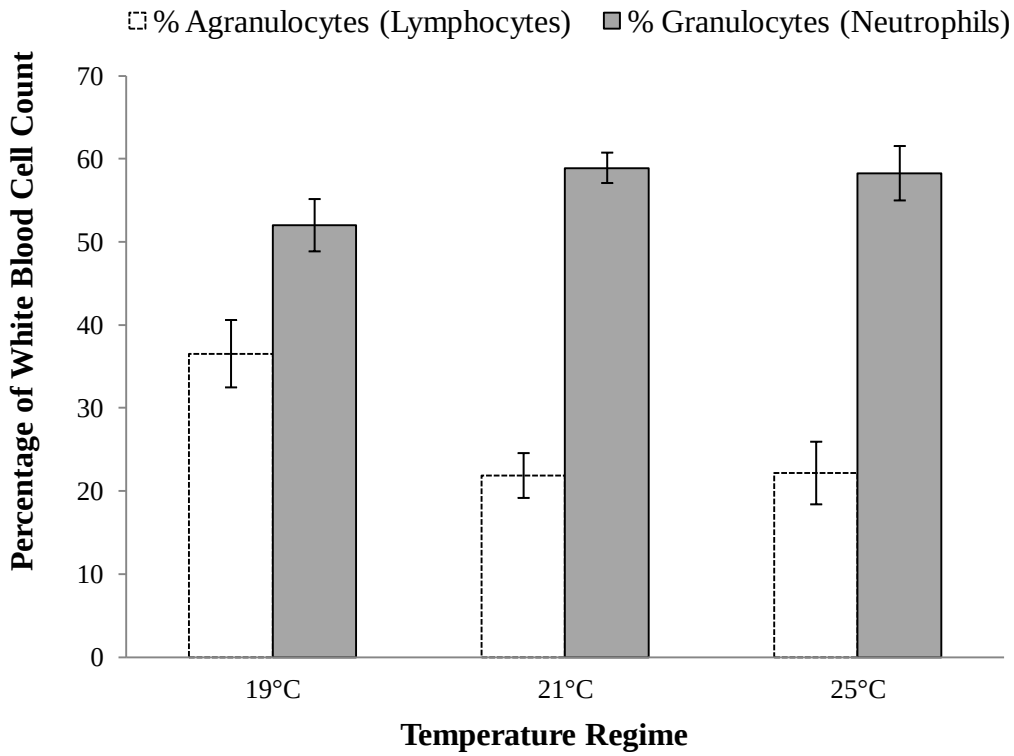


Figure 5.20. Relative percentages of agranulocytes and granulocytes in fish held at different water temperatures. The data for agranulocytes were log10 transformed for normality. A one-way ANOVA (df=2, F=5.7) demonstrated a significant difference between temperatures ($p<0.01$). Tukey's post-hoc test showed a significant difference ($p<0.05$) between 21°C and 25°C compared to 19°C, but no difference between 21°C and 25°C. There were no significant differences in the proportion of granulocytes between treatments.

Figure 5.20 shows that as the water temperature increased from 19°C to 21°C there was a significant decrease in the percentage of agranular cells. As the temperature increased further, the number of agraunlar cells (representing the

lymphocyte population) did not decline further. Conversely the percentage of granular cells representing neutrophils was not affected by water temperature.

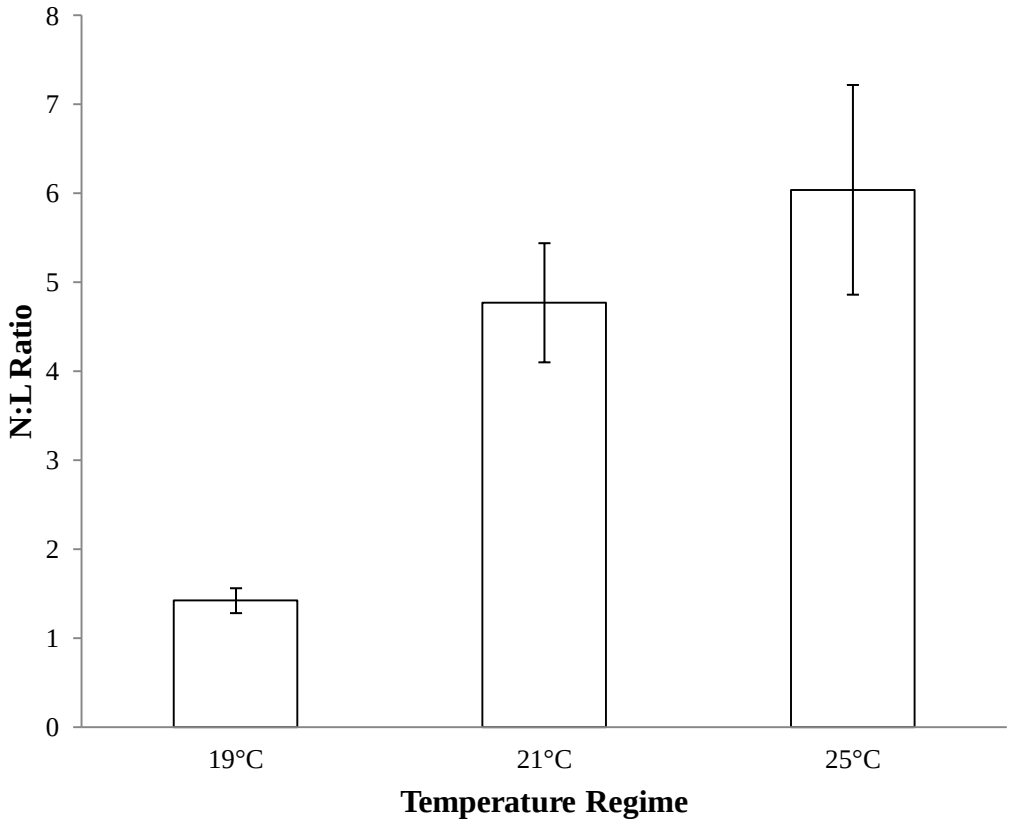


Figure 5.21. The N: L ratio based on the percentage of agraunulocytes and granulocytes determined by flow cytometry (mean ±SEM bars). The data were log10 transformed for normality. A one-way ANOVA indicated a significant difference at the $p<0.05$ level ($df=2$, $F=5.1$). Tukey's post-hoc test ($p<0.05$ level) showed the data for 21°C and 25°C to be significantly different from that of 19°C. There was no significant difference between the 21°C and 25°C treatments.

The N: L ratio is the ratio of neutrophils (granulocytes) to lymphocytes (agranulocytes). No statistical differences were detected in the N: L ratio between tanks within each temperature regime. However, due to the significant decrease in lymphocytes, there was a significant increase in the N: L ratio between 19°C and 21°C (Figure 5.21). Similarly to the data shown in figure 5.19, there was no difference between 21°C and 25°C. Therefore a small chronically elevated temperature increase of 2°C caused a significant response at the cellular level.

5.7.6 Whole Organism Response

5.7.6.1 Ventilation Rate

Ventilation rates, based on the time it took to observe 30 opercula movements, were calculated for fish at the start of the experiment (when all tanks were at 19°C) and after 3 weeks at the experimental temperatures. 30 observations were carried out per tank providing a total of 90 observations per temperature regime at the beginning and 90 observations at the end of the experiment were recorded. Due to the nature of this methodology and the inherent risk of human error, the data were not normally distributed, neither when each tank considered was alone or when the three tanks for each temperature regime were combined. Statistical differences between tanks in each temperature range ($p<0.05$) were found at both the start and end of the experiment, therefore non-parametric testing was conducted. A Kruskal-Wallis test showed that there were differences in the ventilation rate at the start of the experiment between thermal regimes ($H(2) = 9.03$, $p<0.05$), the mean rank values of 116.14 (19°C), 140.22 (21°C) and 150.14 (25°C). Consequently, despite all tanks being held at 19°C at the start of the experiment, the fish in the 3 tanks designated to increase to 25°C had a higher respiration rate. All of these rates are thought to be resting, since fish were given a 10 minute period to adjust to the presence of an observer (see section 5.6.4.1). There were also significant differences in the mean ventilation rate at each temperature regime at the end of the 3 week period ($H(2) = 103$, $p<0.01$) with mean rank values of 67.3 (19°C), 161.23 (21°C) and 177.34 (25°C). A Mann-Whitney U-test was applied to each temperature regime to test for differences between the start and the end ventilation rate. For the fish that remained at 19°C throughout the experiment, there was no significant change in ventilation rate between the start and end of the experiment ($p>0.05$). However, in the tanks where the temperature was increased to 21°C there was a significant rise in ventilation rate ($U=2315$, $p<0.01$) and for 25°C there was also a significant rise in the mean ventilation rate ($U=2467$, $p<0.01$).

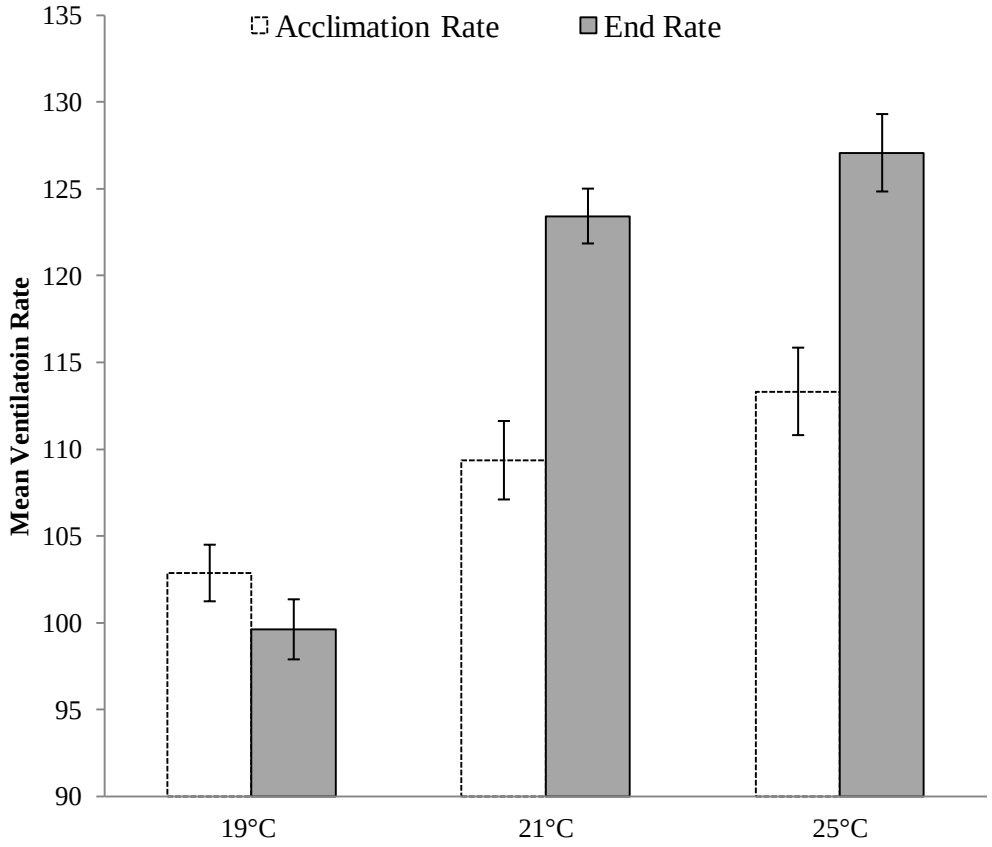


Figure 5.22.Ventilation rates of fish maintained in each temperature regime (90 observations per temperature regime) at the beginning and end of the experimental period (mean ±SEM). There was a significant difference in ventilation rates prior to raising temperature (Kruskal- Wallis, H (2), 9.03 p<0.05) and at the end of the 3 weeks (H(2)= 103, p<0.01). There were significant differences between the acclimation rate and the end rate for both the 21°C and 25°C temperature regimes (Mann- Whitney test, p<0.01).

5.7.6.2 Hepatosomatic Index

For Hepatosomatic Index (HSI) and Condition Factor, a 2 x 3 Factorial ANOVA was used in order to incorporate the effects of gender. This was only possible for these endpoints, as gender was known only at the end of experimentation. No statistical differences were detected for HSI and condition

factor between tanks within each temperature regime. There were no significant effects of temperature, gender, or any significant interaction between the two, for HSI ($p>0.05$).

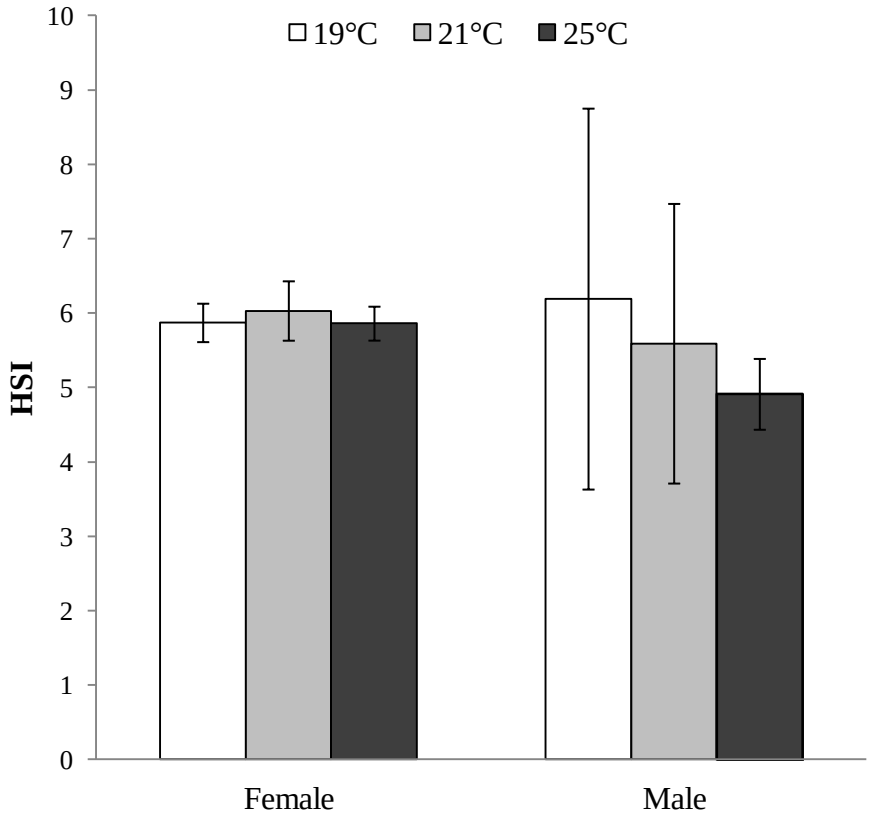


Figure 5.23. The HSI of both male and female sticklebacks held at 19°C, 21°C and 25°C for 3 weeks (mean± SEM). There were no significant differences between gender, temperature or gender x temperature (two-way ANOVA, $p>0.05$).

In times of sexual development, females produce vitellogenin. This egg yolk precursor protein is produced in the liver. Given that higher temperatures can initiate breeding, it was thought that differences in the hepatosomatic index were more likely to exist in females than in males. However, this was not the case, and no significant differences were seen in either sex as the temperatures increased (Figure 5.23). However, males did display greater variability in the HSI than females, particularly at lower temperatures.

5.7.6.3 Condition Factor & Growth Rates

The condition factor (K) was significantly negatively affected by warming the water. There was a significant main effect of temperature on condition factor ($F(2, 0.4) = 5.1$, $p<0.01$), and a significant main effect of gender on condition factor ($F(1, 0.31) = 3.95$, $p=0.05$). A Fisher's Least Significant Difference (LSD) pair-wise post-hoc test showed that there was a significant decrease in condition factor between the control temperature (19°C) and the highest temperature (25°C) and also between 21°C and 25°C. There was no significant interaction between temperature and gender ($F(2, 0.001) = 0.304$, $p>0.05$).

Whilst an increase of 2°C, from 19°C to 21°C, did not have an effect, there was a considerable decline in condition factor when the water temperature was increased to 25°C. Condition factor is an indicator of the growth potential of an individual and so Figure 5.23 indicates that at higher temperatures, the growth of *G.aculeatus* may be reduced.

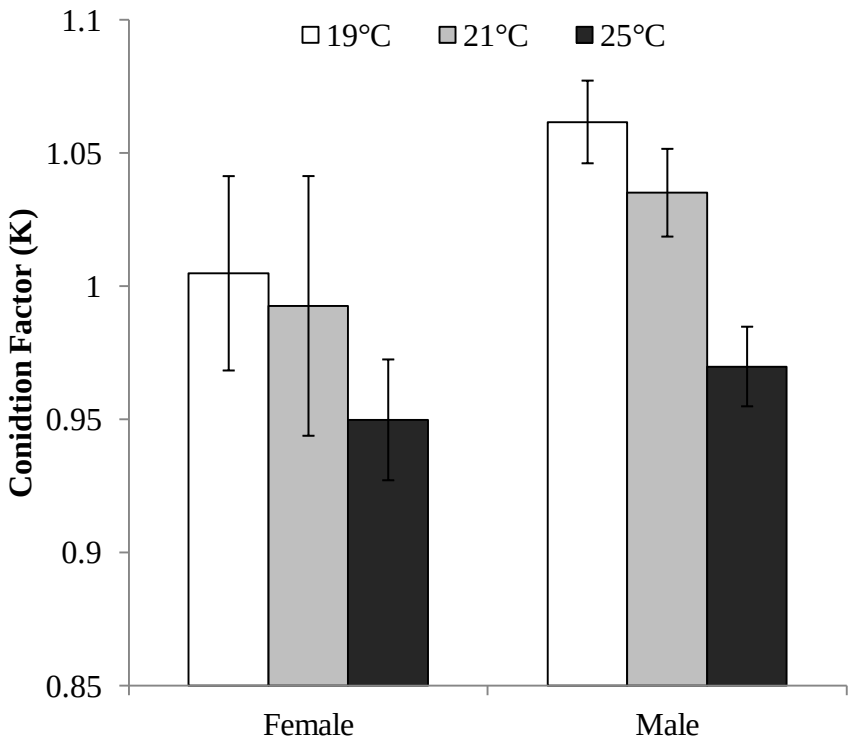


Figure 5.24. The Condition Factor (mean±SEM) of both male and female sticklebacks held at 19°C, 21°C and 25°C for 3 weeks. A two-way ANOVA showed that both temperature (F ([2, 0.4]=5.1, p<0.01) and gender (F[1, 0.31]= 3.95, p=0.05) are significant main factors. LSD post-hoc test showed that the condition factor at 25°C was significantly different from those at 21°C and 19°C (p<0.05). There was no significant interaction between temperature and gender.

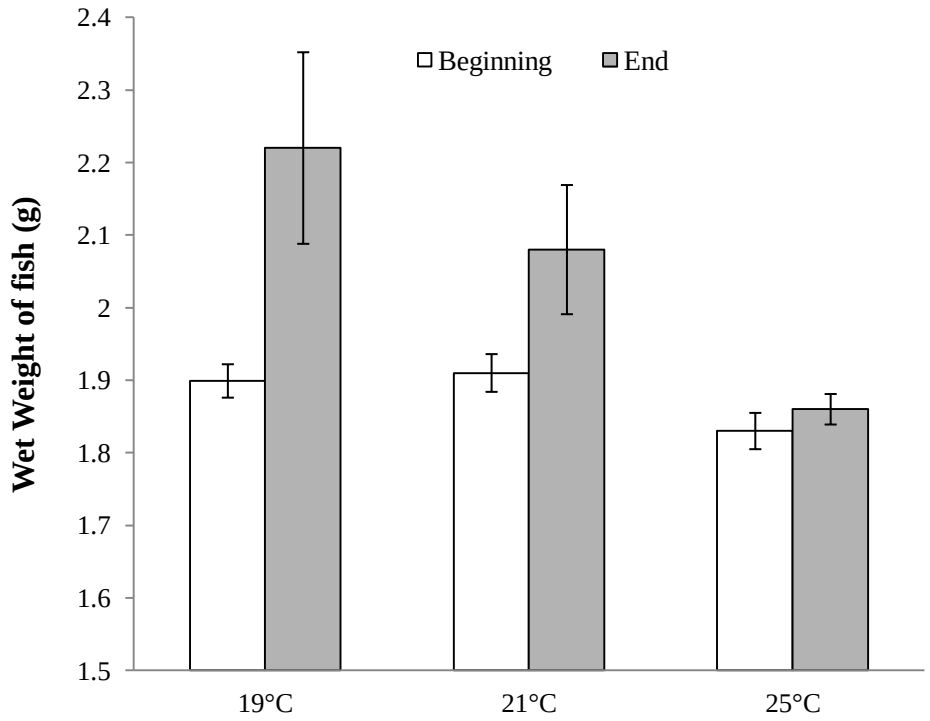


Figure 5.25. The mean (±SEM) beginning and end wet weights of fish held at each temperature regime. A one-way ANOVA showed a significant increase in wet weights of fish held at 19°C ($F[1,16.59]$, $p<0.05$) and 21°C ($F[1, 18.17]$, $p<0.05$). There was no change in wet weight for fish held at 25°C.

The initial weights were determined on 18th January, 2011, and the end weights on 11th April, 2011. No statistical differences were detected in weights of fish between tanks within each temperature regime, either at the start or the end of the experiment. Therefore over this 3 month period there was a significant increase in the average weight of fish held at both 19°C and 21°C, this being greatest at the lower temperature. Conversely, there was no significant increase in weight of those fish held at 25°C, despite them being fed *ad libitum*. This further supports the data shown in figure 5.24, where condition factor (i.e. the potential for growth) had declined in those fish held at the highest water temperature.

Table.5.6 Summary of biological endpoints investigated and their significance.

Organisation level	***End point***	***Significant***	***Effect seen first at 21°C or 25°C***
Biochemical	Cortisol	Significant	25°C
	Glucose	Significant	25°C
Cellular	N:L Ratio	Significant (due to significant reduction in agranulocytes)	21°C
Whole Organism	Ventilation Rate	Significant	21°C
	Hepatosomatic Index	Not Significant	N/A
	Condition Factor	Significant	25°C
	Start and end weights	Significant	25°C

5.8. Discussion

This study is the first known where small increases in water temperature, of only 2°C, elicit a stress response from the biochemical through to the whole organism. Even without the threat of climate change, the water temperatures investigated currently occur in very hot summers and so fish may already be experiencing them. However, these high temperatures may at the moment only be experienced for a short period of time. With climate change, it is anticipated that they will become the average temperatures in summer, and so if prolonged, the implications of this chronic stress are far greater.

It was vital in this study to ensure that the water temperatures in each of the three regimes (19°C, 21°C and 25°C) remained distinctly separate and that there were minimal fluctuations. Whilst in the wild there are circadian variations in water temperature, this study investigated the effects of chronically raised temperature and due to the small differences in experimental thermal regimes, it was important to ensure no overlap between thermal regimes, and therefore to keep fluctuations at a minimum. Using individual thermostatic heaters, insulating the tanks and close monitoring of temperatures allowed water temperature fluctuations to be keep to a minimum, with never more than 0.3°C fluctuation from the desired temperature. Whilst water temperature was successfully controlled, the dissolved oxygen concentration was harder to maintain and there were some differences between tanks, with slightly lower oxygen concentrations in the warmer tanks. Since warmer water can hold less oxygen, oxygen supply was increased in the warmer tanks; however this did not have a significant effect. Even when all the tanks were at the same temperature at the start of the experiment, the oxygen concentrations varied. Attempts to quantify the amount of oxygen supply by counting the number of air bubbles being released from the air stone into each tank were not successful. That being said, the concentration of dissolved oxygen in all tanks was sufficiently high to not pose a stress *to G.aculeatus* (Pottinger *et al*., 2011). Therefore, despite variability in oxygen concentrations, it should not have had an effect on stress levels of the fish.

Temperature Dependent Sex Determination (TSD) is a phenomenon long known to exist in reptiles, and more recently in some species of fish. In fish that are

gonochoristic (i.e. they have separate sexes), sex is usually determined by genotype, i.e. chromosomes (Genetic Sex Determination, GSD). However, environmental parameters, such as temperature, can also affect sex ratios in some species of fish. Given that there were a significantly higher proportion of females in the warmest tanks, it raises the question as to whether the temperature played a role in skewing the sex ratios. Whilst it has been suggested that TSD occurs in 59 species of fish, this is only proven in laboratory studies and not in the wild (Ospina- Alverez & Piferrer, 2008). Furthermore, of all these species, only one true response has been convincingly shown, and that is that higher temperatures produce more males (Goto-Kazeto *et al.*, 2006; Ospina- Alverez & Piferrer, 2008). In these species where TSD has been proved, a 4°C rise in temperature will produce a sex ratio of males to females of 3:1. Genetically female Medaka has been shown to change to phenotypic males at high water temperatures, as a result of elevated cortisol levels (Hayashi *et al*., 2010). However, the opposite was seen with the sticklebacks in this study, with higher water temperatures being linked to a higher proportion of females. Therefore it is unlikely that temperature did in fact alter the sex ratio. Further supporting evidence that this skewed sex ratio was nothing more than chance is that there is only a very small critical period when temperature can alter the sex. This critical period, or the Temperature-Sensitive Period (TSP) usually occurs in the larval stages (Conova & Kynard, 1981; Goto- Kazeto *et al.*, 2006; Ospina- Alverez & Piferrer, 2008). The sticklebacks used in this study were 6 months old at the time of increasing water temperature, and so the TSP had passed. Therefore, it is concluded that the differences in the sex ratios were a result of chance and not water temperature.

Measures were taken to remove or minimise any additional potential stresses. The study was designed to prevent fish from reproducing, as this in itself in an energy demanding process that may have masked the effects of temperature on some endpoints. The study was scheduled to be carried out in the winter months of January and February. However, due to unforeseen delays the experiment did not finish until the beginning of April. Breeding in *G.aculeatus* in the wild begins in mid-April and continues until mid-July (Baggerman, 1985). It is thought that lengthening photoperiod has a stronger control on reproduction than temperature. However, despite advice that a winter photoperiod would be enough to prevent

reproduction (personal communication with Drs T. Pottinger and I. Katsiadaki), the sticklebacks in all tanks did appear to enter sexual maturity. In most tanks, it was possible to identify several sexually maturing males, as indicated by the presence of a developing red throat (never more than orange in this study). Also nest building was evident in all tanks, with the dominant male using food debris and faecal matter to build a nest and using spiggin to glue it together. This nest building behaviour is the point at which a male is considered to be sexually mature (Baggerman, 1985). The males showed territorial behaviour by chasing off any fish, presumably other males that came close to the nest. Females are considered sexually mature when they approach nests to lay eggs (Baggerman, 1985). The aggressive behaviour by nesting males could have contributed to stress levels in other fish in the tanks. Whilst I attempted to avoid this situation by using a winter photoperiod and regular cleaning of tanks, the innate reproductive cycle came into play with the onset of spring. Since sticklebacks are relatively short lived (16-18months [Davies *et al*., 2004]), they may only have one year in which to reproduce, and thus their innate system may prevent them from missing any opportunity, even if environmental conditions may not be optimal.

There was evidence of a stress response at the biochemical, cellular and whole organism level in response to elevated water temperature. At the biochemical level, concentrations of the stress hormone, cortisol, were elevated in fish that were exposed to higher temperatures, suggesting that warmer water was stressful for the fish. Despite the apparent variable nature of cortisol concentrations (Figure 5.16), temperature had a significant effect on the release rates of the stress hormone. The cortisol concentrations in the water were variable over time, with the deviation being greater as temperature increased (SEM values for 19°C: ±0.02, 21°C=± 0.03 and 25°C= ±0.05). Although water samples were taken at the same time of day and prior to feeding, these fluctuations over time reflect the variable nature of cortisol production (Lorenzi *et al*., 2008) and are one of the limitations of using cortisol as a stress indicator. However, despite the increased variation with temperature, higher cortisol concentrations in the water were seen at higher temperatures (Figure 5.17), indicating that the fish held at 25°C were significantly more stressed than those at 19°C. These higher levels of cortisol were present even after 2 weeks after the temperature was increased, suggesting that acclimation to this higher temperature

was not possible. In contrast, at 21°C the cortisol levels were elevated 1 week after the temperature increase but had returned to the basal levels after 2 weeks, suggesting that the fish had acclimated. Water cortisol levels were only significantly elevated 1 week after the temperature was increased for those held at 21°C; therefore it may be possible that the cortisol was elevated not due to temperature, but instead due to another stressor that was present at this time. Temperature and dissolved oxygen concentration records do not appear to have shown any significant discrepancies at this time, and so it is unlikely that this elevated cortisol concentration was due to the experimental set-up. One explanation may be that there was competition or aggression between the fish in these tanks and that this led to a stress response. The results presented in figure 5.16 suggest that a 2°C increase in temperature above the current day summer mean does not elicit a significant cortisol stress response over the experimental period. Given that water temperatures of 21°C are already currently experienced in the River Thames, this lack of cortisol elevation is encouraging, as it indicates that native species may not find these warm temperatures stressful. However, a greater temperature increase of 6°C to 25°C did elicit a stress response at the biochemical level and this was sustained for 2 weeks. There was a 3.4-fold increase in the cortisol concentration in the water of those fish held at this higher temperature. The pattern seen at 25°C (Figure 5.17) to some extent reflects the GAR Model proposed by Martinez-Porchas *et al.* (2009), whereby between 6-48 hours after the initial stress there is an increase in the blood cortisol levels. This is known as the "General Alarm Reaction" (GAR). If, as in chronic stress situations, these conditions continue, the blood cortisol concentration will return to near normal levels but will peak again if the adverse conditions persist further. This second peak in 'GAR' symptoms usually occurs due to energy depletion caused by continued stress.

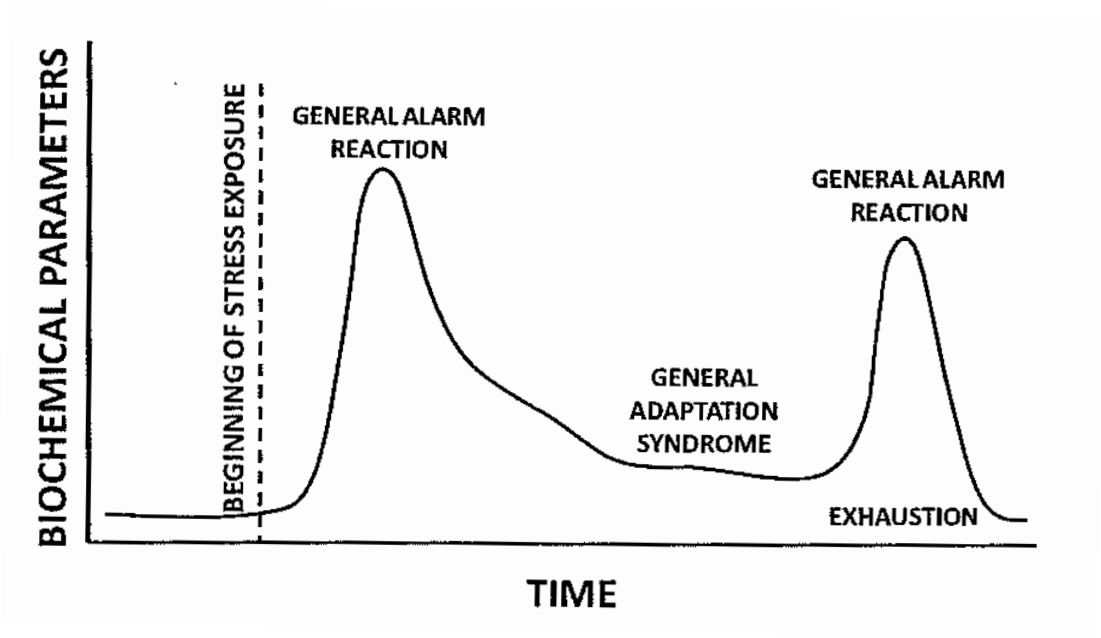


Figure. 5.26. The GAR Model: Biochemical responses for fish exposed to a chronic stress, showing the biochemical processes to restore homeostasis and depletion of energy over time (Martinez- Porchas *et al*., 2009).

The peak in water cortisol concentrations after 1week from those fish held at 21°C may actually be representative of the second GAR symptoms caused by depletion of resources and not by interactions between fish in the tanks. However, this is thought unlikely, since there was no significant peak in cortisol concentration between 1 hour and 24 hours after the initial temperature increase to 21°C. However, this GAR model is more clearly seen in those held at 25°C, where there was an initial peak after 1hour and then again at 1week after the temperature was elevated. Therefore, it may not be that cortisol levels are fluctuating sporadically over this time period, but rather that levels are following the classic patterns of a chronic stress response. Chronic stress and therefore chronically elevated levels of cortisol in the blood are known to have 'knock-on' effects on many other processes.

The first effect of elevated cortisol levels is the release of glucose stored in the liver to provide energy to regain homeostasis. This was evident, as there was a significant increase in the plasma glucose concentrations of fish held at 25°C. A 2°C rise in temperature above the basal water temperature (19°C) did not result in an increase in plasma glucose concentrations. Since the cortisol concentration was not significantly elevated at 21°C, it is logical that the glucose concentration was also

not elevated. Glucose is released from the liver into the blood in response to the production of cortisol in times of stress. This glucose provides the organism with an energy substrate with which to fight the stress and regain homeostasis. There have been some conflicting studies regarding the suitability of glucose concentration as an indicator of stress. For example, Pottinger *et al*., (2002) found that chronically stressed sticklebacks had a reduced concentration of whole body glucose. In contrast, other studies have found that there was an increase in plasma glucose concentration by two to three-fold in fish fed cortisol (Barton *et al*., 1987) or exposed to a synthetic corticosteroid in the water (Kugathas & Sumpter, 2011), although it has been reported to be as high as 30-fold (Martinez-Porchas *et al.,* 2009). The difference can probably be explained by the fact that in the study by Pottinger *et al*. (2002), whole body glucose concentrations were recorded, whereas other studies have used only plasma concentrations. In times of stress, glucose is mobilised from the liver and muscle and released into the blood (Martinez- Porchas *et al*., 2009) and also 'used' to satisfy the increased metabolic demand. Therefore whilst there may be an overall reduction, there will be an increase in the plasma reflecting the influx from liver to blood.

The results from the biochemical endpoints, cortisol and glucose, provide evidence that a chronic increase in water temperature of 6°C above the current summer mean water temperature is stressful to the three-spined stickleback. The results from the cellular endpoints, the N: L ratio, also demonstrate a typical stress response at 25°C, but also at 21°C. Based on the proportions of granular and agranular cells as determined by the flow cytometer, it appears that the circulating number of lymphocytes in the blood declined with increasing water temperature. This lymphopenia or 'trafficking' of lymphocytes out of the blood and into other body components in times of stress is well documented (Davis *et al*., 2008; Dhabhar, 2002). It has also been reported that in times of stress, the number of neutrophils in the blood increases; however this was not evident in this study. The relative proportions of leukocytes (largely, lymphocytes and neutrophils) provide information on the state of the immune system (Dhabhar, 2002). The neutrophil to lymphocyte ratio (N: L) is a reliable indicator of high cortisol levels and therefore a good indicator of stress. (Davis *et al.,* 2008). In this study, the N: L ratio significantly increased with only a 2°C rise in water temperature, even though

increases in cortisol concentrations were only significant at 25°C. This indicates that with high water temperatures already occurring in the summer months for short periods, a stress response at the cellular level could occur. It is unclear why the N: L ratio should have increased when there was no evidence of a significant biochemical stress response at 21°C. The N: L ratio does not explicitly provide information on the ability of an individual to fight infection or disease without challenging the fish to an infection. However, it is still extremely important, as it provides an indication as to an individual's susceptibility to infection compared to another, and can be used as a predictor of an individual's immune-competence (Davis *et al.*, 2008). Based on this, it is concluded that both a 2°C and a 6°C rise in water temperature above current mean summer temperatures are enough to decrease the number of circulating lymphocytes in the blood, potentially weakening the immune system and rendering the fish susceptible to disease.

Cabagma *et al.* (2005) found that there was a high N: L ratio in reproducing fish, providing evidence that reproduction in itself can be a 'stressful' process, presumably due to the energy demands of developing gonads and courtship. Since the fish in this study did enter sexual maturity, there is the possibility that the N: L ratios were affected not only by temperature but also by reproduction. However, sexually mature males were evident in all tanks, even those at 19°C, which had the lowest N: L ratio. Therefore it can be concluded that the higher N: L ratios at higher temperatures were a result of stress from the water temperature and not reproduction.

The negative effects of higher temperatures were also seen at the whole organism level. There were significant differences in the ventilation rates between the different temperature regimes. When all the tanks were set to 19°C, the fish in the three tanks designated to increase to 25°C had a higher respiration rate even at the beginning of the experiment. There are several possible explanations for this. Firstly, the tanks which were set to be raised to 25°C were closest to the door. Therefore, they may have been affected by more interruptions when the door of the room was opened, or that they were nearer the source of noise (coming from the corridor) that may have caused them some stress. However, prior to raising water temperatures, the cortisol release rate was actually lowest in the tanks which were to be raised to 25°C, suggesting that these fish were not experiencing stress. Another possibility is that oxygen availability in these tanks was lower and so the fish had to

respire quicker. Figure 5.16 shows that oxygen availability prior to raising the temperature was significantly lower in the 21°C and 25°C designated tanks, but all tanks had dissolved oxygen levels well above those known to be stressful. However, although the three tanks that were to be raised to 21°C had a lower dissolved oxygen concentration, the ventilation rate of these fish was no different to those in tanks maintained at 19°C. This suggests that more than dissolved oxygen was a factor for the high ventilation rates in the 25°C tanks. A contributing explanation may lie with gender differences. The 25°C tanks had a higher proportion of females in them than did the other 6 tanks (which were male dominated) (Table 5.6). It could be that females have a naturally higher respiration rate, or that the social hierarchies in these tanks posed a stress without additional stresses of temperature. It has been reported that social subordinates have a higher basal metabolic rate (Sloman *et al*., 2000), which could be an explanation here. Before the temperatures were raised to 25°C, there was some evidence that males were reaching sexual maturity, as mentioned above. Territorial behavioural was displayed by some males in all tanks, but it could be that the females were more stressed by this behaviour than other males, therefore having higher ventilation rates. Alternatively, the females themselves could have been entering sexual maturity, which is an energy demanding process, and hence had higher respiration rates. Given that cortisol concentrations were not higher at the start in these tanks, it is most likely that the higher respiration rates are due to females having higher resting metabolic needs.

Some caution should be taken when interpreting the results from the ventilation rates, due to the variable nature as demonstrated by the significant differences between tanks within each thermal regime. However, when the means of each temperature range where considered, as expected, there was no significant change in the ventilation rate of the fish that remained at 19°C. On the contrary, in both sets of tanks where the temperature was increased, there was a significant increase in respiration rate. There was a decrease in dissolved oxygen concentration in tanks at 25°C, but this was only a small decrease, and oxygen levels were still well within the safe limits for sticklebacks, and so it is unlikely that they had an effect. At higher temperatures, fish have a faster metabolism and so need to breathe faster. This study measured the resting rate of respiration, not the maximal. In chapter two, it was demonstrated that in coral reef fish which are living near their

thermal maximum, fish were not able to increase their maximal uptake of oxygen (Nilsson *et al.*, 2009), but whether the same is true for *G.aculeatus* is not known. In these tropical species, the aerobic scope, and therefore the ability of a fish to have sufficient energy for growth, was dramatically reduced with increasing water temperature, due to the increases in resting respiration rate. That is to say, as temperature was increased by 2°C, the fish had to respire much faster in order to maintain normal physiological demands, therefore expending more energy on respiration and leaving less for growth. *G.aculeatus* also displayed this higher resting respiration rate at higher temperatures, and therefore expended more energy on survival and less on growth. Therefore, although this increase in respiration rate may be due to a higher metabolism, if sustained it could result in decreased growth or in a reduced reproductive output.

Condition factor and the HSI declined with increasing temperature; however this was only significant for condition factor. Even with a relatively small increase in temperature of just a 2°C to 21°C (which is currently seen in the warmest days in the River Thames), there was a significant decline in condition factor. Additional increases in temperature did not significantly reduce the condition factor any further. Not only were reductions in condition factor seen, but the actual growth of fish held at the highest water temperature ceased. Usually, higher water temperatures are associated with high assimilation efficiency and therefore higher scope for growth, providing sufficient food is available. However, assimilation efficiency is not always higher in warmer waters (Chinnery & Williams, 2003). Assimilation efficiency was not measured in this experiment, but the sticklebacks were fed 4 times daily until satiation. Whilst it is a possibility that food was limiting, as it was not actually measured and quantified, it is unlikely insufficient food was placed in each tank or that assimilation efficiency was dramatically reduced so as to prevent growth. Therefore it is concluded that fish held at the highest temperature had higher basal metabolic demands and sufficiently stressed to prevent energy being available for growth.

5.9 Conclusions

This study has provided evidence from the biochemical to the whole organism level that a small chronically raised temperature of 2°C is enough to elicit a stress response in *G.aculeatus.* For almost all endpoints there were no significant differences between tanks within each thermal regime. Having three tanks at each temperature regime offers some repeatability in the study, adding strength to these findings and confidence that small increases in temperature were indeed stressful for this species. Whilst a temperature of 21°C is already experienced in the River Thames, it does not last for periods as long as three weeks, and so fish may not suffer the effects of chronic stress. However, with climate change, it is likely that 21°C will become the normal summer mean temperature and not an extreme. A 6°C rise in water temperature to 25°C further increased the stress response, with marked reductions in growth and the efficiency of the immune system. Whilst it is unlikely that 25°C will become the mean summer water temperature, even in a worst-case scenario, there may well be summers when these temperatures are experienced, perhaps even for a chronic period.

The three-spined stickleback is a hardy species, and is not particularly sensitive to temperature in comparison to many other UK fish. If the stickleback is negatively affected by these temperatures, there is concern that more sensitive species, such as the perch, dace or chub to name a few, will be more severely affected. The results of this study suggest that the water temperatures predicted to occur as a consequence of climate change will pose a significant stress to fish in the River Thames, potentially increasing their susceptibility to disease, reducing growth and reproductive output, and therefore having consequences at the population level.

Chapter 6. Summary and Conclusions

Climate change is a truly global concern, and will likely impact all ecosystems, whether terrestrial, freshwater, marine, temperate or tropical. Understanding how species will respond to changes is of paramount importance if they are to be adequately protected. The work presented herein assessed whether small increases in temperature are likely to become stressful for a range of fish, from both marine fish on the Great Barrier Reef to freshwater fish in the River Thames. This thesis has also examined whether or not there is evidence of climate change in the Thames region and whether the fish inhabiting the River Thames have responded to any changes.

Climate change is expected to be greater in the higher latitudes, and hence mainly affect countries such as Britain, but the Great Barrier Reef is considered to be particularly sensitive to changes in climate. Some families of fish, such as cardinalfish, on the Great Barrier Reef are already living near their upper thermal limits and are particularly sensitive to small increases in temperature. In four of the five species of coral reef fish tested, their aerobic scope was significantly reduced by as little as 2°C rise in water temperature (31, 32 and 33°C, compared to the current summer mean of 29°C). The reduced aerobic scope was due to increased resting oxygen consumption and an inability to increase the maximal oxygen uptake. However, there were interfamilial differences, with the two species of cardinalfish, *Ostorhinchus cyanosoma* and *O.doederleini* being more sensitive to increases in temperature than the three species of damselfish tested (*Dasyllus aruanus*, *Chromis atripectoralis* and *Acanthochromis polyacanthus*). The differences in ability to cope aerobically with warming waters will likely lead to a change in the community structure on coral reefs.

The anthropogenic inputs of CO_2 into the atmosphere are absorbed by the oceans, leading to a lowering of pH, an effect known as Ocean Acidification. Even at control temperatures (29°C), a lowered pH of 0.3 units caused the same percentage loss in aerobic scope as did a 3°C warming. When coupled with warming water, low pH poses a significant physiological challenge to some coral reef fish. These results

provide evidence that climate change will directly affect coral reef fish, whereas until now it was considered that reef fish would be primarily impacted indirectly, through a loss of coral cover due to mass coral bleaching events. It is likely that over the coming century there will be a loss of some species, such as cardinalfish, in tropical coral reefs resulting in potentially less diverse and productive environments.

Concerning Britain, analysis of a 150 year dataset provided evidence for gradual warming in the Thames region and increased rainfall in winter months, suggesting that the fish in the River Thames are already experiencing climate change. Analysis of a 15 year dataset on fish populations in the River Thames showed that fish have been exposed to some extreme weather events, events that are likely to become more frequent and intense. Cyprinid species all displayed a similar pattern in density and biomass over this time period. This pattern was different to those of all the non-cyprinid fish population, suggesting that families of fish may respond differently to changes in the climate. A Naive Bayes Feature Selection identified that water temperature has a greater affect on the fish population of the River Thames than flow rates. Bayesian Networks were able to correctly identify key relationships in the network, both between fish and the physical environment and also the interactions between different species of fish. Bayesian Networks also indicated that cyprinid species may benefit from the warm-and-dry summers that are predicted to become typical with climate change.

This study also examined the effects of chronically elevated water temperature, realistic of that expected as a consequence of climate change, on the stress response system of the three-spined stickleback, *Gasterosteus aculeatus,* which is native to Britain. Temperature regimes were selected based on the current day summer mean of 19°C as a control and a best case scenario (B1, +2°C) and a worst case scenario (A1F1, +6°C) temperature prediction by the end of 2080 (IPCC, 2007). For a period of 3 weeks, fish were held in tank water of either 19°C, 21°C or 25°C. Even a small increase of 2°C (above current summer mean) resulted in a stress response at the cellular and whole organism level. A 6°C rise in temperature resulted in a stress response at the biochemical level (higher cortisol and glucose concentrations), cellular level (higher neutrophil: lymphocyte ratio) and whole organism level (higher ventilation rate and lowered condition factor and growth). Therefore, even a chronic increase of just 2°C above the current day summer mean

temperature, which can occur already in very warm summers, is enough to elicit a stress response. Further warming to 25°C, which although unlikely to become the 'normal' summer mean, may well be experienced in warmer years, could have a negative effect from the biochemical to the whole organism level, with reductions in condition factor and growth rates. *G. aculeatus* is considered to be temperature tolerant and a hardy species. Therefore, these results indicate that climate change may indeed prove to be stressful, even for resilient species, with dire consequences for more temperature-sensitive species.

This research has confirmed that there is evidence of climate change in the Thames region and therefore that fish in the River Thames will already have been exposed to warming. Despite the many differences in environmental conditions, habitat types and fish community structures between the River Thames and the Great Barrier Reef, this thesis has demonstrated that fish from both ecosystems are sensitive to increases in temperature. A chronic increase of just 2°C above the current summer mean was enough to prove stressful for all species of fish tested. Given the inertia in the climate system, we will undoubtedly see rises in air and therefore water temperature in the coming century. For important ecosystems such as the Great Barrier Reef and the River Thames, whilst fish communities may still exist in 100 years from now, the assemblages of fish may be very different and potentially much less diverse. Given the results from these *in-vivo* studies and the Bayesian networks, it is clear that the threat of climate change is real and its impacts on fish, regardless of whether freshwater, marine, temperate or tropical, needs serious consideration.

This thesis has attempted to highlight some of the key impacts that climate change will likely have on fish. However, in this large field there is undoubtedly much more research to be covered. Following on from this study there are a number of pathways that could be followed. Firstly, whilst the respirometry techniques used in chapters two and three provided strong evidence that warming waters reduced the aerobic scope of coral reef fish, the impacts of experimental stress were not taken into consideration. Despite the fact that this technique has been widely used and accepted (Östlund-Nilsson & Nilsson, 2004; Nilsson *et al.*, 2007; Urbina *et al.*, 2012) and that fish were left to acclimate to the respirometry chamber for 30 minutes before recording oxygen consumption, it cannot be denied that the fish were in an

unnatural environment and therefore the results are not necessarily a true picture of their natural ability to adapt (Martins *et al.*, 2011). Ideally, the respiromtery experiments should be repeated in conjunction with a stress response technique to quantify the amount of stress each fish is subjected to, as this may impact their ability to cope with confinement (Martins *et al.*, 2011). Using the technique employed in chapter five of measuring the concentration of cortisol in the water, in this case in the water held in the respirometer, would clarify whether fish are indeed stressed, and if so, to what extent. This is important in order to estimate how far the readings may differ from their natural respiration rates.

Secondly, it would be of great interest to examine whether there have yet been any changes in the fish population structure at Lizard Island, specifically whether there has been a decline in thermally sensitive species such as the cardinalfish. There has already been warming off the coast of Queensland and so waters in the lagoon over the last century will have increased. It might be the case that presently, the waters are still within the thermal ranges for coral reef fish and so no changes in fish community structure have occurred. However, it may also be the case that fish species, or indeed, families have already been lost from the reefs. There needs to be a benchmark set of what the coral reef fish assemblage is at specific sites on the Great Barrier Reef, in order for future studies to assess the extent of any change. What we consider now as being the benchmark, may already be significantly different from the fish communities on the reefs of yesteryear.

The use of Bayesian Networks in modelling ecological datasets is indeed a useful tool; however, the work from chapter 3 could be progressed to develop the networks to allow more detailed predictive information about the likely effects of small increases in temperature on the fish population in the non-tidal River Thames. The networks used in this thesis are still in their early days of development and it would warrant an entire thesis to properly develop and understand their usefulness in these types of datasets. Additionally, the networks produced did not take into consideration the effects of extreme, one-off events, and the impacts of these should not be underestimated. Building on this study, it would be useful to develop the model further to predict what the implications of wet-and-mild winters and hot-and-dry summers are on the entire fish population of the non-tidal River Thames. Once these general trends have been modelled, Bayesian Networks could then be used to

predict what impacts ‘big events’ will have on the fish population (e.g. extreme high summer temperatures, droughts and flood events). Only once both general trends and extreme events are accounted for can one begin to predict the responses of fish populations to climate change.

The final data chapter on the three-spined stickleback could be repeated using the same endpoints, both using the temperatures used in this thesis and also at smaller increments, such as 1°C above the current summer mean. If the results presented herein are consistently reproducible, it would be of interest to know if even smaller increases in temperature also elicit a stress response. It would also be interesting to investigate whether stress responses are detected at the genome level, such as through changes in the expression for Heat Shock Proteins (HSPs). HSPs are a family of highly conserved proteins that are rapidly encoded for in times of thermal stress. HSPs help cells regain homeostasis in times of stress by binding to denatured proteins and acting as ‘molecular chaperones’ aiding protein synthesis (Basu *et al.*, 2002). The changes in gene expression for HSPs occur at levels far below lethal thresholds (Karouna-Renier & Zehr, 1999) allowing HSPs to be good indicators of not just acute but also chronic stress, such as can be anticipated with climate change.

This study could also be developed to determine whether the stress responses detected would, in fact, lead to declines at the population level due to reductions in fecundity and susceptibility to disease. Therefore, one might want to expand upon this research and allow the fish to reproduce to enable egg counts to be carried out and also to expose the fish to a pathogen and test the immune-competence of the fish. By allowing the fish to reproduce would also permit further studies on genetic adaptation, to determine whether or not offspring from fish held at higher temperatures are able to tolerate higher temperatures themselves. Although it has been widely accepted that climate change is occurring at a rate faster than evolution, recent research by Donelson *et al*. (2012) has demonstrated that at least one species of fish on the Great Barrier Reef is able to produce offspring that are more thermally suited to a warmer environment. This gives some hope that future generations of fish will be able to cope with our changing climate. Whether this is true of freshwater fish in Britain, is currently not known and merits investigation.

Given that most freshwater fish in the non-tidal River Thames live well within their thermal range, it could be thought that increases in temperature prove to be beneficial for most species. If the predictions of climate change hold true, and Britain experiences more frequent warm-dry summers, we may well see an increase in the cyprinid population, such as roach and bleak. However, this may be accompanied by a decline in the population of non-cyprinid species, such as perch. It appears that the perch population in the non-tidal River Thames is already declining. Increasing competition from a growing roach population may result in further declines and a potentially unsustainable number of perch. Therefore, 100 years from now, the non-tidal River Thames may still support a healthy number of fish; nevertheless, it is likely that it will less diverse. However, I believe that the warming waters have the potential to be stressful for even the coarse fish population, given the results from the study on the three-spined stickleback. If, in the coming years, there are particularly warm summers, fish populations both on the Great Barrier Reef and River Thames may be negatively impacted. This could lead to reduced reproductive output which if occurs over several consecutive years, could result in localised population crashes.

Predictions are also that spring will arrive 1-3weeks (FSBI, 2007) earlier which could result in fish spawning earlier. Whilst this may seem beneficial, since it would give fry a longer summer in which to grow before winter, thus reducing over-winter mortality rates, this can only happen if the appropriate food sources are available. Therefore, there is a risk that there will be a mis-match between prey abundance and predator requirements. Many larvae are known to display diapauses, whereby adverse environmental conditions prevent development and instead the animal enters a state of dormancy. Given that environmental conditions induce and conclude these periods of diapause, a changing climate and extreme events, could cause many invertebrate larvae to develop at the wrong time. It has been reported that photoperiod is the main driver that induces the summer diapause in the copepod, *Acartia bifilosa* (Chimnery & Williams, 2003). Whilst it has also been reported that photoperiod is the main driver in sexual development for the three-spined stickleback (Baggerman, 1985), this did not hold true in this thesis. *G. aculeatus* entered sexual maturity at the onset of spring, regardless of temperature and photoperiod. Their innate reproductive cycle caused them to initiate breeding,

despite environmental conditions being unfavourable. Therefore, this suggests that that there may be discrepancies between the responses of invertebrates and fish to environmental cues. This could have profound effects on the chances of survival of fish, particularly in the first year of life.

Invertebrates are particularly sensitive to their physical environment; therefore changes in the climate are likely to have just as great an effect, if not more so, on a plethora of organisms. A decline in the food source therefore may have more of a profound effect on fish populations than changes in the temperature, pH or flow rates. This study did not take into account the food supply and the effects that climate change would have on them, and how this would impact fish populations. This is a major limitation to the study and one that should be addressed in future studies.

Another potential problem with climate change is the introduction of new, non-native species. For example zander, *Stizostedian lucioperca*, a warm water species, originally from mainland Europe which was illegally introduced into British rivers in the 1980's. The presence of zander has been reported in the River Trent and Warwickshire Avon, and they are known to be able to depress juvenile populations of fish in rivers (Nunn *et al*., 2007a). Zander recordings were also made by the Environment Agency for the lower reaches of the non-tidal River Thames in years 2007-2009. Although at present they are not a great risk, given that zander are a warm water species, it is likely to do well as waters warm and could therefore pose a threat to our native species through increased predation and competition. This is just one species of introduced fish that could be a potential threat to an already stressed ecosystem, but there are likely to be more. Thereby further complicating the ability to predict what the fish populations of the future will look like.

There is clearly much more work to be done on the subject of this thesis, all beyond the scope of this PhD. However, this should not detract from the findings presented herein, but instead it would support the conclusion that fish, whether freshwater, marine, temperate or tropical will undoubtedly be affected by climate change and that much more research is needed in order to adequately protect these important and precious ecosystems.

Chapter 7. References

Adams, S. (1990) Status and use of biological indicators for evaluating the effects of stress on fish. In: *Biological Indicators of Stress in Fish. American Fisheries society Symposium*, 8, pp.1-8.

Allan, G., & Maguire, G. (1992) Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon* Fabricius. *Aquaculture*, 107, pp. 33-47.

Anderson, D. (1990) Immunological indicators: Effects of environmental stress on immune protection and disease outbreaks. In: *Biological Indicators of Stress in Fish. American Fisheries society Symposium*, 8, pp. 38-50.

Araujo, F., Williams, W., & Bailey, R. (2000) Fish assemblages as indicators of water quality in the middle Thames Estuary, England (1980-1989). *Estuaries*, 23, pp. 305-317.

Arnell, N. (1998) Climate change and water resources in Britain. *Climate Change*, 39, pp. 83-110.

Arnell, N., & Reynard, N. (1996) The effects of climate change due to global warming on river flows in Great Britain. *Journal of Hydrology*, 183, pp. 397- 424.

Baggerman, B. (1985) The role of biological rhythms in the photoperiodic regulation of seasonal breeding in the sticklebacks *Gasterosteus aculeatus*, L. *Netherlands Journal of Zoology*, 35, pp. 14-31.

Barrionuevo, W., & Fernandes, M. (1998) Time-course of respiratory metabolic adjustments of a South American fish, *Prochilodus scrofa*, exposed to low and high temperature. *Journal of Applied Ichthyology*, 14, pp. 37-41.

Barton, B., Schreck, C., & Barton, L. (1987) Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions and stress responses in juvenile rainbow trout. *Diseases of Aquatic Organisms*, 2, pp. 173-185.

Basu, N., Todgham, A., Ackerman, P., Bibeau, M., Nakano, K., Schulte, P. & Iwama, G. (2002) Heat shock proteins genes and their functional significance in fish. *Gene*, 295, pp.173-183.

Bellwood, D., Hoey, A., & Choat, H. (2003) Limited functional redundancy in high diversity systems: resilience and ecosystem function on coral reefs. *Ecology Letters*, 6, pp. 281-285.

Biro, P., Post, J., & Booth, D. (2007) Mechanisms for climate-induced mortality of fish population in whole-lake experiments. *Proceedings of the National Academy of Sciences*, 104, pp. 9715-9719

Bouck, G. (1980) Etiology of gas bubble disease. *Transactions of the American Fisheries Society*, 109 (6), pp703-707.

Brashares, J.S. (2010). Filtering wildlife. *Science*, 329, pp. 402-403.

Brian, J., Harris, C., Runnalls, T., Fantinati, A,, Pojana, G., Marcomini, A., Booy, P., Lamoree, M., Kortenkamp, A., & Sumpter, J. (2008) Evidence of temperature-depended effects on the estrogenic response of fish: implications with regard to climate change. *Science of the Total Environment*, 397, pp. 72-81.

Brian, J., Beresford, N., Walker, J., Pojana, G., Fantinati, A., Marcomini, A., & Sumpter, J. (2009) Hypoxia does not influence the response of fish to a mixture of estrogenic chemicals. *Environmental Science and Technology*, 43, pp.214-218.

Bry, C. (1982) Daily variations in plasma cortisol levels of individual female rainbow trout *Salmo gairdneri*: evidence for a post-feeding peak in well-adapted fish. *General and Comparative Endocrinology*, 48, pp. 462-468.

Burel, C., Person-Le Ruyet, J., Gaumot, F., Le Roux, A., Severe, A., & Boeuf, G. (1996) Effects of temperature on growth and metabolism in juvenile turbot. *Journal of Fish Biology*, 49, pp. 678-692.

Cabagma, M., Lajmanovich, R., Stringhini, G., Sanchez-Hernandez, J., & Peltzer, M. (2005). Hematological parameters of health status in the common toad *Bufo arenarum* in agroecosystems of Santa Fe Province, Argentina. *Applied Herpetology*, 2, pp.373-380.

Caldeira. K., & Wickett. M.E. (2003) Oceanography: Anthropogenic carbon and ocean pH. *Nature*, 425, pp. 365.

Chaui-Berlinck, J., Monteiro, L., Navos, C., & Bicudo, J. (2002) Temperature effects on energy metabolism: a dynamic system analysis. *Proceedings of the Royal Society of London, B*, 269, pp. 15-19.

Chellappa, S., Huntingford, F., Strang, R., & Thomson, R. (1995) Condition factor and hepatosomatic index as estimates of energy status in male three-spined sticklebacks. *Journal of fish biology*, 47, pp. 775-787.

Chezhian, A., Kabilan, N., Kumar, S., & Selvan, S. (2011). Influence of different calcium levels and low pH of water on the plasma electrolyte regulation of a fresh water teleost fish *cyprinus carpio* Var. communities (*Linnaeus*, 1958). *Current research journal of biological sciences*, 3(2), pp. 147-154.

Chimnery, F., & Williams, J. (2003). Photoperiod and temperature regulation of diapauses egg production in *Acartia bifilosa* from Southampton Water. *Marine Ecology Progress Series*, 263, pp.149-157.

Claiborne, J., Edwards, S., & Morrison-Shetlar, A. (2002) Acid-base regulation in fishes: Cellular and molecular mechanisms. *Journal of Experimental Zoology*, 293, pp. 302-319.

Clarke, A., & Johnston, N. (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, 68, pp. 893-905.

Conover, D., & Kynard, B. (1981) Environmental sex determination: interaction of temperature and genotype in fish. *Science*, 213, pp. 577-579.

Crampton, V., Berheim, A., Gausen, M., Næss, A., & Hølland, P. (2003) Effects of low oxygen on fish performance. Oxygen levels in commercial salmon farming conditions. *EWOS Perspective*, 2, pp8-13.

Crick, H. (2004) Impact of climate change of birds. *Ibis*, 146 (1), pp. 48-56.

Crick, H., Dudley, C., Glue, D., & Thomson, D. (1997) UK birds are laying eggs earlier. *Nature*, 388, pp. 526.

Currie, S., Reddin, K., McGinn, P., McConnell, T., & Perry, J. (2008) β Adrenergic stimulation enhances the heat shock response in fish. *Physiology and Biochemical Zoology*, 81(4), pp. 414-425.

Daufresne, M., Roger, M., Capra, H., & Lamouroux, N. (2003) Long-term changes within the invertebrate and the fish communities of the upper Rhone River: effects of climatic factors. *Global Change Biology*, 10, pp. 124-140.

Davies, C., Shelley, J., Harding, P., McLean, I., Gardiner, R., & Peirson, G. (2004) *Freshwater fishes in Britain; the species and their distribution*. Denmark, Apollo Books.

Davis, A., Maney, D., & Maerz, J. (2008) The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*, 22, pp. 760-772.

DeWitt, T., Sih, A., & Wilson, D. (1998) Costs and limits of phenotypic plasticity. *Trends in Biochemical Science*, 13, pp. 77-81.

Dhabhar, F. (2002) Stress induced augmentation of immune function- the role of stress hormones, leukocyte trafficking and cytokines. *Brain, Behaviour and Immunity*, 16, pp. 785-798.

Donelson, J., Munday, P., McCormick, M., & Nilsson, G. (2011) Acclimation to predicted ocean warming through developmental plasticity in a tropical reef fish. *Global Change Biology*, 17, pp. 1712-1719.

Donelson, J., Munday, P.L., McCormick, M., & Pitchet, C. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, 2, pp. 30-33.

Dose, V., & Menzel, A. (2004) Bayesian analysis of climate change impacts in phenology. *Global Change Biology*, 10, pp. 259-272.

Dulvy, N., Rogers, S., Jennings, S., Stelzenmüller, V., Dye, S., & Skjoldal, H. (2008) Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. *Journal of Applied Ecology*, 45, pp. 1029-1039.

Eastman, J., & Devries, A. (1997) Biology and phenotypic plasticity of the Antarctic notothenild fish *Trematomus newnesi* in McMurdo Sound. *Antarctic Science*, 9, pp. 27-35.

Eaton, G., & Scheller, R. (1996) Effects of climate warming on fish thermal habitat in streams of the United States. *Limnology & Oceanography*, 41, pp. 1109-1115.

Ege, R., & Krogh, A. (1914) On the relation between the temperature and the respiratory exchange in fishes. *Internationale Revue Gesamten Hydrobiologie*, 7, pp. 48-55.

ENDS Report. (2009) Thames Water fined £150,000 for bleach spill. *ENDS Report*, 409, pp. 61-62.

Ellis, T., North, B., Scott, A., Bromage, N., Porter, M., & Gadd, O. (2002) The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology*, 61, pp. 493-531.

Ellis, T., James, J., Sundh, H., Fridell, F., Sundall, K., Scott, A. (2007). Non-invasive measurements of cortisol and melatonin in tanks stocked with seawater Atlantic Salmon. *Aquaculture*, 272, pp. 698-706.

European Environment Agency, (2012) *Climate Change, Impacts and Vulnerability in Europe, 2012. An Indicator- Based Report*.

Evans, D. (1987) The Fish Gill: site of action and model for toxic effects of environmental pollution. *Environmental Health Perspectives*, 71, pp. 47-58.

Fanouraki, E., Papandroulakis, N., Ellis, T., Mylonas, C., Scott, A., & Pavlidis, M. (2008) Water cortisol is a reliable indicator of stress in European sea bass, *Dicentrarchus labrax. Behaviour*, 145, pp. 1267-1281.

Feely, R., Sabine, C., Lee, K., Berelson, W., Kleypas, J., Fabry, V., Millero, F. (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science*, 305, pp. 362-366.

Feunteun, E. (2002) Management and restoration of European eel populations (*Anguilla Anguilla*): an impossible bargin. *Ecological Engineering*, 18, pp. 575-591.

Fishelson, L. (1998) Behaviour, socio-ecology and sexuality in damselfishes (Pomacentridae). *Italian Journal of Zoology*, 65 (1), pp. 387-398.

Fisheries Society of the British Isles, FSBI. (2007) Climate change and the fishes of Britain and Ireland: Briefing paper 4. Fisheries Society of the British Isles, Granta Information Systems, 82A High Street, Sawston, Cambridge, CB2 4H, UK.

Freda, J., & McDonald, D. (1988) Physiological correlates of interspecific variation in acid tolerance in fish. *Journal of Experimental Biology*, 136, pp. 243-258.

Fromm, P. (1980) A review of some physiological and toxicological responses of freshwater fish to acid stress. *Environmental Biology of Fishes*, 5 (1), pp. 79-93.

Gardiner, N., & Jones, G. (2005) Habitat specialism and overlap in a guild of coral reef fishes (Apogonidae). *Marine Ecology Progress Series*, 205, pp. 163-175.

Gardiner, N., Munday, P., & Nilsson, G. (2010) Counter-gradient variation in respiratory performance of coral reef fishes at elevated temperatures. *PLoSOne*, 5 (10): e13299

Gattuso, J-P., Frankignoulle, M., Bourge,I., Romaine, D., & Buddemeier, R. (1998) Effect of calcium carbonate saturation of seawater on coral calcification. *Global and Planetary Change*, 8, pp. 37-46.

Glas, M., Fabricius, K., de Beer, D., Uthicke, S., (2012) The O2, pH and CA2+ microenvironment of benthic formaminfera in a high CO2 world. *PLoS ONE*, 7 (11) e50010. doi:10.1371/journal.pone.0050010

Goto-Kazeto, R., Abe, Y., Masai, K., Yamaha, E., Adachi, J., & Yamauch, K. (2006) Temperature-dependent sex determination in goldfish: establishing the temperature-sensitive period & effect of constant and fluctuating water temperatures. *Aquaculture*, 254, pp. 617-624.

Graham, C., & Harrod, C. (2009) Review paper: Implications of climate change for the fishes of the British Isles. *Journal of Fish Biology*, 74, pp. 1143-1205.

Green, B., & Fisher. R. (2004) Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. Journal of Experimental Marine Biology & Ecology, 299, pp. 115-132.

Grenouillet, G., Hugueny, B., Carrel, G., Oliver, J., & Pont, D. (2001) Large-scale synchrony & inter-annual variability in roach recruitment in the Rhone River: the relative role of climatic factors & density-dependent processes. *Freshwater Biology*, 46, pp. 11-26.

Guinotte, J., & Fabry, V. (2008) Ocean acidification and its potential effects on marine ecosystems. *Annals of the New York Academy of Sciences*, 1134, pp. 320-342.

Guinotte, J., Buddemeier, R., & Kleypass, J. (2003) Future coral reef habitat marginality: temporal and spatial effects of climate change in the Pacific Basin. *Coral reefs*, 22, pp. 551-558.

Havenhand, J., Buttler, F., Thorndyke, M., & Williamson, J. (2008) Near-future levels of elevated pCO_2 reduce fertilisation success in a sea urchin. *Current Biology*, 18, pp. 651-652.

Hayashi, Y., Kubira, H., Yamaguchi, T., shiraishi, E., Yazawa, T., Hirai, T., Kamei, Y & Kitano, T. (2010) High temperature causes masculinisation of genetically female medaka by elevation of cortisol. *Molecular Reproduction & Development*, 77, pp. 679-686.

Hirata, T., Kaneko, T., Ono, T., Nakazato, T., Furukawa, N., Hasegawa, S., Wakabayashi, S., Shigekawa, M., Chang, M-H., Romera, M., & Hirose, S. (2003) Mechanism of acid adaptation of a fish living in a pH 3.5 lake. *American Journal of Physiology- Regulatory, Integrative and Comparative physiology*, 284, pp. 1199-1212.

Hoegh-Guldberg, O. (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research*, 50, pp. 839-866.

Hoegh-Guldberg, O., Mumby, P., Hooten, A., Steneck, R., Greenfield, P., Gomez, E., Harvell, C., Sale, P., Edwards, A., Caldeira, K., Knowlton, N., Eakin, C., Iglesias-Prieto, R., Muthiga, N., Bradbury, R., Dubi, A., & Hatziolos, M. (2007a)

Coral reefs under rapid climate change and ocean acidification. *Science*, 318, pp. 1737-1742.

Hoegh-Guldberg, O., Anthony, K., Berkelmans, R., Dove, S., Fabricus, K., Lough, J., Marshall, P., Van Open, M., Negri, A., & Willis, B. (2007b) Vulnerability of reef-building corals on the Great Barrier Reef to climate change. In: Johnson, J., & Marshall, P. Eds. *Climate Change and the Great Barrier Reef*. Great Barrier Reef Marine Park Authority and Australian Greenhouse Office, Australia.

Hughes, S. (1998) A mobile horizontal hydroacoustic fisheries survey of the River Thames, United Kingdom. *Fisheries Research*, 35, pp. 91-97.

Hughes, T., Baird, A., Bellwood, D., Card, M., Connolly, S., Folke, C., Grosbery, R., Hoegh-Gulderh, O., Jackson, J., Kleypas, J., Lough, J., Marshall, P., Nyström, M., Palumbi, S., Pandolfi, J., Rosen, B., & Roughgarden, J. (2003) Climate change, human impacts and the resilience of coral reefs. *American Association for the Advancement of Science*, 301, pp. 929-933.

Hutchings, P., Ahyoung, S., Byrne, M., Przeslawski, R., & Wörheide, G. (2007) Vulnerability of benthic invertebrates of the Great Barrier Reef to climate change. In: Johnson, J., & Marshall, P. Eds. *Climate Change and the Great Barrier Reef*. Great Barrier Reef Marine Park Authority and Australian Greenhouse Office, Australia.

Inoue, T., Moritomo, T., Tamira, Y., Mamiya, S., Fujino, H., & Nakanishi, T. (2002) A new method for fish leukocyte counting and partial differentiation by flow cytometry. *Fish & Shellfish Immunology*, 13, pp. 379-390.

IPCC. (2007) *Climate Change 2007: Synthesis Report*. An Assessment of the Intergovernmental Panel on Climate Change.

Ishimatsu, A., Hayashi, M., Lee, K., Kikkawa, T., & Kita, J. (2005) Physiological effects on fishes in a high-CO_2 world. *Journal of Geophysical Research*, 110, C09S09 doi: 101029/2004JC002564.

Iwama, G., Afonso, L., Todgham, A., Ackerman, P., & Nakano, K. (2004) Are hsps suitable for indicating stressed states in fish? *The Journal of Experimental Biology*, 207, pp. 15-19.

Iwama, G., Thomas, P., Forsyth, R., & Vijayan, M. (1998) Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries*, 8, pp. 35-56.

Jobling, M. (1997) Temperature and growth: modulation of growth rate via temperature change. In: Wood, C., & McDonald, D. Eds. *Global warming: Implications for freshwater and marine fish*. Society for Experimental Biology, seminar series, 61, pp. 225-255.

Johnson, A., Acreman, M., Dunbar, M., Feist, S., Giacomello, A., Gozlan, R., Hinsley, S., Ibbotson, A., Jarvie, H., Jones, I., Longshaw, M., Maberly, S., Marsh, T., Neal, C., Newman, J., Nunn, M., Pickup, R., Reynard, N., Sullivan, C., Sumpter, J., & Williams, R. (2009) The British river of the future: How climate change and other social and economic pressures might affect 2 contrasting lowland river ecosystems. *Science of the Total Environment*, 407, pp. 4787-4798.

Johnston, I., Clarke, A., & Ward, P. (1991) Temperature and metabolic rate in sedentary fish from the Antarctic, North Sea and Indo-West Pacific. *Oceanography and Marine Biology*, 109, pp. 191-195.

Kakuta, I., & Murachi, S. (1997) Physiological response of Carp, *Cyprinus carpio*, exposed to raw sewage containing fish processing wastewater. *Environmental Toxicology and Water Quality*, 12, pp. 1-9.

Karouna-Renier, N., & Zehr, J. (1999). Ecological implications of molecular biomarkers: assaying sub-lethal stress in the midge *Chironomus tentans* using heat shock protein 70 (HSP-70) expression. *Hydrobiologia*, 401, pp.255-264.

Knutzen, J. (1981) Effects of decreased pH on marine organisms. *Marine Pollution Bulletin*, 12, pp. 25-29.

Kuchorczyk, D., Luczynski. M., Kujawa. R., & Czerkies. P. (1997) Effect of temperature on embryonic and larval development of bream (*Abramis brama*, L.). *Aquatic Sciences*, 59, pp. 214-224.

Kugathas, S. & Sumpter, J. (2011) Synthetic glucocorticoids in the environment: first results on their potential impacts on fish. *Environmental Science & Technology*, 45, pp. 2377-2383.

Kurihara, H., Shimode, S., & Shirayama, Y. (2004) Sub-lethal effects of elevated concentrations of CO_2 on planktonic copepods and sea urchins. *Journal of Oceanography*, 60, pp. 743-750.

Lake, P. (2003) Ecological effects of perturbation by drought in flowing waters. *Freshwater Biology*, 48, pp. 1161-1172.

Langley, P., & Sage, S. (1994) Induction of selective Bayesian classifiers. *Proceedings of the Tenth Conference on Uncertainty in Artificial Intelligence* , pp. 399-406. Seattle, WA: Morgan Kaufmann.

Leclercq, N., Gattuso, J., & Jaubert, J. (2002) Primary production, respiration and calcification of a coral reef mesocosm under increased CO_2 partial pressure. *Limnology and Oceanography*, 47, pp. 555-564.

Le Morvan-Rocher, C., Troutaud, D., & Deschaux, P. (1995) Effects of temperature on carp leukocytes mitogen-induced proliferation and non-specific cytotoxic activity. *Developmental and Comparative Immunology*, 19, pp. 87-95.

Lorenzi, V., Earley, R., Rodgers, E., Pepper, D., & Grober, M. (2008) Diurnal patterns and sex differences in cortisol, 11-ketotestosterone, testosterone and 17β-estradiol in the bluebanded goby (*Lythryprius dalli*). *General and Comparative Endocrinology*, 155, pp. 438-446.

Lough, J. (2007) Climate and climate change on the Great Barrier Reef. In: Johnson, J., & Marshall, P. Eds. *Climate Change and the Great Barrier Reef.* Great Barrier Reef Marine Park Authority and Australian Greenhouse Office, Australia.

Lucentini, L., Lorenzoni, M., Panara, F., & Mearelli, M. (2002) Effects of short- and long-term thermal stress in perch (*Perca fluviatilis*) determined through fluctuating asymmetry and HSP70 expression. *Italian Journal of Zoology*, 69, pp. 13-17.

Magnuson, J., & Destasio, B. (1997) Thermal niche of fishes and global warming. In: Wood, C., & McDonald, D. Eds. *Global Warming: implications for freshwater and marine fish*. Cambridge: Cambridge University Press.

Marcot, B., Holthausen, R., Raphael, M., Rowland, M., & Wisdom, M. (2001) Using Bayesian belief networks to evaluate fish and wildlife population viability under land management alternatives from an environmental impact statement. *Forest Ecology & Management*, 153, pp. 29-42.

Marnane, M., & Bellwood, D., (2002) Diet and nocturnal foraging in cardinalfishes (Apogonidae) at One Tree Reef, Great Barrier Reef, Australia. *Marine Ecology Progress series*, 231, pp. 261-268.

Martinez-Porchas, M., Martinez-Cordora, L., & Ramos-Enriquez, R. (2009) Cortisol and Glucose: reliable indicators of fish stress? *Pan- American Journal of Aquatic Sciences*, 4 (2), pp. 158-178.

Martins, C., Castanheira, M., Engrola, S., Costas, B., & Conceição, L.C. (2011) Individual differences in metabolism predict coping styles in fish. *Applied Animal Behaviour Science*, 130, pp. 135-143.

Maunder, R., Matthessen, P., Sumpter, J., & Pottinger, T. (2007) Rapid bioconcentration of steroids in the plasma of three-spined sticklebacks, *Gasterosteus aculeatus*, exposed to waterborne testosterone and 17β-oestradiol. *Journal of Fish Biology*, 70, pp. 678-690.

McCormick. M., & Molony, B. (1995) Influence of water temperature during the larval stage on size, age and body condition of a tropical reef fish at settlement. *Marine Ecology Progress Series*, 118, pp. 59-68.

McFarlane, J., & Curtis, S. (1989) Multiple concurrent stressors in chicks: Effects of plasma corticosteroids on the heterophil: lymphocyte ratio. *Poultry Science*, 68 (4), pp. 522-527.

Michaelidis, B., Ouzounis, C., Paleras, A., & Pörtner, H. (2005) Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Marine Ecology Progress Series*, 293, pp. 109-118.

Michaelidis, B., Spring, A., & Pörtner, H. (2007) Effects of long-term acclimation to environmental hypercapnia on extracellular acid-base status and metabolic capacity in Mediterranean fish *Sparus aurata*. *Marine Biology*, 150, pp. 1417-1429.

Milns, I., Beale, C., & Smith, V. (2010) Revealing ecological networks using Bayesian network inference algorithms. *Ecology*, 91(7), pp. 1892-1899.

Mohseni, O., Stefan, H.G., & Eaton, J. (2003) Global warming and potential changes in fish habitat in U.S streams. *Climatic Change*, 59, pp. 389-409.

Mora, C., & Ospina, A. (2001) Tolerance to high temperatures and potential impact of sea warming on reef fishes of Gorgona Island (tropical Eastern Pacific). *Marine Biology*, 139, pp. 765-769.

Moran, R., Harvey, I., Moss, B., Feuchtmayr, H., Hatton, K., Heyes, T., & Atkinson, D. (2010) Influence of stimulated climate change and eutrophication on three-spined stickleback populations: A large scale mesocosm experiment. *Freshwater Biology*, 55, pp. 315-325.

Mulholland, P., Best, G., Coutant, C., Hornberger, G., Meyer, J., Robinson, P., Stenberg, J., Turner, R., Vera-Herrera, F., & Wetzel, R. (1997) Effects of climate change on freshwater ecosystems of the south-eastern United States and the Gulf coast of Mexico. *Hydrological Processes*, 11, pp. 949-970.

Munday, P., Jones, G., Sheaves, M., Williams, A., & Goby, G. (2007) Vulnerability of fishes of the Great Barrier Reef to climate change. In: Johnson, J., & Marshall, P.Eds. *Climate Change and the Great Barrier Reef*. Great Barrier Reef Marine Park Authority and Australian Greenhouse Office, Australia.

Munday, P., Jones, G., Pratchett, M., & Williams, A. (2008a) Climate change and the future for coral reef fishes. *Fish and Fisheries*, 9, pp. 261-285.

Munday, P., Kingsford, M., O'Callaghan, M., & Donelson, J. (2008b) Elevated temperature restricts growth potential of a coral reef fish. *Coral Reefs*, 27, pp. 927-931.

Nilsson, G., Crawley, N., Lunde, I., & Munday, P. (2009) Elevated temperature reduces the respiratory scope of coral reef fishes. *Global Change Biology*, 15, pp. 1405-1412.

Nilsson, G., Östlund-Nilsson, S., Penfold, R., & Grutter, A. (2007) From record performance to hypoxia tolerance: respiratory transition in damselfish larvae settling on a coral reef. *Proceedings of the Royal Society*, 274, pp. 79-85.

Nunn, A., Bolland, J., Harvey, J., & Cowx, I. (2007a). Establishment of self-sustaining populations of non-native fish species in the River Trent and Warwickshire Avon, UK, indicated by the presence of 0+ fish. Aquatic Invasions, 2 (3), pp.190-196.

Nunn A., Cowx, I., Frear, P., & Harvey, J. (2003) Is water temperature and adequate predictor of recruitment success in cyprinid fish populations in lowland rivers? *Freshwater Biology*, 48, pp. 579-588.

Nunn, A., Frear, P., Lee, M., & Cowx, I. (2010) Is there evidence for a shift in fish growth and recruitment success linked to climate change. *Journal of Fish Biology*, 77, pp. 1780-1792.

Nunn, A., Harvey, J., Britton, J., Frear, P., & Cowx, I. (2007b) Fish, climate and the Gulf Stream: the influence of abiotic factors on the recruitment success of cyprinid fishes in lowland rivers. *Freshwater Biology*, 52, pp. 1576-1586.

Nyström, M., Folke, C., & Moberg, F. (2000) Coral reef disturbance and resilience in a human-dominated environment. *Trends in Ecology and Evolution*, 15, pp. 413-417.

Orr, J., Fabry, V., Aumont, O., Bopp, L., Doney, S., Feely, R., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjor, R., Plattner, G-K., Rodges, K., Sabire, C., Sarmiento, J., Schlitzer, R., Slater, R., Totterdel, I., Weing, M., Yamanaka, Y., & Youi, A. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, 437, pp. 681-686.

Ospina-Àlverez, N., & Piferrer, F. (2008) Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern and possible effects of climate change. PLOS ONE, 3.

Östlund-Nilsson, S., Mayer, I., & Huntingford, F. (2007) *Biology of the Three-Spined Stickleback.* Florida, CRC Press.

Östlund-Nilsson., S., & Nilsson, G. (2004) Breathing with a mouth full of eggs: respiratory consequences of mouth-brooding in cardinalfish. *Proceedings of the Royal Society, B,* 271, pp. 1015-1022.

Owen, S., Huggett, D., Hutchinson, T., Hetheridge, M., McCormack, P., Kinter, L., Ericson, J., Constantine, L., & Sumpter, J. (2010) The value of repeating studies & multiple controls: replicated 28-day growth studies of rainbow trout exposed to clofibric acid. *Environmental Toxicology & Chemistry,* 29, pp. 2831-2839.

Parker, R., & Dixon, R. (1998) Changes in a North Carolina reef fish community after 15 years of intense fishing- global warming implications. *Transactions of the American Fisheries Society*, 127, pp. 908-920.

Parmesan, C., & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, pp. 37-42.

Pérez-Casanova, J., Afonso, L., Johnson, S., Currie, S., & Camperl, A. (2008) The stress and metabolic responses of juvenile Atlantic cod *Gadus morhua* L. to an acute thermal challenge. *Journal of Fish Biology*, 72, pp. 899-916.

Perry, S., & Gilmour, K. (2006) Acid-base balance and CO2 excretion in fish: unanswered questions and emerging models. *Respiratory Physiology and Neurobiology*, 154, pp. 199-215.

Perry, A., Low, P., Ellis, J., & Reynolds, J. (2005) Climate change and the distribution shifts in marine fishes. *Science*, 308, pp. 1912-1915.

Petherick, A. (2010) Cold blamed for Bolivia's mass fish deaths. *Nature*, 467, p 617.

Pörtner, H., & Farrell, A. (2008) Physiology and climate change. *Science,* 322, pp. 690-692.

Pörtner, H., & Knust, R. (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, 315, pp. 95-97.

Pörtner, H., Van Dijk, P., Hardewig, I., & Sommer, A. (2000) Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In: Davidson, W., Howard-Williams, C., & Broady. P. Eds. *Antarctic Ecosystems: Models for*

wider ecological understanding. Christchurch, New Zealand. Caxton Press: pp109-122.

Pörtner, H., Langenbuch, M., & Michaelidis, B. (2005) Synergistic effects of temperature extremes, hypoxia and increases in CO_2 on marine animals. From Earth history to global change. *Journal of Geophysical Research*, 110: CO9S10 doi: 101029/2004JC002561

Pottinger, T., Cook, A., Jurgens, M., Rhodes, G., Katsiadaki, I., Balaam, J., Smith, A., & Matthiessen, P. (2011) Effects of sewage effluent remediation on body size, somatic RNA:DNA ratio and markers of chemical exposure in three-spined sticklebacks. *Environment International*, 37, pp. 158-169.

Pottinger, T., Garrick, T., & Yeomans, W. (2002) The three-spined stickleback as an environmental sentinel: effects of stressors on whole-body physiology indices. *Journal of Fish Biology*, 61, pp. 207-229.

Pratchett, M., Munday, P., Wilson, S., Graham, N., Cinner, J., Bellwood, D., Jones, G., Polunin, N., & McClanahan, T. (2008) Effects of climate-induced coral bleaching on coral reef fishes- ecological and economic consequences. In: Gibson, R., Atkinson, R., Gordon, J. Eds. *Oceanography and Marine Biology: An Annual Review,* 46, pp. 251-296.

Randall, D., & Bauner, C. (1991) Effects of environmental factors on exercise in fish. *Journal of Experimental Biology*, 160, pp. 113-126.

Reynaud, S., Leclercq, N., Romaine-Lioud, S., Ferrier-Pages, C., Jaubert, J., & Gattuso, J. (2003) Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Global Change Biology*, 9, pp. 1660-1668.

Rice, J. (1990) Bioenergetics modelling approaches to evaluation of stress in fishes. In: *Biological Indicators of Stress in Fish. American Fisheries society Symposium*, 8, pp. 80-92.

Ricker, W. (1975) Computation and interpretation of biological statistics of populations. *Bulletin of the Fisheries Research Board of Canada*, 191, p382.

Robertson, O., Hane, S., Wexter, B., & Rinfret, A. (1963) The effect of hydrocortisone on immature rainbow trout (*Salmo gairdnerii*). *General and Comparative Endocrinology*, 3, pp. 422-436.

Rombough.P. (1997) The effects of temperature of embryonic and larval development. In: Wood, C., & McDonald, D. Eds. *Global Warming: Implications for freshwater and marine fish.* Society for Experimental Biology, seminar series 61, pp. 177-224.

Roy, D., & Sparks, T. (2000) Phenology of British butterflies and climate change. *Global Change Biology*, 6, pp. 407-416.

Ruane, N., & Komen, H. (2003) Measuring cortisol in the water as an indicator of stress caused by increased loading density in common carp (*Cyprinus carpio*). *Aquaculture*, 218, pp. 687-693.

Saeys, Y., Inza, I., & Larrañaga, P. (2007) A review of feature selection techniques in bioinformatics. *Bioinformatics*, 23, pp. 2507-2515.

Scharsack. J., Kalbe, M., Derner, R., Kurtz, J., & Milinski, M. (2004) Modulation of granulocyte responses in three-spined stickleback *Gasterosteus aculeatus* infected with the tapeworm *Schistocephalus solidus*. *Diseases of Aquatic Organisms*, 59, pp. 141-150.

Schreck, C. (1990) Physiological, behavioural and performance indicators of stress. In: *Biological Indicators of Stress in Fish. American Fisheries society Symposium*, 8, pp. 29-37.

Scott, A., & Ellis, T. (2007) Measurement of fish steroids in water. A Review. *General and Comparative Endocrinology*, 153, pp. 392-400.

Scott, A., Hirschenhauser, K., Bender, N., Oliverira, R., Earley, R., Sebire, M., Ellis, T., Pavlidis, M., Hubbard, P., Huertas, M., & Cancino, A. (2008) Non-invasive measurement of steroids in fish-holding water: important considerations when applying the procedure to behaviour studies. *Behaviour*, 145, pp. 1307-1528.

Sebire, M., Katsiadake, I., & Scott, A. (2007) Non-invasive measurements of 11-Ketotesterone, cortisol and androstenedione in male three-spined stickleback (*Gasterosteus aculeatus*). *General and Comparative Endocrinology*, 152, pp. 30-38.

Sefton, C., & Boorman, D. (1997) A regional investigation of climate change impacts on UK stream flows. *Journal of Hydrology*, 195, pp. 26-44.

Shaw, P. (2003) *Multivariate statistics for the Environmental Sciences*. London. Arnold.

Shirayama.Y., & Thornton. H. (2005) Effects of increased atmospheric CO_2 on shallow water marine benthos. *Journal of Geophysical Research*, 110: CO9508. Doi: 1029/2004JC002618.

Shuter, B., & Meisner, J. (1992) Tools for assessing the impact of climate change on freshwater fish populations. *Geojournal*, 28, pp. 7-20.

Silbergeld, E. (1974) Blood glucose: A sensitive indicator of environmental stress in fish. *Bulletin of Environmental Contamination & Toxicology*, 11 (1), pp. 20-25.

Sloman, K., Motherwell, G., O'Connor, K., & Taylor, A. (2000) The effect of social stress on the standard metabolic rate (SMR) of Brown trout (*Salmo trutta*). *Fish Physiology & Biochemistry*, 23, pp. 49-53.

Sollid, J., Weber, R., & Nilsson, G. (2005) Temperature alters the respiratory surface area of crucian carp *Carassius carassius* and goldfish *Carassius auratus*. *Journal of Experimental Biology*, 208, pp. 1109-1116.

Soloman, S., Plattner, G., Knutts, R., & Friedlingsten, P. (2008) Irreversible climate change due to carbon dioxide emissions. Proceedings of the National Academy of Science of the United States of America, 106 (6), pp1704-1709.

Steffensen, J. (2002) Metabolic cold adaptation of polar fish based on measurements of aerobic oxygen consumption: Fact or artefact? Artefact! *Comparative Biochemistry and Physiology, Part A*, 132, pp. 789-795.

Taylor, E., Egginton, S., Taylor, S., & Butler, P. (1997) Factors which may limit swimming performance at different temperatures. In: Wood, C., & McDonald, D.

Eds. *Global warming: Implications for freshwater and marine fish.* Society for Experimental Biology, seminar series 61, pp. 105-134.

Teh, S., & Hinton, D. (1998) Gender-specific growth and hepatic neoplasia in medaka (*Oryzias latipes*). *Aquatic Toxicology,* 41, pp. 141-159.

Tewksbury, J., Huey, R., & Deutsch, C. (2008) Putting the heat on tropical animals. *Science,* 320, pp.1296-1297.

Thomas, P. (1990) Molecular and biochemical responses of fish to stressors and their potential use in environmental monitoring. In: *Biological Indicators of Stress in Fish. American Fisheries society Symposium,* 8, pp. 9-28.

Tullis, A., & Baillie, M. (2005) The metabolic and biochemical responses of tropical whitespotted bamboo shark *Chiloscyllium plagiosum* to alterations in environmental temperature. *Journal of Fish Biology,* 67, pp. 950-968.

Urbina, M., Glover, C., & Forster, M. (2012) A novel oxyconforming response in the freshwater fish *Galaxias maculates*. *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology,* 161, pp. 301-306.

Van Dijk, P., Tesch, C., Hordewig, I., & Pörtner, H. (1999) Physiological disturbances at critically high temperatures: A comparison between stenothermic Antarctic and eurythermal temperate eelpouts (Zoarcidae). *Journal of Experimental Biology, 202*, pp. 3611-3621.

Verkberk, W., Bilton, D., Calosi, P., & Spicer, J. (2011) Oxygen supply in aquatic ectotherms: partial pressure and solubility together explain biodiversity and size patterns. *Ecology*, 92, pp. 1565-1572.

Vogel, G. (2010). Europe tries to save its eels. *Science*, 329, pp. 505-507.

Wachenfeld, D., Johnson, J., Skeat, A., Kenchington, R., Marshall, P., & Innes, J. (2007) Introduction to the Great Barrier Reef and climate change. In: Johnson, J., & Marshall, P. Eds. *Climate Change and the Great Barrier Reef*. Great Barrier Reef Marine Park Authority and Australian Greenhouse Office, Australia.

Weatherley, N., Campbell-Lendrun, E., & Ormerod, S. (1991) The growth of brown trout (*Salmo trutta*) in mild winters and summer droughts in upland Wales: model validation and preliminary predictions. *Freshwater Biology*, 26, pp. 121-131.

Webb, B., & Walsh, A. (2004) Changing UK river temperatures and their impact on fish populations. *Hydrology: Science and practice for the 21st Century*, 11, pp. 177-191.

Westfall., B. (1945) Coagulation film anoxia in fishes. *Ecology*, 26, pp. 283-287.

Wheeler, A. (1969) *The fishes of the British Isles and North-West Europe*. Macmillian.

Williams, W. (1967) The growth and mortality of 4 species of fish in the River Thames at Reading. *Journal of Animal Ecology*, 36, pp. 695-720.

Winn, C. (2010). *I never knew that about the River Thames*. Edbury Press.

Winter, M., Lillicrap, A., Caunter, J., Schaffner, C., Alder, A., Ramil, M., Ternes, T., Giltrow, E., Sumpter, J., & Hutchinson, T. (2008) Defining the chronic impacts of atenolol on embryo-larval development and reproduction in the fathead minnow (*Pimephales promelas*). *Aquatic toxicology*, 86, pp. 361-369.

Wood, C., & McDonald, D.G. (1997) *Global Warming: implications for freshwater and marine fish*. Cambridge, UK, Cambridge University Press.

Xenopoulos, M., Lodge, D., Alcamo, J., Marker, M., Schulze, K., & Vuurens, D. (2005) Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biology*, 11, pp. 1557-1564.

Yvon-Durocher, G., Montoya, J., Trimmer, M., & Woodward, G. (2010) Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. *Global Change Biology*, 17, pp. 1681-1694.

Zarate, J., & Bradley, T. (2003) Heat shock proteins are not sensitive indicators of hatchery stress in salmon. *Aquaculture*, 223, pp. 175-187.

Chapter 8. Appendices

8.1. Appendix 1. James Cook University Ethical Approval

JAMES COOK UNIVERSITY
Townsville Qld 4811 Australia

Tina Langford, Ethics Officer, Research Office. Ph: 07 4781 4342; Fax: 07 4781 5521

ETHICS REVIEW COMMITTEE
Animal Ethics Committee
APPROVAL FOR ANIMAL BASED RESEARCH OR TEACHING

PRINCIPAL INVESTIGATOR	**Miss Natalie Crawley**				
CO-INVESTIGATOR(S)	Goran Nilsson (University of Oslo)				
SUPERVISOR(S)	Prof Geoffrey Jones & Dr Philip Munday (Marine & Tropical Biology)				
SCHOOL	Marine & Tropical Biology				
PROJECT TITLE	**Effects of ocean temperature and pH on larval fish respiration and lactate production**				
APPROVAL DATE	04 Dec 07	**EXPIRY DATE**	31 Dec 2010	**CATEGORY**	**5**

This project has been allocated Ethics Approval Number with the following conditions:	**A**	**1270**

1. All subsequent records and correspondence relating to this project must refer to this number.
2. That there is NO departure from the approved protocols unless prior approval has been sought from the Animal Ethics Committee.
3. The Principal Investigator is to advise the responsible Ethics Monitor appointed by the Ethics Review Committee:
   - periodically of the progress of the project;
   - when the project is completed, suspended or prematurely terminated for any reason.
4. In compliance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*, and the *Queensland Animal Care and Protection Act 2001*, it is **MANDATORY** that you provide an annual report on the progress of your project. This report must also detail animal usage, and any unexpected event or serious adverse effect that may have occurred during the study.

NAME OF RESPONSIBLE MONITOR	Schwarzkopf, Dr Lin
EMAIL ADDRESS	lin.schwarzkopf@jcu.edu.au
ASSESSED AT MEETING **APPROVED** **Professor Phillip Summers** **Chair, Animal Ethics Committee**	**Date:** 4 Dec 2007 **Date:**
Tina Langford Ethics Officer **Research Office** Tina.Langford@jcu.edu.au	Date: 12 December 2007

8.2. Appendix 2. Great Barrier Reef Marine Park Authority Research Permit

Authorisation to collect using the School GBRMPA Permit (GO6/20234.1)

Surname	Other name	Species	Location	Dates of collection	Number Proposed	Number Actual
Crawley	Natalie	Cirrhilabrus punctatus	Lizard Island	4-22/1/2008	20	
Crawley	Natalie	Paraperus cylindrica	Lizard Island	4-22/1/2008	10	
Crawley	Natalie	Acanthochromis polyacanthus	Lizard Island	4-22/1/2008	40	
Crawley	Natalie	Dasyellus aranus	Lizard Island	4-22/1/2008	40	
Crawley	Natalie	Apogon doederleini	Lizard Island	4-22/1/2008	40	
Crawley	Natalie	Chromis viridis	Lizard Island	4-22/1/2008	40	
Crawley	Natalie	Pomacentrus nagasakiensis	Lizard Island	4-22/1/2008	40	
Crawley	Natalie	Pomacentrus amboinensis	Lizard Island	4-22/1/2008	40	

Purpose of collection: Respritory rates of larval fish

		Tick box
1	Are you aware of the conditions of collection on this GBRMPA Permit?	✓
2	Have you read the conditions of the QDPI permit?	✓
3	It is critical that you notify QDPI within 48 hrs before field work commences.	✓
4	Ensure you have a hard copy of both the DPI and GBRMPA permit on your boat	✓
5	You must be diving as a JCU diver (with appropriate approvals)	✓
6	Ensure you have suitable ID with you in the field	✓

Applicant Name: Natalie Crawley **Signed**

HOS Approval

18/12/07

Date

8.3. Publications resulting from this research

Nilsson G., **Crawley, N.**, Lunde, I., & Munday, P. (2008). Elevated temperature reduces the respiratory scope of coral reef fishes. *Global Change Biology*, 15 (6), pp. 1405-1412.

Munday, P., **Crawley, N**., & Nilsson, G. (2009). Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Marine Ecology Progress Series*, 388, pp. 235-242.

Conferences:

Poster presented at the Post-Graduate Research Poster Conference, Brunel University, March 2012: Assessing the effects of chronic thermal stress on the stickleback, *Gasterosteus aculeatus.*