# The Impacts of Climate Change on Freshwater Fish, Macroinvertebrates, and their Interactions

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

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## Abstract

Climate change has been described as one of the biggest threats to biodiversity and ecosystem functioning with average global temperatures having increased by 0.6° over the past century. Freshwater ecosystems, especially those already suffering from anthropogenic nutrient loadings, are vulnerable to climatic change. Many organisms inhabiting these ecosystems are ectothermic and therefore will be influenced by increasing temperatures either directly through thermal impacts or indirectly through the impact of temperature on resources such as oxygen or food. By utilising a large scale mesocosm approach, this project aims to investigate the influence of a 4°C rise in temperature under realistic nutrient loadings on populations of a small freshwater fish (3 spined-stickleback; *Gasterosteus aculeatus*), macroinvertebrate populations and the trophic interactions between the two (predator-prey relationships).

This study found that warming reduced stickleback population biomass by 60% and nutrient addition reduced biomass by around 80%. Nutrients and warming together resulted in extinction of the stickleback populations. These losses were mainly attributed to the increased likelihood of severe hypoxia in heated and nutrient-addition mesocosms. Macroinvertebrate populations varied in response to warming and nutrient addition dependent upon the taxa, though there was an overall 25% reduction in total biomass of invertebrates in heated mesocosms at final harvest. Five taxa showed a decrease in biomass in response to heating: Chaoboridae, Ephemeroptera, Corixidae, Notonectidae and Gastropoda, whilst biomass of Oligochaeta and Amphipoda increased. Of the taxa that decreased, four can be classed as containing very active species and their total biomass was shown to be directly related to levels of dissolved oxygen. Seasonal data showed similar population trends with no evidence of more severe effects of warming in summer.

The search rate and handling time of sticklebacks preying on four species of macroinvertebrate showed only small variations with a 4°C increase in summer temperature. However, even small changes in handling time produce a change in rank of prey profitability. Optimal diet of sticklebacks preying on invertebrates that were not cryptic was correctly predicted from applying Optimal Diet Theory to the results of laboratory experiments. However, invertebrate populations in the mesocosms were too small to examine whether increasing temperature caused a change in diet breadth as invertebrate numbers were always below critical switch points.

Warming of nutrient-rich waters can thus have dire consequences for freshwater ectotherm populations. The loss even of such a hardy fish species suggests a precarious future for many less tolerant species in such eutrophic systems under current climate change predictions. Population changes can result from lack of resources or alterations of trophic interactions i.e. predator-prey interactions.

# Chapter 1

## Introduction
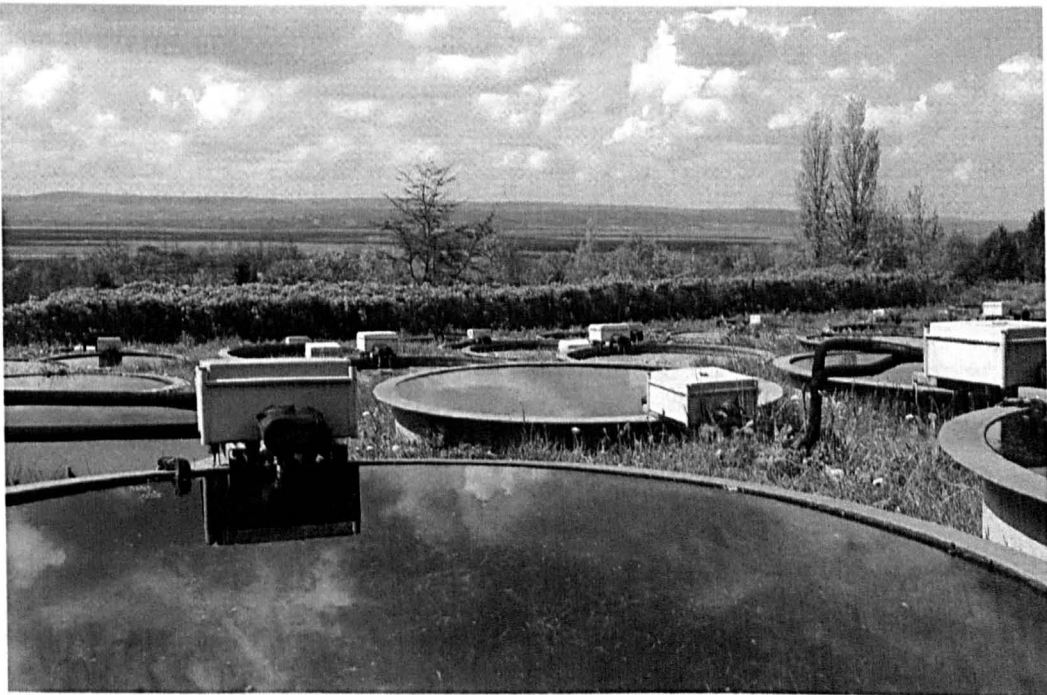
## 1.1 Introduction

### 1.1.1 Climate change

Throughout the history of planet Earth, climate has continually changed. What is unusual about the current change is the rapid rate at which it is occurring and the underlying anthropogenic causes (McDonald & Wood, 1997). Current models predict an increase in average temperature in temperate regions of 3 to 5°C over the next 100 years, with an associated increase in frequency of extremely hot periods and generally warm years (IPCC, 2007). The rapidity of the change leaves little time for the adaptation of organisms that have spent millions of years evolving to their current form and has been described as one of the most important threats to biodiversity and ecosystem functioning (McCarthy et. al., 2001). The individual responses of species are difficult enough to predict but the potential impacts of climate change on communities with complex interactions are seemingly impossible to fathom (Pimm, 2009). Yet steps must be taken, albeit small ones, in understanding how changing temperature will alter individual populations and their interactions.

Aquatic ecosystems contain a diverse range of ectothermic organisms whose metabolic rate and physiological processes are determined by their surrounding temperature. This renders them vulnerable to changing temperatures and could lead to the loss of whole unique ecosystems such as the coral reefs (Reaser, Pomerance et. al., 2000). Despite making up only 0.8% of the Earth's surface, freshwater aquatic ecosystems contain an estimated 6% of all described species (Dudgeon et. al., 2006). Extinction rates of some freshwater fauna are believed to be as rapid as the loss of biodiversity from the rainforests (Ricciardi & Rasmussen, 1999). Shallow lakes in particular are susceptible to changes in climate due to their large surface area to volume ratio resulting in increased

exposure to solar radiation. They remain mixed, even in warmer months, thus lacking deep cool water refuge for organisms. They are also vulnerable to other anthropogenic stressors such as nutrient loading from surrounding land use which may compound the impact of increasing temperature. Current research has shown warming induced changes in lakes such as: shifts in spring peaks of phytoplankton (Scheffer et. al., 2001); changes in concentrations of soluble phosphate, total nitrogen and conductivity (McKee et. al., 2003); increases in plant biomass (Kankaala et. al., 2002) and alterations to macroinvertebrate and fish assemblages (Baulch et. al., 2005; Graham & Harrod 2009). Impacts on individual organisms will not occur in isolation and interactions among members of the whole community need to be examined in order to make any form of prediction about freshwater communities under climate change regimes.

To assess the potential impacts of climate change on shallow lake ecosystems, large-scale replicated experiments which encompass realistic environmental variables are required (Carpenter, 1989). Such studies exist but are costly and therefore few in number. Eutrophication caused by external nutrient loading is an extensive problem in freshwater ecosystems and the conditions it produces, such as low oxygen levels, may be exacerbated by warming. Studies therefore need to incorporate nutrient levels currently entering freshwaters in order to make realistic predictions about climate change. They also need to take into account multiple stressors that may be produced by warming such as a possible increase in cyanobacteria (Elliot et. al. 2006) and alterations to water chemistry including decreased pH (Mckee et. al. 2003) which could in turn facilitate the release of soluble reactive phosphorus from the sediment (Wang et. al.2008).

*1.1.2 Impacts on fish*

Freshwater fish may respond to climate change through a number of interacting factors including increased temperature, changes in precipitation and hydrological regimes and the impact of increasing temperature on other abiotic factors such as oxygen availability. Any response of fish populations is likely to be complex and linked to changes in the entire freshwater community (Graham & Harrod, 2009). Rijnsdorp *et. al.,* (2009), suggest four interlinked climate-driven mechanisms behind changes in fish populations.

1. **Physiological responses.** Temperature has a strong influence on the physiology of fish as it directly influences metabolic rates and growth (Brander, 1995; Blanck *et. al.,* 2007). As temperature increases so will metabolic rates and it is conceivable that at high temperature there will not be sufficient resources, such as oxygen, to fuel this increased rate (Clarke & Johnston, 1999).

2. **Behavioural responses.** Where possible, fish will seek out favourable conditions. This may produce a shift in range for a species, especially for populations that inhabit areas at the edge of their thermal range (Sagarin *et. al.,* 2006). However, fish that inhabit small isolated water bodies such as ponds and small shallow lakes may lack thermal refuge or the ability to escape unfavourable conditions produced by climate change.

3. **Changes in population dynamics through alterations in mortality, reproduction and growth rates.** Early life stages of fish are particularly sensitive to environmental perturbations (Gumaa, 1978) as eggs and embryos cannot escape unfavourable conditions.

4. **Ecosystem level changes and/or trophic interactions.** Climate change will not only act directly on fish but also through alterations in freshwater communities and interactions between fish and their prey/food source. Shifts in timing of life-history events can lead to an uncoupling, or 'mismatch', of predator and prey phenologies, affecting predator-prey dynamics and food web structure and function (Durant *et. al.*, 2007).

In contrast to the marine environment there is currently only a small body of literature examining the impact of climate change on freshwater fish (Graham & Harrod, 2009), with populations showing varying responses dependent upon the species and ecosystem in question. The Arctic charr (*Salvelinus alpinus* (L.)) is one of the most cold-adapted of salmonids (optimal growth 15 – 16°C; (Larsson & Berglund, 1998) and has shown delayed reproduction by 3 – 4 weeks and increased egg mortality when exposed to temperatures greater than 12°C (Jungwirth & Winkler, 1984). Perch (*Perca fluviatilis*) is common in slow moving and standing water across Europe. Embryonic development is most successful between 8 and 18°C in this species (Saat & Veersalu, 1995) and reproductive success could therefore be reduced at increased summer temperatures. As many temperate species have fixed spawning times that are controlled by factors other than changing climate (e.g. photoperiod) it is predicted that they will be vulnerable to reduced reproductive success caused by climate-induced shifts in the timing of food supply such as the spring peak in phytoplankton and zooplankton (Durant *et. al.*, 2007). In contrast, the abundance of seven species of fish in Perialpine lakes demonstrated no significant response to climate change over the course of thirty-one years (Massol *et. al.*, 2007). Major predictions for the freshwater fishes of the British Isles under climate change regimes include the increased abundance of warm-adapted and invasive

species with a reduction in abundance of cold-adapted salmonids (Graham &
Harrod, 2009).

The three-spined stickleback (*Gasterosteus aculeatus* (L.)) inhabits a range of
temperate freshwater ecosystems and meets the criteria for fish as early
indicators of climate change (Holmes, 1990), such as being widely distributed, in
stable abundance, and showing a graded response to a range of temperatures,
thus making it suitable for climate-impact studies. They have a relatively high
thermal tolerance compared with many British species (28°C dependent on
acclimation, salinity and presence of lateral plates; Heuts, 1947; Jordan &
Garside, 1972) and can withstand particularly low oxygen levels without
exhibiting any behavioural signs of distress (1.6 – 2.0 mg $L^{-1}$; Giles, 1987). Their
reproduction is particularly sensitive to warming-related oxygen shortages as the
survival of the embryos is dependent on the male fanning the eggs with
oxygenated water. Even at temperatures well below their thermal tolerance
limit, warming can reduce the amount of time the male spends engaged in
parental care behaviour and therefore the survival of the embryos (Hopkins,
2006). They are therefore likely to show changes in population dynamics
through impacts on reproductive success and physiology at extremes of
temperature and oxygen availability.

### 1.1.3 Impacts on macroinvertebrates

The majority of research on climate impacts on invertebrates has concentrated
on terrestrial species (Heino *et. al.*, 2009). Aquatic invertebrates are much less
studied; a Web of Science search on 'climate change' and 'freshwater
invertebrates' returned only twenty-three results. As previously mentioned,
climate change impacts on freshwater macroinvertebrates are likely to be
difficult to predict due to freshwater ecosystem being exposed to multiple

stressors including changing land use and introduction of non-native species (Sala *et. al.*, 2000). Climate change will not only act on individual species but also on their trophic interactions and therefore the ecosystem. Increased metabolic rates with warming will lead to an increased demand for resources such as oxygen and food which, if not met, may result in increased mortality. An increase in movement, along with metabolic rate will cause alterations in trophic interactions especially between active predators and their prey as encounter frequencies will be altered. Populations may also be altered through shifts in range and changes in phenology (e.g. earlier emergence of chironomid larvae).

### 1.1.4 Impacts on interactions

It is not enough to simply examine the response of a particular organism to climate change in isolation. There is also a range of complex trophic interactions that might be altered due to differential responses at different trophic levels (Freitas *et. al.*, 2007). Changes in population size due to increased mortality or reproduction and changes in the timing of peak reproduction will all have impacts on the next trophic level. Temperature may also act directly upon the interaction itself. Ectothermic predator-prey interactions are particularly vulnerable to changes in temperature as their metabolic rate and speed of movement are directly linked to temperature. Previous studies on freshwater ectotherms (vertebrate and invertebrate) have shown that increasing temperature decreases the time it takes for a predator to search for and handle a prey item (Thompson, 1978; Persson, 1986). Again, this response will not be uniform as prey themselves may increase their ability to escape at higher temperatures. By altering the components of a predator's response to prey, temperature may also alter which prey items are most profitable in terms of energy gained per unit of energy expended. Warming thus has the potential to

cause shifts in a predator's optimal diet with associated consequences for prey assemblages.

## 1.2 Aims

The objective of this project is to investigate the response of three-spined stickleback and macroinvertebrate populations to warming under environmentally realistic conditions. Additionally, the temperature sensitivity of the interaction between the stickleback and its invertebrate prey will be investigated. These aims will be achieved by:

- Monitoring populations of three-spined sticklebacks and macroinvertebrates exposed to combinations of 4°C warming above ambient temperatures and increased, but environmentally realistic nutrient loadings in a large-scale mesocosm experiment.

- Correlating biotic and environmental variables from the mesocosms to assess the likely causes of any changes in populations, i.e. direct effects of temperature or indirect effects through other abiotic or biotic factors such as oxygen levels or increased competition.

- Performing laboratory experiments to assess the impact of increasing temperature on stickleback predation of macroinvertebrates and to predict the optimal diet of sticklebacks at different temperatures.

- Testing laboratory-derived predictions of optimal diet breadth at different temperatures using data collected from the Ness mesocosms

# Chapter 2

Influence of simulated

climate change and eutrophication

on three-spined stickleback

populations: a large scale

mesocosm experiment

## 2.1 Introduction

Climate change will be extremely important in freshwaters owing to effects on hydrology, flux of nutrients from catchments, and the temperature-sensitivity of most freshwater animals. Current models predict an increase in average temperature in temperate regions of 3 to 5°C over the next 100 years, with an associated increase in frequency of extremely hot periods and generally warm years (IPCC, 2007). Predicting the impact of such warming on freshwater ectotherms is important for assessing how climate change will alter freshwater ecosystem structure and functioning, and affect fisheries and other ecosystem services.

Shallow lakes are particularly susceptible to changes in temperature owing to their high surface area to volume ratio. They usually remain mixed even in the warmer months and thus lack cool deep-water refuges for fish. Moreover, warming reduces solubility of oxygen in water, which may pose a problem for ectotherms whose metabolism, and therefore demand for oxygen, increases with temperature. Oxygen limitation is particularly important for aerobic heterotrophs because oxygen, unlike energy and organic nutrients, cannot be amply stored. Consequently, even brief periods of severe hypoxia can be lethal. Aquatic ectotherms subjected to such reduced oxygen supply, prefer lower temperatures when refuges are available, and at increased temperatures make physiological adjustments to increase oxygen uptake and delivery and reduce demand (Kramer, 1987; Atkinson, Morley & Hughes, 2006). But these adjustments are not always possible or sufficient. Pörtner et al. (2004) argue that the upper thermal limit of aquatic ectotherms is set by their inability to obtain sufficient oxygen to meet their increased metabolic demands, leading to temperature-induced reduction in aerobic scope and ultimately anaerobiosis.

Warming, therefore, is expected generally to lead to a progressively inadequate oxygen supply relative to demand.

However, warming may reduce dissolved oxygen (DO) much below the concentrations expected from this physical effect on oxygen solubility if warming increases net heterotrophy of the freshwater community. This effect of respiration is seen most clearly at night when there is respiration without any photosynthesis, but can also occur during the day when light penetration into the water column is low because of shade by terrestrial vegetation, floating plants or algae, especially in cloudy conditions. Macrophyte-dominated shallow freshwater mesocosms under a 3 °C warming regime have shown a higher frequency of hypoxic episodes than under ambient temperature conditions (McKee et al., 2003). Warming of freshwaters may be even more problematic when coupled with other anthropogenically altered processes such as nutrient loading, which remains a major problem throughout the world. Increased nutrient concentrations have been shown to increase the density of shade-producing algae or floating macrophytes, and hence the extent of hypoxia in freshwaters (Correll, 1998). The coupling of the ectotherms' decreasing ability to supply themselves with oxygen at higher temperatures, and the reduced availability of oxygen, is likely to lead to loss of prey items and fish kills (Pörtner, Peck & Hirse, 2006). For example, the ability of the three-spined stickleback (*Gasterosteus aculeatus* (L.)) to survive hypoxic conditions was greatly reduced at 20°C compared with 13°C (Jones, 1952). A major question is the extent to which fish populations will be impacted by various realistic combinations of warming and nutrient loading.

Different life cycle stages have varied sensitivities to alterations in the physical environment. Eggs and embryos that cannot escape unfavourable conditions

may be particularly vulnerable.  The timing of reproduction could therefore be

critical.  To ensure maximum recruitment to the next generation the peak

production at one trophic level should occur at the same time as the peak

production at the lower trophic level.  Climate change can lead to a shift in timing

of life-history events leading to an uncoupling, or 'mismatch' of predator and

prey phenologies, affecting predator-prey dynamics and food web structure and

function (Fortier, Ponton & Gilbert, 1995; Visser et al., 1998; Adrian, Wilhelm &

Gerten, 2006; Durant et al., 2007).  Zooplankters have been shown to shift their

peak productivity as temperature increases (Scheffer et al., 2001; De Senerpont

Domis et al., 2007; Feuchtmayr et. al., unpublished data) resulting in an

alteration of the timing of the spring clear water phase in lakes.  Zooplankton

forms an important component of many food chains that will be adversely

affected if warming does not induce a similar shift in life history timing.  Thus, it

is important to distinguish the relative influences of warming not just on

population changes but also on the timing of reproduction.

Here we give results of a large-scale experiment in which temperature and

nutrient loading were manipulated in large outdoor mesocosms.  Laboratory

studies often lack sufficient realism as they do not incorporate correlated

environmental changes (e.g. nutrient inputs), whereas field correlations have to

deal with complex patterns of multiple varying and co-varying conditions.  Three-

spined sticklebacks (*Gasterosteus aculeatus* (L.)) were used as study

organisms.  They have a relatively high thermal tolerance compared with many

British species (28°C dependent on acclimation, salinity and presence of lateral

plates; Heuts, 1947; Jordan & Garside, 1972) and can withstand particularly low

oxygen levels without exhibiting any behavioural signs of distress (1.6 – 2.0 mg

$L^{-1}$; Giles, 1987).  Their reproduction is particularly sensitive to warming-related

oxygen shortages as the survival of the embryos is dependent on the male

fanning the eggs with oxygenated water. Even at temperatures well below the thermal tolerance limit, warming can reduce the amount of time the male spends engaged in parental care behaviour and therefore the survival of the embryos (Hopkins, 2006).

This chapter reports the impacts in 2006/07 of combinations of 4°C warming above ambient temperatures and increased, but environmentally realistic nutrient loadings on the availability of oxygen, the state of stickleback populations and the timing of their breeding.

## 2.2 Methods

### 2.2.1 Mesocosm establishment

Forty-eight shallow lake mesocosms were established at Ness Botanical Gardens on the Wirral Peninsula, UK (53°16'N, 3°02'W) in fibreglass tanks (diameter 2m, depth 1m) sunk into the ground for insulation. Twenty-four tanks contained pipework connected to a hot water system through which hot water was pumped, controlled by a sensor to maintain a continuous 4°C difference between the heated and the twenty-four ambient tanks. The ambient tanks had similar 'dummy' pipework unconnected to the hot water system. Technical specifications are given by McKee et al. (2000). Each tank was floored with a standard sediment (50% by weight garden loam, 47.5% chopped organic oat straw, 2.5% rotted organic cow manure), 20cm deep, and filled with local borehole water. Inocula of zooplankton and macroinvertebrates from local ponds were added in October 2005. Aquatic plants established naturally from the pond inocula, and after checks in July 2006 to ensure that tanks were seeded with the same complement of plant species, plants were left to grow unhindered. Three nutrient regimes, reflecting the range of inputs in intensive agricultural

landscapes (James et al., 2005), were applied (N0, no nutrient addition; N1 treatment: 250 µg L$^{-1}$ N and 50 µg L$^{-1}$ P; N2 treatment: 2500 µg L$^{-1}$ N and 50 µg L$^{-1}$ P, added as Na NO$_3$ and KH$_2$PO$_4$ every two weeks). In May 2006 when the systems had stabilised and oxygen concentrations were adequate two adult male and two adult female three-spined sticklebacks (*Gasterosteus aculeatus* (L.)), obtained from an organic-rich pond less than a kilometre from the mesocosm site, were added to half of the tanks in a randomised block design. The 12 treatments (heating, nutrients, fish addition and their combinations) were applied in a randomised block design resulting in 4 blocks of 12 tanks. The block design was used to reduce any bias between the upper and lower parts of the slightly inclined site.


## 2.2.2 Mesocosm monitoring

Temperature in the tanks was continuously monitored by sensors, and oxygen readings were taken at a depth of 35cm every fortnight (monthly in winter) using a YSI Model 85 oxygen probe between 10.30 and 12.30 hours (hereafter, "mid-day" samples). Two-hourly sampling of oxygen levels was carried out during three 24-hour periods in 2007 on the 27-28 June (16 mesocosms), 4-5 July (8 mesocosms) and 16-17 July 2007 (16 mesocosms). Submerged and floating plants and filamentous algae were monitored every 2-3 weeks in 2007 as proportion of water volume infested (PVI). During the final harvest, in September 2007, all plants were removed, sorted and had their fresh weight determined following spin-drying for a standard 5 minute period to remove surplus water.


Fish populations were monitored monthly (two-monthly between October and February) by trapping with four bottle traps per tank which were checked at least daily over a 2-day period. Each bottle trap, based on a minnow trap, comprised

a two-litre plastic bottle with the open end cut off, inverted and inserted into the bottle. After weighing the fish to the nearest 0.1 g with an electronic balance and measuring their standard length to the nearest 0.1 mm with Vernier callipers, they were returned to their respective mesocosms. In July 2006, following a particularly hot period we re-stocked the stickleback populations after concluding that the fish had failed to establish due to high temperatures and low oxygen. To help ensure that the populations established on this occasion, each tank was re-stocked with 27 fish. The fish were broadly size-matched with the same number of juveniles and adults being placed in each tank with a total biomass of 13.45g (SEM ± 1.07). Assessment of the onset of stickleback breeding season was carried out by recording the Julian day (1st January being day 1) upon which the first coloured male was seen. The experiment ended in September 2007 when the contents of the tanks were harvested and the masses and lengths of all fish were recorded.

## 2.2.3 Statistics

Data from fish final harvest biomass was log (n + 1) transformed and analysed using Kruskal-wallis tests across the 24 mesocosms. Post-hoc Bonferroni-corrected Mann-Whitney tests allowed comparison between pairs of nutrient treatments. Population size-class structure was tested for normality using probability plots, then analysed using Mann-Whitney and Kruskal-Wallis tests with post-hoc Bonferroni-corrected Mann-Whitney tests on pairs of nutrient treatments. As sampling efficiency was insufficient to show detailed population dynamics, seasonal mean fish biomasses were calculated for the winter season (October – March: 5 samples) and summer season (April – September: 6 samples) and analysed using a linear mixed model with season as a repeated measure and block as a random factor. The same analysis was also carried out on winter and summer seasonal DO means which were based on 9 and 10

samples for winter and summer respectively. Data for regression analysis was tested for normality with Shapiro-Wilkes tests, and where linear regression was used was tested for linearity using residual plots and curve estimation in SPSS. Effects of treatment on maximum and minimum diel oxygen concentrations during two days in June and July 2007 were analysed using linear mixed models with date as a repeated measure. Plant data was analysed using non-linear regression and balanced ANOVA. Analysis was carried out using the Minitab 15 statistical package, SPSS 16 and SigmaPlot 11. No block effect was found in any test.

## 2.3 Results

### 2.3.1 Impacts of warming and nutrients on stickleback populations

Final harvest fish biomass was reduced by both warming (P = 0.046) and nutrient addition (P = 0.006) (Fig. 2.1). At the final harvest, fish were absent from the combined heating and nutrient addition tanks regardless of the level of nutrient addition (Fig. 2.1). Warming alone reduced the final biomass by approximately 60% (76% in population size) whilst unheated nutrient-loaded tanks showed an even more marked reduction of over 80% (95% in population size). Among nutrient treatments, final harvest fish biomass differed significantly (Post-hoc Bonferroni-corrected Mann-Whitney tests: P = 0.006) only between tanks with no added nutrients and each of the nutrient treatments: no difference was observed between N1 and N2. Data from winter and summer (Table 2.1; Fig. 2.2) show that the negative effects of warming and nutrients on stickleback population biomass occurred throughout the experimental period. The apparently stronger effect of warming on biomass from April to September (Fig. 2.2) was marginally non-significant (P = 0.065, Table 2.1). Final harvest mean fish size was significantly affected by heating and nutrients with an increase in

average fish length with heating (Ambient = 23.79mm; Heated = 28.02mm;

Mann-Whitney: P < 0.001) and nutrients (A = 23.79mm; AN1 = 26.20mm; AN2 =

39.21mm; Kruskal-Wallis : P < 0.001).  The reductions in final harvest population

biomass due to heating and nutrients were therefore less than the reduction in

numbers due to the greater mean size of fish in the heated and nutrient regimes.


### 2.3.2 Impacts of warming and nutrients on the availability of oxygen

No interaction between heating and nutrient addition on the mid-day DO

concentration was observed, but the treatment warming alone did reduce the

mean mid-day concentration of DO in the mesocosms (Table 2.2; Fig. 2.3a).

Mid-day DO concentrations were also lower in summer (March – August

inclusive) than in winter (September to February inclusive) across all treatments

(Table 2.2).  Furthermore mid-day DO concentrations at 35 cm depth in heated

mesocosms fell below two critical oxygen levels (as identified by the European

Inland Fisheries Advisory Commission (EIFAC)) on a greater percentage of

sampling occasions than in ambient mesocosms (Table 2.3).  Regression


**Table 2.1.** Effects of warming and nutrient loading on the biomass of sticklebacks in a mesocosm experiment during 2006 and 2007. Repeated measures ANOVA was performed on means (g) which had been Log n + 1 transformed from 5 winter (Oct -Mar) and 6 summer (Apr-Sep) samples.

| Effect | d.f. | $F$ | P |
|---|---|---|---|
| Warming | 1,18 | 4.80 | 0.042 |
| Nutrients | 2,18 | 3.45 | 0.054 |
| Season (Repeated measure) | 1,18 | 3.86 | 0.065 |
| Warming x Nutrients | 2,18 | 0.59 | 0.563 |
| Warming x Season | 1,18 | 3.83 | 0.066 |
| Nutrients x Season | 2,18 | 1.93 | 0.174 |
| Warming x Nutrients x Season | 2,18 | 1.49 | 0.251 |
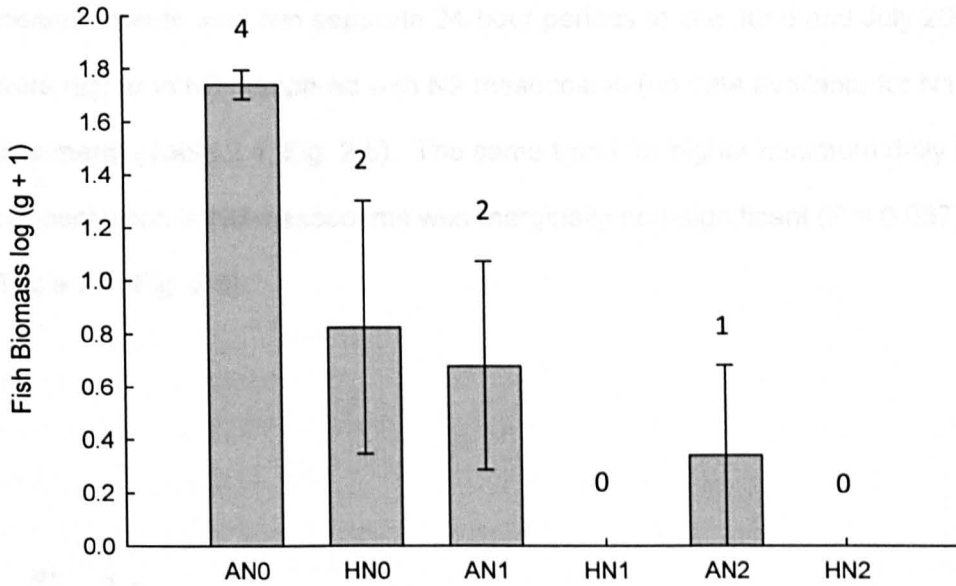
**Figure 2.1.** Total biomass of sticklebacks from the final harvest of a set of mesocosms subjected to heating and nutrient treatments for 16 months. Values are means ± SE. Abbreviations are: A, Ambient; H, Heated; N0, No added Nutrients; N1, Low Nutrients; and N2, High Nutrients (see methods for treatment details) and n = 4. Numbers represent the number of populations still extant per treatment. Kruskal Wallis test adjusted for ties: Heating (P = 0.046); Nutrients (P = 0.006). Pairwise comparison with Bonferroni-corrected Mann-Whitney tests revealed the significant differences (P < 0.017) was only between N0 and each of the nutrient addition treatments (N1 and N2).

analysis showed a significant relationship between seasonal means of fish biomasses and seasonal oxygen means (Fig. 2.4). A balanced ANOVA carried out on the residuals of the regression relationships found that neither heating nor nutrient addition accounted for any of the remaining variation in fish biomass. The three winter extinctions were in fact artefacts resulting from small populations remaining undetected during sampling. By contrast, the summer extinctions were confirmed as actual extinctions during final harvest. While the trend towards lower mean mid-day DO concentrations with increased nutrient loading seen in winter (October 2006 to March 2007 inclusive) and summer (April 2007 to September 2007 inclusive) (Fig. 2.3b) was not statistically

significant (Table 2.2), maximum daily DO concentrations based on two-hourly measurements over two separate 24-hour periods in late June and July 2007, were higher in N0 compared with N2 mesocosms (no data available for N1 treatment) (Table 2.4; Fig. 2.5). The same trend for higher minimum daily DO concentration in N0 mesocosms was marginally non-significant (P = 0.067, Table 2.4; Fig. 2.5).
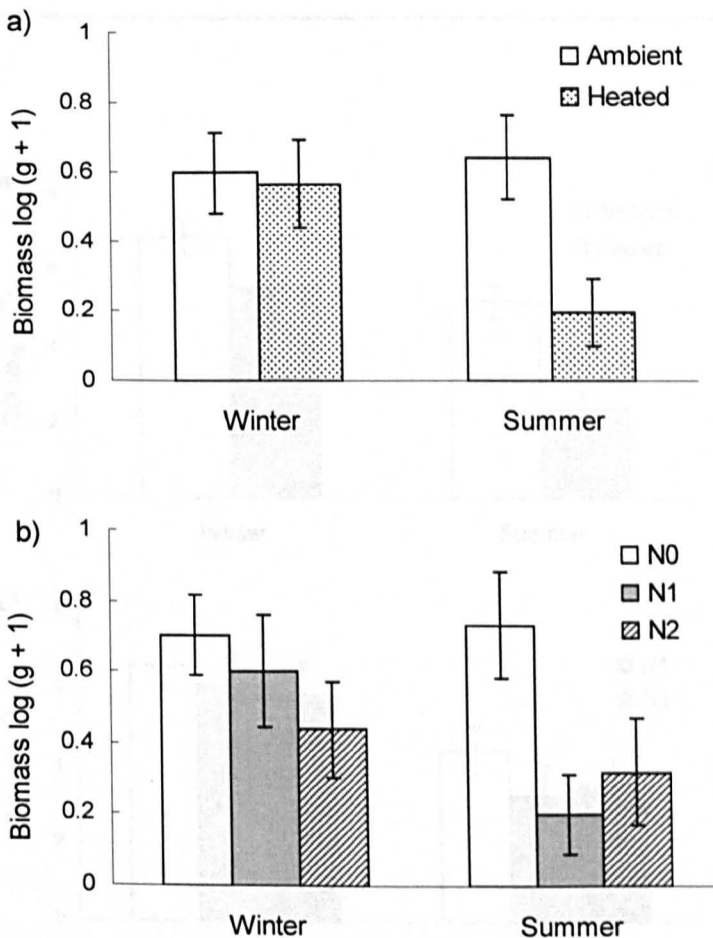


**Figure 2.2.** The effect of (a) heating and (b) three levels of nutrient addition on winter (October to March) and summer (April to September) fish biomass in a mesocosm experiment. N0, N1, and N2 represent a sequence of increasing nutrient loading. Values are means ± SE.

**Table 2.2.** Effects of warming and nutrient loading on the dissolved oxygen (DO) concentrations recorded during 2006 and 2007 in a mesocosm experiment. Repeated measures ANOVA was performed on means from 9 winter (Oct -Mar) and 10 summer (Apr-Sep) samples (mg $L^{-1}$)

| Effect | d.f. | $F$ | P |
|---|---|---|---|
| Warming | 1,18 | 14.52 | **0.001** |
| Nutrients | 2,18 | 0.616 | 0.551 |
| Season (Repeated measure) | 1,18 | 89.49 | **<0.001** |
| Warming x Nutrients | 2,18 | 0.75 | 0.486 |
| Warming x Season | 1,18 | 0.90 | 0.357 |
| Nutrients x Season | 2,18 | 0.41 | 0.672 |
| Warming x Nutrients x Season | 2,18 | 0.76 | 0.481 |
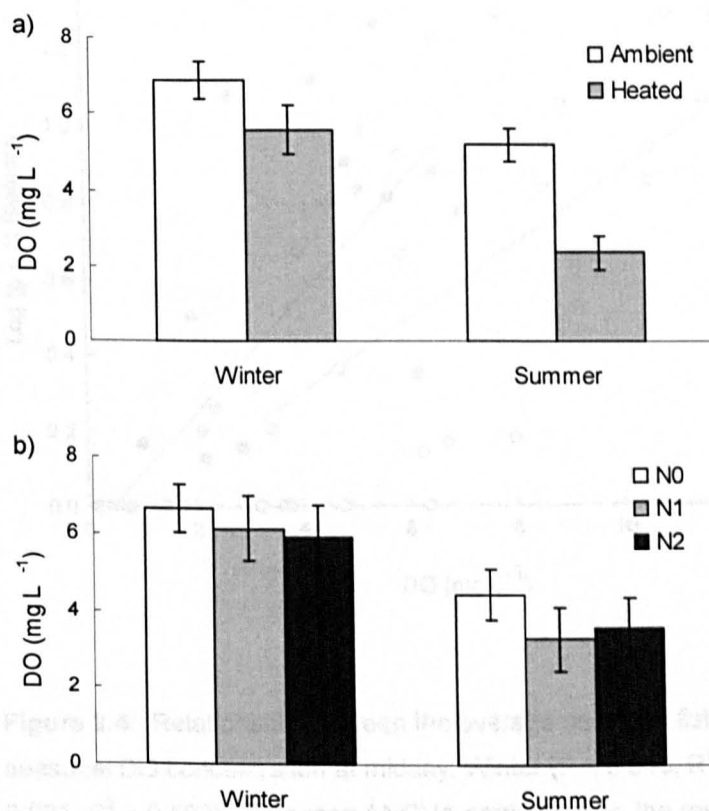


**Figure 2.3.** The effect of (a) heating and (b) three levels of nutrient addition on winter (October to March) and summer (April to September) DO in a mesocosm experiment. N0, N1, and N2 represent a sequence of increasing nutrient loading. Values are means ± SE.

**Table 2.3.** The annual percentage of fortnightly samplings where mid-day dissolved oxygen concentrations in ambient and heated mesocosms had fallen below two critical DO levels (5 mg L$^{-1}$ and 2 mg L$^{-1}$) as identified by the European Inland Fisheries Advisory Commission (EIFAC) and below a critical DO level for stickleback survival (1.6 mg L$^{-1}$; Giles, 1987). Values are percentages ± SE. Balanced ANOVA was performed after arcsin transformation of percentages. No effect of nutrients was observed.

| Oxygen Level | Ambient | Heated | d.f. | F | P |
|---|---|---|---|---|---|
| < 5 mg L$^{-1}$ | 53.23 ± 5.06 | 72.02 ± 7.28 | 1,18 | 11.43 | 0.035 |
| < 2 mg L$^{-1}$ | 25.99 ± 4.55 | 50.66 ± 5.27 | 1,18 | 13.05 | 0.002 |
| < 1.6 mg L$^{-1}$ | 23.24 ± 4.60 | 45.16 ± 4.35 | 1,18 | 5.23 | 0.003 |
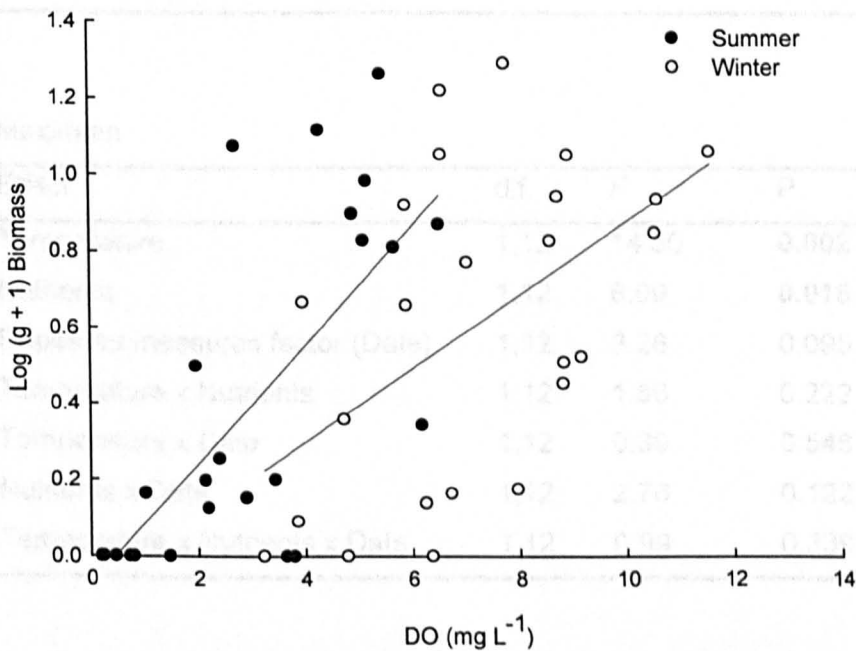


**Figure 2.4.** Relationship between the average seasonal fish biomass and average seasonal DO concentration at midday; Winter (P = 0.010, R$^2$ = 0.266), Summer (P < 0.001, R$^2$ = 0.502). Balanced ANOVA carried out on the residuals of the regression relationship found no significant effect of heating (P = 0.845) or nutrient addition (P = 0.066).

**Table 2.4.** Repeated measures mixed model analysis of effects of warming and two nutrient regimes on minimum and maximum daily oxygen concentrations (mg $O_2$ $L^{-1}$) derived from 2-hourly observations over 24 hours on 27-28 June and 16-17 July 2007 (16 mesocosms). "Nutrients" refers to addition of nutrients at the N2 level (see text) compared with the N0 level, in which no additional nutrients were provided.

Minimum

| Effect | d.f. | F | P |
|---|---|---|---|
| Temperature | 1,12 | 11.89 | **0.005** |
| Nutrients | 1,12 | 4.06 | 0.067 |
| Repeated measures factor (Date) | 1,12 | 1.53 | 0.240 |
| Temperature x Nutrients | 1,12 | 3.01 | 0.108 |
| Temperature x Date | 1,12 | 1.44 | 0.253 |
| Nutrients x Date | 1,12 | 0.29 | 0.600 |
| Temperature x Nutrients x Date | 1,12 | 0.166 | 0.691 |

Maximum

| Effect | d.f. | F | P |
|---|---|---|---|
| Temperature | 1,12 | 14.50 | **0.002** |
| Nutrients | 1,12 | 8.09 | **0.015** |
| Repeated measures factor (Date) | 1,12 | 3.28 | 0.095 |
| Temperature x Nutrients | 1,12 | 1.66 | 0.222 |
| Temperature x Date | 1,12 | 0.39 | 0.546 |
| Nutrients x Date | 1,12 | 2.76 | 0.122 |
| Temperature x Nutrients x Date | 1,12 | 0.99 | 0.339 |
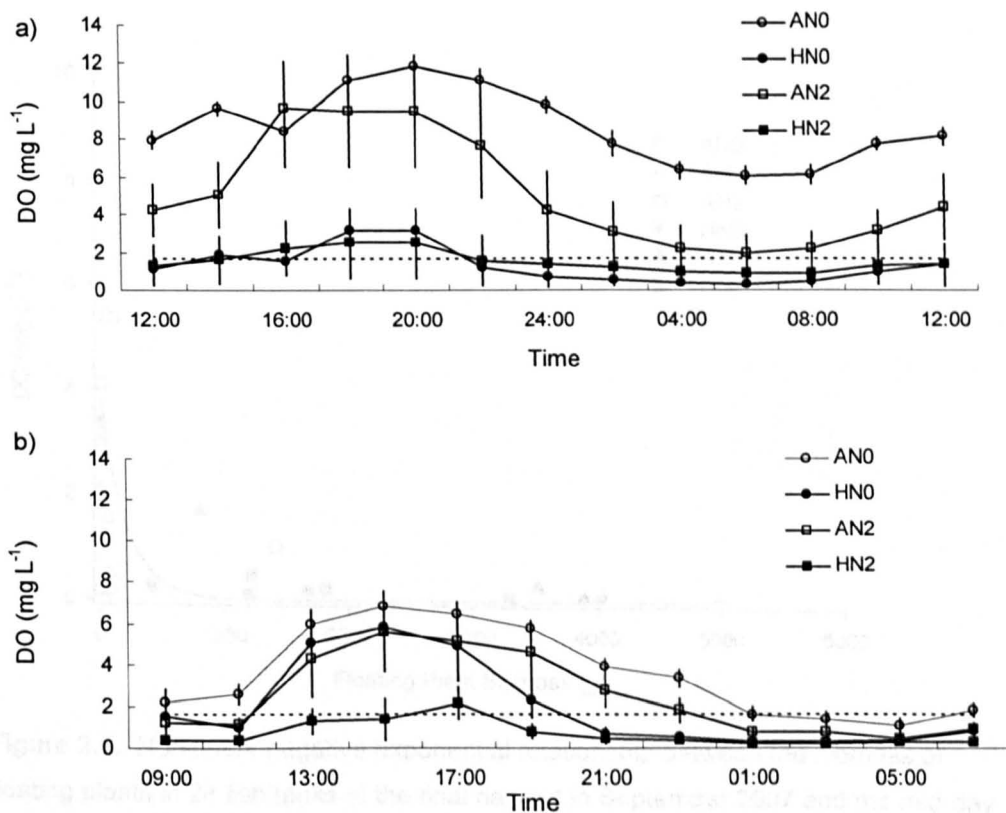
**Figure 2.5.** Diel DO concentrations recorded over 24-hours at 2 hourly intervals on two days in summer 2007. Each of the four treatments (heating or ambient x no added nutrients or high nutrient loading, see Methods for details) were replicated four times, a) 27-28 June and b) 16-17 July 2007. Values are means ± SE. The dashed line represents a level of 1.6 mg $L^{-1}$ below which sticklebacks struggle to survive (Giles, 1981). For abbreviations, see Figure 2.1.

## 2.3.3 Impacts of warming and nutrients on macrophyte abundance

All tanks developed substantial populations of aquatic plants with final harvested biomass in September 2007 averaging 2.2 kg fresh weight $m^{-2}$. Submerged plants did not show a significant response to any of the treatments whilst floating plant biomass increased with warming but not nutrient addition (Fig. 2.7). Low DO levels immediately prior to final harvest in September 2007 were associated not just with mesocosm warming, but also with a canopy of floating plants (Fig. 2.6).
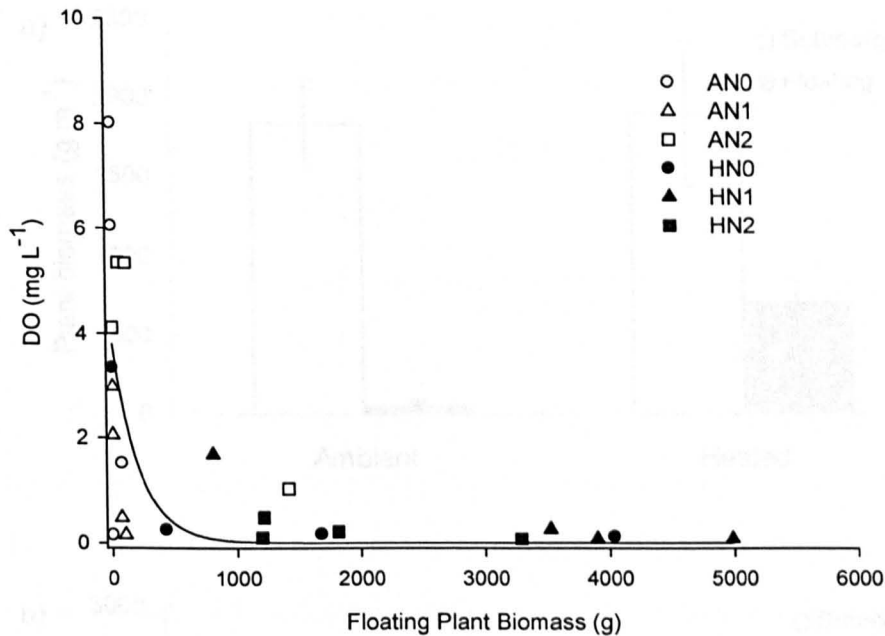
**Figure 2.6.** Non-linear negative exponential relationship between the biomass of floating plants in 24 fish tanks at the final harvest in September 2007 and the mid-day DO concentration at 35cm depth recorded 12 days prior to the final harvest ($R^2 = 0.433$, $P < 0.001$)

### 2.3.4 Impacts of warming on stickleback breeding

Visual inspection of the sticklebacks was only possible during the monthly samplings so was insufficiently sensitive to pick up minor shifts in breeding onset. Timing of colour change and therefore breeding condition in male sticklebacks was relatively insensitive to a 4°C rise in temperature. The average day of the first sighting of a coloured male was on day 58.6 in ambient tanks and day 58.1 in heated and the difference between the two was not statistically significant (Students T-Test; $P = 0.978$). Successful breeding was recorded in 10 ambient mesocosms and 5 heated mesocosms out of 12 by observation of presence of new juveniles. Heating reduced the number of mesocosms with juveniles present though this was not statistically supported ($X^2 = 2.794$; $P = 0.095$).

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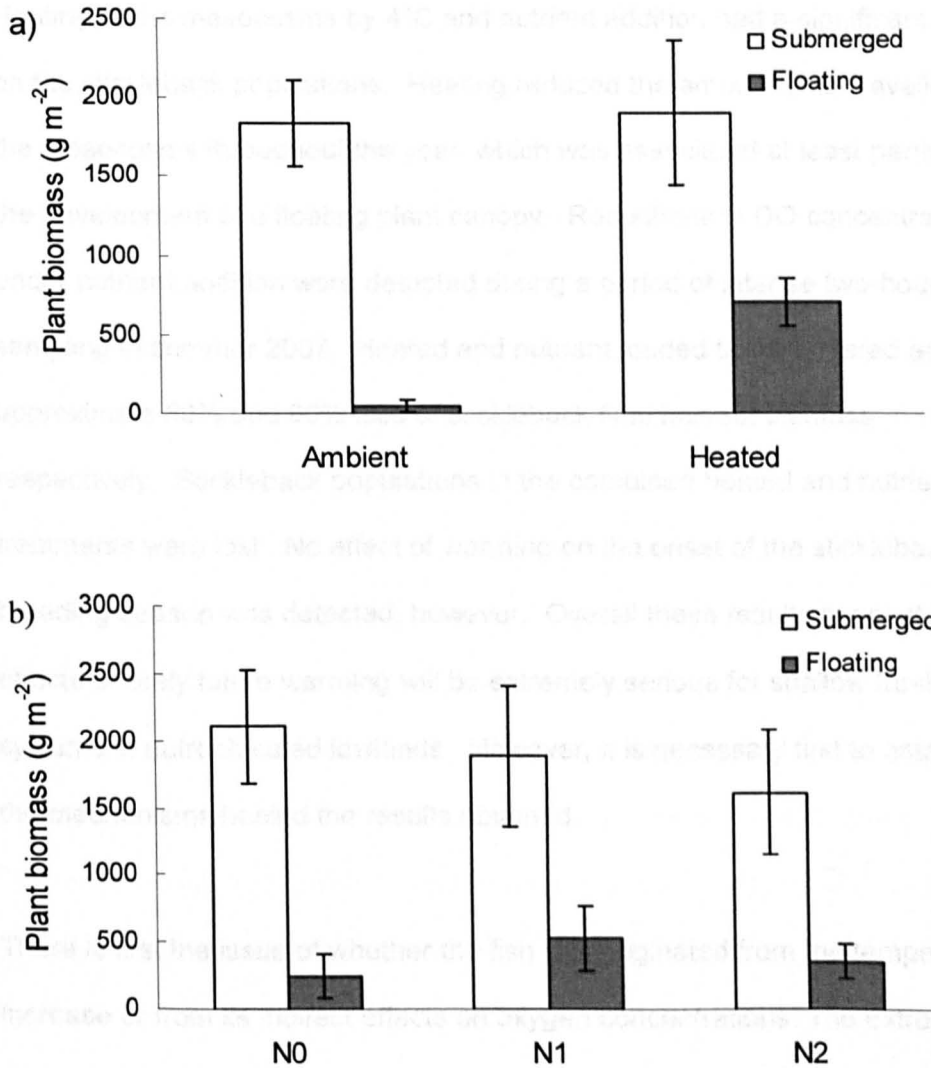
**Figure 2.7.** The effect of (a) heating and (b) three levels of nutrient addition on the biomass of submerged and floating plants in the final harvest. Balanced ANOVA: Heating; submerged (P = 0.911) and floating (P < 0.001). Nutrients submerged (P = 0.781) and floating (P = 0.315). No interactions were found. For abbreviations, see Figure 2.1.

## 2.4 Discussion

Heating of the mesocosms by 4°C and nutrient addition had a significant impact

on the stickleback populations. Heating reduced the amount of DO available in

the mesocosms throughout the year, which was associated at least partly with

the development of a floating plant canopy. Reductions in DO concentrations

under nutrient addition were detected during a period of intense two-hourly

sampling in summer 2007. Heated and nutrient loaded tanks suffered an

approximate 60% and 80% loss of stickleback final harvest biomass

respectively. Stickleback populations in the combined heated and nutrient

treatments were lost. No effect of warming on the onset of the stickleback

breeding season was detected, however. Overall these results suggest that

effects of likely future warming will be extremely serious for shallow freshwater

systems in eutrophicated lowlands. However, it is necessary first to establish

the mechanisms behind the results obtained.

There is first the issue of whether the fish kills originated from the temperature

increase or from its indirect effects on oxygen concentrations. The extreme

maximum midday temperature recorded for the heated mesocosms during the

experimental period was $24.7 \pm 0.8°C$ (mean $\pm$ SD for the 12 mesocosms). This

is below the upper lethal temperature of 28°C reported for sticklebacks from

laboratory studies (Heuts, 1947). This warming is unlikely to have reached the

extreme thermal limit throughout the whole of the water volume of any

mesocosm, but may have caused some thermal stress. We suggest that this

stress was exacerbated by the warming-induced reduction in DO concentration.

Warmed mesocosms contained less DO than ambient mesocosms especially

during the summer months (Fig. 2.3). Also, with 4 °C warming, a greater

percentage of samples throughout the year had DO concentrations below a

critical value the 1.6 mg $L^{-1}$ (Table 2.4) below which point sticklebacks begin to

exhibit signs of distress (Giles, 1987). This creates a mismatch between the increasing requirements of the animal and the decreasing availability of the resource, which can result in ectotherms dying at lower temperatures than would otherwise be lethal in fully oxygenated water (Jones, 1952; Pörtner, Mark & Bock, 2004; Ficke, Myrick & Hansen, 2007). There is also further evidence that the effect of warming on fish biomass acted through its effect on DO concentrations. Stickleback seasonal biomass was shown to be significantly related to seasonal DO concentrations (Fig. 2.4), and analysis of the residuals from this regression relationship showed that neither heating nor nutrient addition significantly accounted for any of the remaining variation.

Warming the mesocosms by 4°C resulted in an average mid-day summer 2007 DO concentration of 2.35 mg $L^{-1}$ in summer 2007 which is near to the lowest critical DO concentration identified by the European Inland Fisheries Advisory Commission for a moderately tolerant species (Table 2.4). Although saturated DO concentrations are inversely related to water temperature due to reduced solubility, heating the mesocosms by 4°C would only produce a maximum drop in DO concentration of 1.51 mg $L^{-1}$ (Mortimer, 1956). This accounts for only about a third of the 4.7 mg $L^{-1}$ mean difference in maximum DO concentrations between ambient and heated mesocosms in the diel recordings in 2007 (Table 2.3). The rest of the reduction in oxygen concentration was due to net community respiration. This raises the question of whether the oxygen depletion was simply a function of microbial respiration in the sediments or linked with the temperature-induced changes in the macrophyte community composition.

Several mechanisms can lead to net ecosystem heterotrophy rather than autotrophy under warming. Increasing temperature can increase the growth of macrophytes in a similar manner to the effect of nutrient addition with the same associated consequences. Kankalla et al. (2002) showed an increase of 300-

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500% in the maximum shoot biomass of emergent macrophytes with an increase of 2–3 °C above ambient temperature.  In our experiment, floating plants, which can absorb oxygen through their submerged roots but release all photosynthetically produced oxygen direct to the atmosphere, increased with warming (Fig. 2.6).  Through these mechanisms the gross primary production (GPP) of oxygen no longer matches the increased community respiration (CR) leading to an effectively more heterotrophic system.  Autotrophic oxygen production is also less thermally sensitive than aerobic respiration by heterotrophs.  This was suggested by Allen et al. (2005) for terrestrial systems with C3 photosynthesis but appears also to have support in the metabolic balance of the oceans (López-Urrutia et al., 2006).  Finally, the shade cast by the leaves of floating plants may reduce photosynthetic oxygen replenishment by submerged plants and algae, but it is not clear whether this reduction will be balanced by a reduction in respiratory oxygen consumption by the community due to oxygen limitation of respiration. The effect of shade on net community metabolic balance is thus unclear.

The reduction in final-harvest fish population size and biomass in nutrient-loaded ecosystems (Fig. 2.1) was also likely to have been related to low oxygen levels created by eutrophic conditions, though significant effects of nutrients on DO concentrations were only detected during the intensive sampling period in summer 2007. Eutrophication through excessive nutrient loading is considered one of the major global threats to fish biodiversity (Leveque et al., 2008) because the increased nutrient levels result in excessive plant and algal growth and hence decay (Correll, 1998).  Crowding from macrophytes or dense surface algal growth can reduce the amount of light penetrating the water body and also inhibit photosynthesis through self-shading.  The result is a more deoxygenated, eutrophic system with associated loss of species richness (Jeppesen et. al.,

2000). When data from the 24 mesocosms were combined with data from the 24 matching but fishless mesocosms (Feuchtmayr et. al., 2009; Appendix 1) floating plant biomass at final harvest showed a significant increase with nutrient addition of 120% and 164% in N1 and N2 treatments respectively. Thus, the possibility of an effect of nutrients on DO concentrations via shading from floating plants may be possible, though is not evident from the relationship between floating plant biomass and both nutrient treatment and reduced DO levels in the 24 mesocosms around the time of final harvest (Fig. 2.6, 2.7). In addition, the possibility of high nutrient levels leading to algal shading and/or high (especially night-time) community respiration around the period of peak phytoplankton abundance in the spring, may also have been implicated in acute falls in DO concentrations, hence fish survival. But the frequency of sampling was insufficient to test this idea.

Aside from the direct effects of warming and nutrient addition on the ability of sticklebacks to survive, these treatments may also alter populations by affecting the success of reproduction. The timing of reproduction should coincide with sufficient resources for successful recruitment to the next generation. The reduction in DO concentrations was greatest between March – August (Table 2.2; Fig. 2.3) which is also the stickleback breeding season in the U.K. (Baker, 1994). In the mesocosms where DO concentration was not reduced to levels immediately lethal to the fish it may still have adversely altered populations by preventing the adults from breeding. Without sufficient male stickleback fanning with oxygenated water the eggs cannot survive (Wootton, 1976). Low DO levels cause the male to spend more time at the surface to obtain sufficient oxygen for his own survival thus reducing the amount of time he can commit to parental care (Giles, 1987; Hopkins, 2006). In this experiment a negative impact of

warming on the reproductive success was suggested, but not statistically significant.

The phenologies of predator (e.g. fish) and prey (e.g. zooplankton) are usually closely coupled to ensure that peak production of predators is matched with that of prey availability ( Fortier, Ponton & Gilbert, 1995; Visser et al., 1998; Durant et al., 2007). Warming by 4°C shifted peak zooplankton numbers from May 16 to April 18 in the first spring (2006) of the experiment before fish were added (Feuchtmayr et al., Unpublished data). Typically, sticklebacks occurring at latitudes similar to the mesocosms start breeding around late April – May (Baker, 1994). In this experiment there was no significant difference in the date of the first-observed coloured male between ambient and heated mesocosms. This is consistent with the consensus that photoperiod is the most important external determinant of breeding condition in male sticklebacks (Wootton, 1976). Trials using high temperatures combined with short day-lengths have shown inhibited spermatogenesis in male sticklebacks (Ahsan & Hoar, 1963). Sexual maturation in females is thought to be similarly dependent on photoperiod though the interplay with temperature is more complex (Wootton, 1976). If photoperiod is the main external determinant of the onset of stickleback breeding then it is unlikely that an increased number of years with warmer spring weather will cause a phenotypically plastic earlier peak production in the same manner as that of their zooplankton prey. However, the benefits to genotypes that do breed early may lead to a longer term evolutionary shift to earlier mean reproduction in those populations that survive warming.

The three-spined stickleback is one of Britain's hardiest native species. Given the impact of warming and nutrient addition on our mesocosm populations, what can we expect for other freshwater fish species? According to the European

Inland Fisheries Advisory Commission (EIFAC), a general rule for maintaining all stages of the lifecycle of moderately tolerant species such as roach (*Rutilus rutilus*) is that the DO concentration should drop no lower than 5 mg L$^{-1}$ for 50% of the year and no lower than 2 mg L$^{-1}$ for 5% of the year (Alabaster & Lloyd, 1982). In our mesocosms a 4°C increase in temperature increased the annual percentage of time the mid-day DO concentrations dropped below these critical levels (Table 2.4). Pike (*Esox lucius*), requires oxygen levels of well over 4 mg L$^{-1}$ for normal larval development whilst roach have an optimal maximum temperature for spawning of only 20°C (Alabaster & Lloyd, 1982). If climate changes as predicted, these species might face serious problems in eutrophic lakes (Winfield, Fletcher, & James, 2006). The few fish that are tolerant of such extremes of temperature and oxygen are mostly introduced species such as the grass and common carp as well as the native crucian carp (*Carassius carassius*) that can tolerate complete anoxia for a few days at room temperature (Nilsson & Renshaw, 2004). Grass carp (*Ctenopharyngodon idella*) can survive temperatures of up to 38°C and is known for its ability to destroy native vegetation and dramatically alter freshwater ecosystems (McKnight & Hepp, 1995). Common carp (*Cyprinus carpio*), widely stocked in freshwaters for angling purposes, is tolerant of a range of conditions and reduces macroinvertebrates, macroalgae and macrophytes with associated loss of water birds (Haas et al., 2007). These characteristics are not exclusive to carp and other commonly stocked tolerant species include tench (*Abramis brama*) and bream (*Tinca tinca*) (www.fishbase.org.uk). With native species already under threat from the conditions climate change will produce, the alteration of habitat by introduced species could exacerbate their decline. However, in contrast to our experiments, wild systems are not likely to be isolated and therefore have potential to be re-colonised. They are also more likely to contain microhabitats of more favourable conditions to provide refuge for fish. These factors may

prevent local extinctions but are unlikely to prevent populations being adversely affected by changing conditions.

In summary, climate change looks set to have a large impact on fish populations in waters that are already nutrient-rich. Temperatures are predicted to increase for many decades unless urgent measures are taken to halt this trend. However, reducing the nutrient loading in eutrophic freshwaters may help to alleviate future poor conditions produced by warming and help reduce the loss of fish populations.

# Chapter 3

The seasonal impacts of
warming, nutrients and fish on
freshwater macroinvertebrates

## 3.1 Introduction

Macroinvertebrates play important and varied roles in freshwater ecosystems. Despite this, there is little research into how these thermally sensitive animals will respond to changes in global temperature and how alterations in community composition will alter ecosystem functioning. Their sensitivity to climate change is likely to depend upon the species in question and is likely to vary seasonally as the impacts of warming may be most pronounced at the extremes, i.e. summer. Specific research is needed on impacts of warming on freshwater taxa in whole communities in order to understand and predict the responses of freshwater ecosystems to climate change. This chapter will mainly examine the impacts of warming, nutrients and season on the invertebrates in the Ness mesocosms and discuss the potential consequences of any alterations in populations. Specific mechanisms behind population changes will be discussed and tested in the following chapter.

The functional roles of invertebrates in freshwater ecosystems are many fold and define how they interact with other trophic levels. As a result of the sheer diversity of both animals and their specific functions, macroinvertebrates are usually divided into functional guilds based upon their feeding behaviour (Cummins, 1974). 'Shredders' refers to the animals that feed mainly on coarse particulate organic matter (CPOM) and their densities tend to be correlated with the spatial and temporal accumulation of organic matter (Graça, 2001). Their importance in the ecosystem lies in their ability to increase the conversion of CPOM to Fine Particulate Organic Matter (FPOM) and Dissolved Organic Matter (DOM)(Cummins & Klug, 1979). 'Grazer-scrapers' are animals that feed by removing food that is attached to a surface, e.g. algae on rocks. A tight coupling of algal abundance and grazer numbers has been observed, with most studies demonstrating a reduction in algal biomass in the presence of grazers (Wallace

& Webster, 1996). Invertebrates feeding on FPOM are either 'collector-gatherers' if they take this from the substrate or 'collector-filterers' if they take it directly from suspension. Some species of 'collector-filterers' are particularly important in that they remove FPOM from suspension and transfer it to the sediment as larger particles in the form of faeces. The final group in the classification of Cummins (1974) is that of the predators which actively kill and feed on live animals. As in other ecosystems, they provide top-down control for their prey populations. Apart from the predators, all the other functional guilds can be regarded as detritivores to some extent and are therefore important in the cycling of nutrients within the ecosystem.

Changes in temperature can alter these trophic interactions and hence ecosystem functioning. They can act directly on metabolic rates and hence the trophic interaction itself, or indirectly through alterations of populations and hence community composition. All freshwater macroinvertebrates are ectotherms and are therefore sensitive to the temperature changes associated with current global warming predictions (3 to 5°C in temperate regions over the next century; IPCC, 2007). Their metabolic rate is closely coupled to that of their external temperature with most invertebrates displaying a $Q_{10}$ of around 2 with $Q_{10}$ defined as the factor by which the rate of the process being studied increases with a 10°C rise in temperature (For further details see Chapter 5; Brockington & Clarke, 2001; Marshall et.al., 2003). Increasing temperature can increase the rates of prey encounter for sit-and-wait predators such as the Odonata (Thompson, 1978), assimilation rates of filter feeding Bivalvia and mineralization by decomposers (Anderson & Sedell, 1979; Menendez et.al., 2003).

There are a number of mechanisms by which temperature change can act on community composition. Some species have been shown to shift their range as temperatures increase (Parmesan et. al., 1999; Hickling et. al., 2005). Changes in life history are widespread (Ward & Stanford, 1982) and include alterations in peak production e.g. zooplankton (Scheffer et. al., 2001; Lehikoinen et. al., 2009), timing of emergence (Hassall et. al., 2007), fecundity (Aston, 1973; Nascimento & Alves, 2009) and growth.

A major aim of this study is to examine how increasing temperature will affect invertebrate populations, and to discuss the likely interactions with the rest of the community. Baulch et al. (2005) found little effect of warming by 4.5 °C on invertebrate communities whilst Mckee et al. (2003) and Feuchtmayr et. al. (2007) found that heating by 3 °C led to an increase in biomass of macrophyte-associated invertebrates such as gastropods. An increase in these grazer-scrapers would be expected to lead to a decrease in algal biomass and have knock-on effects for the rest of the community such as increased macrophyte abundance (Jones et. al., 1999). In a study where a Canadian stream was experimentally warmed by 3.5 – 4°C a reduction in densities of invertebrates, particularly chironomids, was recorded (Hogg & Williams 1996) and this was attributed to a less efficient use of resources by the animals. Field data on invertebrate responses to warming are, therefore, scarce and contradictory (Hogg & Williams, 1996; Mckee et al., 2003; Baulch et al., 2005). To address these issues, this chapter examines the seasonal macroinvertebrate data from a large scale climate change impacts experiment consisting of forty-eight 3000L experimental aquatic ecosystems monitored over the period of one year (September 2006 - August 2007) whilst encompassing realistic additions such as predatory fish and anthropogenic nutrient loading. Experimental manipulations included temperature (ambient and ambient + 4°C) nutrients (no

addition, low addition and high addition) and fish (presence/absence). By

examining abundances and biomass of specific taxa over the course of a year it

is hoped that not only will we be able to detect slight changes in populations that

examination of the final harvest data would be insensitive to (i.e. changes in

phenology), but also that we can also investigate how these impacts vary

seasonally.


## 3.2 Methods

Forty-eight shallow lake mesocosms were established at Ness Botanical

Gardens on the Wirral Peninsula, UK (53°16'N, 3°02'W). A detailed description

of their establishment and the assignment of treatments is provided in Chapter 2

(A randomised block design including combinations of: ambient/heated × three

nutrient regimes × fish presence/absence; four replicates of each).

Invertebrates were collected from local ponds and mixed before being

distributed amongst the tanks in January 2006. Two to three weeks after the

initial inocula, invertebrate communities were cross-mixed between the tanks

using standard net sweeps. After initial problems with severe hypoxia all tanks

were subsequently re-stocked via the same method in May 2006. After re-

stocking, monitoring of the macroinvertebrate populations began with monthly

sampling (bi-monthly from November – March) and continued until September

2007 when the experiment was terminated. After a period to allow settling of the

mesocosm communities, data from a 12-month period, September 2006 –

August 2007, were collected and used in this study. Invertebrates in the water

column were sampled by a standardised net sweep method (Fig. 3.1). Benthic

invertebrates were sampled using an Eckman grab (14.6 × 14.6 cm) to collect

material from alternating sides of the tank so as to allow recovery of the

community between sampling sessions. These samples were washed through

sieves to remove any large organic matter. All invertebrates in the samples were identified, usually to family or species level if practicable, counted, and the first ten individuals of each species were sorted into 5mm length classes excluding appendages e.g. tail filaments on Odonata and Ephemeroptera or antennae in Isopoda. Invertebrates were returned to the tanks after sampling. For details of conversion of numbers into biomasses see Chapter 4.

### 3.2.1 Statistical analysis

Data were divided into seasons; Autumn (September 2006 – November 2006; n = 3), Winter (December 2006 – February 2007; n = 1), Spring (March 2007 – May 2007; n = 4) and Summer (June 2007 – August 2007; n = 3). All data were tested for normaility and log (n + 1) transformed where appropriate. Invertebrate seasonal biomass data was tested using a Linear Mixed Model with block as a random factor and season as a repeated measure. Post-hoc Tukey tests allowed comparisons between pairs of nutrient treatments and seasons where these results were significant. All analysis was carried out using Minitab 15 and SPSS 16.0 statistical packages.
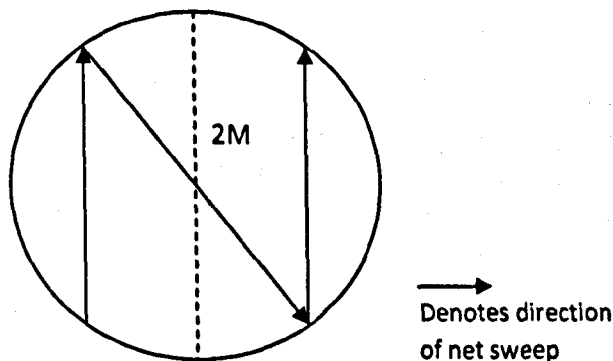


**Figure 3.1.** Schematic plan of mesocosm illustrating the continuous effort net sweep method. The sweep extends equal distances on either side of an imaginary mid-line (dashed) in the direction of the arrow at mid-range depth.

## 3.3 Results

Results will be discussed for each taxon within the major phyla and for the benthic and sweep sample communities separately, where appropriate.

**Table 3.1.** Sweep sample community: The main effects of heating (H), fish addition (F), nutrient addition (N) and season on the invertebrate biomass found in the sampling of 48 mesocosms over a period of 12 months (September 2006 – August 2007). Any interaction effects are described within the text. Symbol (+/–) denotes direction of effect. The following taxa were recorded but showed no significant response to treatments or season and are therefore omitted from the table: Oligochaeta and Bivalvia.

| PHYLUM<br>  CLASS/SUBCLASS<br>    Order/Suborder<br>      Family<br>        *Species* | H | F | N | S |
|---|---|---|---|---|
| **ANNELIDA**<br>  HIRUDINEA | ns | ns | ns | **<0.001** |
| **ARTHROPODA**<br>  ARACHNIDA<br>    **Hydracarina** | ns | ns | ns | **<0.001** |
|   INSECTA |  |  |  |  |
|     **Coleoptera** | ns | ns | ns | 0.001 |
|     **Diptera** |  |  |  |  |
|       Chaoboridae | ns | 0.001 – | ns | ns |
|       Chironomidae | 0.004 – | ns | ns | ns |
|       Culicidae | ns | ns | ns | **<0.001** |
|     **Ephemeroptera** |  |  |  |  |
|       *Cloeon dipterum* | ns | ns | ns | ns |
|     **Hemiptera** |  |  |  |  |
|       Corixidae | 0.016 – | ns | ns | **<0.001** |
|       Notonectidae | 0.047 – | 0.012 – | ns | **<0.001** |
|     **Odonata** | 0.001 + | ns | ns | 0.014 |
|   CRUSTACEA<br>    **Amphipoda** |  |  |  |  |
|       *Gammarus pulex* | ns | 0.006 – | ns | ns |
|     **Isopoda** |  |  |  |  |
|       *Asellus aquaticus* | ns | 0.002 – | 0.047 | **<0.001** |
| **MOLLUSCA**<br>  GASTROPODA | **<0.001 –** | ns | ns | 0.014 |
|   **PLATYHELMINTHES** | 0.019 + | ns | 0.021 | **<0.001** |

**Table 3.2.** Benthic Community: The main effects of heating (H), fish addition (F), nutrient addition (N) and season on the invertebrate biomass found in the sampling of 48 mesocosms over a period of 12 months (September 2006 – August 2007). Any interaction effects are described within the text. Symbol (+/–) denotes direction of effect. The following taxa were recorded but showed no significant response to treatments or season and are therefore omitted from the table: Chaoboridae, Culicidae, *Cloeon dipterum*, Corixidae, Notonectidae and Odonata.

| PHYLUM<br>  CLASS/SUBCLASS<br>    Order/Suborder<br>      Family<br>        *Species* | H | F | N | S |
|---|---|---|---|---|
| **ANNELIDA** | | | | |
|   HIRUDINEA | ns | ns | ns | 0.057 |
|   OLIGOCHAETA | <0.001 + | ns | ns | 0.001 |
| | | | | |
| **ARTHROPODA** | | | | |
|   ARACHNIDA | | | | |
|     **Hydracarina** | <0.001 – | ns | ns | <0.001 |
| | | | | |
|   INSECTA | | | | |
|     **Coleoptera** | ns | ns | ns | ns |
|     **Diptera** | | | | |
|       Chironomidae | 0.033 – | ns | ns | 0.001 |
| | | | | |
|   CRUSTACEA | | | | |
|     **Amphipoda** | | | | |
|       *Gammarus pulex* | ns | <0.001 – | ns | 0.002 |
|     **Isopoda** | | | | |
|       *Asellus aquaticus* | ns | <0.001 – | ns | <0.001 |
| | | | | |
| **MOLLUSCA** | | | | |
|   BIVALVIA | <0.001 + | ns | ns | 0.001 |
|   GASTROPODA | 0.005 – | ns | ns | <0.001 |
| | | | | |
| **PLATYHELMINTHES** | ns | 0.040 – | 0.011 | <0.001 |

**ANNELIDA**

HIRUDINEA

The biomass of Hirudinea varied significantly with season in the sweep sampling

(Table 3.1) with a larger biomass in the Spring sampling compared to Winter

(Post-hoc LSD test P= 0.025) and Summer season when compared with all

other seasons (Post-hoc LSD tests; Autumn P = 0.003; Winter P < 0.01 and

Spring P = 0.004).

OLIGOCHAETA

Oligochaeta were mainly present in the benthic sampling and had a significantly

higher biomass in the Spring and Summer samples than in Autumn (Post-hoc

LSD test; P = 0.003 and P = 0.013 respectively).  Heated mesocosms contained

a greater biomass of Oligochaeta regardless of the season (Fig. 3.2), which over

the entire year was, on average, 703% greater than in ambient mesocosms.  A

three-way interaction between heating, nutrients and season was present but

the biological relevance of this is difficult to interpret.



**Figure 3.2**. The average biomass of Oligochaeta found in the benthic sampling of 48
mesocosms across four seasons in ambient and heated mesocosms.  Data are
biomasses (log (n + 1) transformed) and values shown are means ± SE.

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**ARTHROPODA**

ARACHNIDA

**Hydracarina**

The was less biomass of freshwater mites in the sweep samples in the Winter season compared to all other seasons (Post-hoc LSD tests; $P < 0.001$ for all comparisons). There was a seasonally dependent impact of heating, with heated mesocosms containing a greater biomass of Hydracarina but only in Autumn and Summer (H × S interaction, $P = 0.034$; Fig. 3.3). A heating × fish interaction showed a decrease in Hydracarina biomass with fish addition but only in ambient mesocosms (H × F interaction, $P = 0.023$; Fig. 3.4). Benthic sampling showed a significant increase in the biomass of Hydracarina (Table 3.2) of 445% in heated mesocosms when compared with ambient mesocosms across the 12 month experimental period.
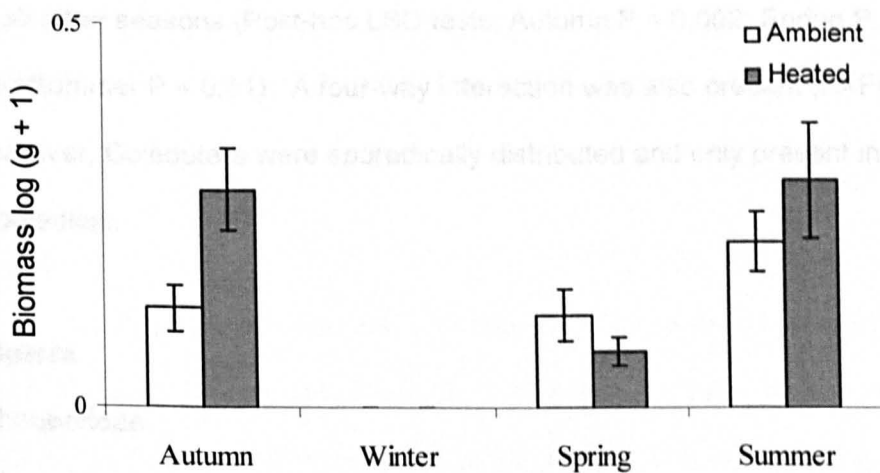
**Figure 3.3**. The average biomass of Hydracarina found in the sweep sampling of 48 mesocosms across four seasons in ambient and heated mesocosms. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
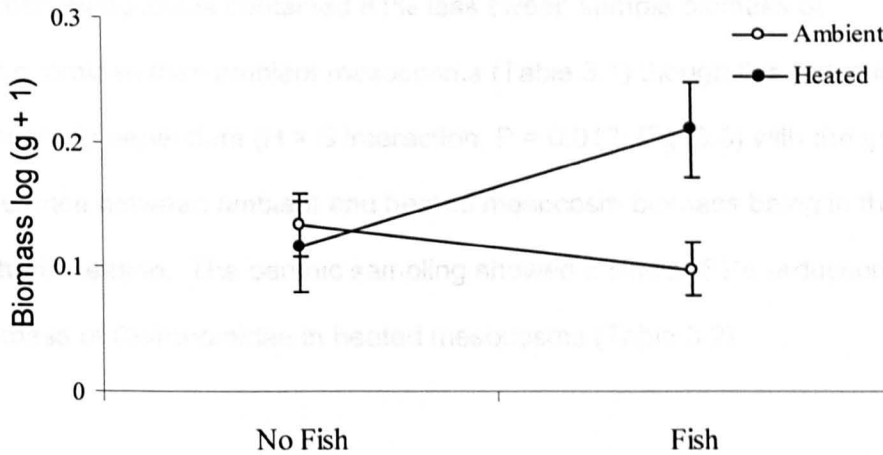
**Figure 3.4**. An interaction plot displaying the effects of heating and fish on the sweep sample biomass of Hydracarina in 48 mesocosms across a 12 month period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.

INSECTA

**Coleoptera**

The sweep sample biomass of Coleoptera (adults and larvae combined) varied significantly with season due to a lower biomass in the winter season compared to all other seasons (Post-hoc LSD tests; Autumn P = 0.002; Spring P = 0.005 and Summer P < 0.01). A four-way interaction was also present (H×F×N×S). However, Coleoptera were sporadically distributed and only present in low biomasses.

**Diptera**

Chaoboridae

Chaoboridae were mainly present in the sweep sampling and showed a significant 75% reduction in biomass in mesocosms containing fish (Table 3.1).

Chironomidae

Heated mesocosms contained 81% less sweep sample biomass of
Chironomidae than ambient mesocosms (Table 3.1) though this reduction was
seasonally dependent (H × S interaction, P = 0.012; Fig. 3.5) with the greatest
difference between ambient and heated mesocosm biomass being in the
Autumn season.  The benthic sampling showed a similar 81% reduction in the
biomass of Chironomidae in heated mesocosms (Table 3.2)

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**Figure 3.5**. The average biomass of Chironomidae found in the sweep  sampling of 48
mesocosms across four seasons in ambient and heated mesocosms.  Data are
biomasses (log (g + 1) transformed) and values shown are means ± SE.

Culicidae

Most culicids were observed in the sweep  samples where the biomass was
lower in the Winter sampling (Table 3.1).  There was a fish × season interaction,
with a reduced biomass in tanks containing fish but only in the Spring season
(F × S interaction, P = 0.007; Fig. 3.6).
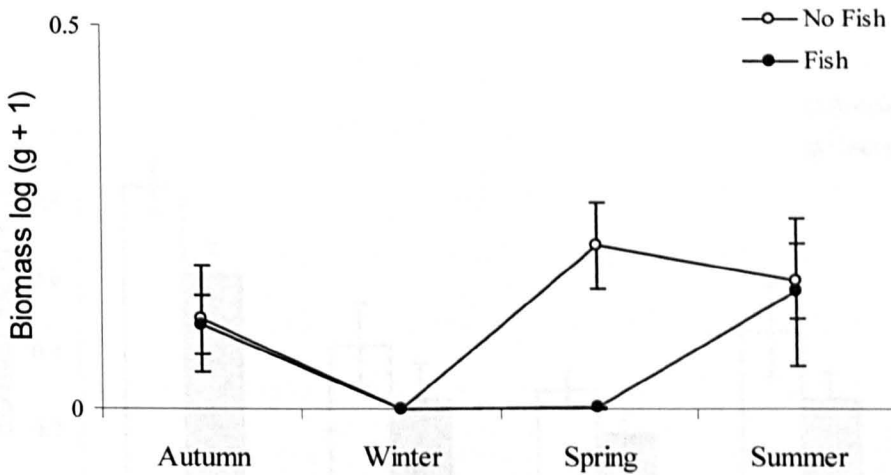
**Figure 3.6**. An interaction plot displaying the effects of fish addition and season on the sweep sampling biomass of Culicidae in 48 mesocosms across a 12 month period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.

## Hemiptera

Corixidae

The sweep  sampling showed a seasonal effect on biomass of Corixidae (Table 3.1) with significantly more biomass present in the Autumn sampling than any other season (Post-hoc LSD tests; $P < 0.001$ in all cases; Fig. 3.7).  There was a lower corixid biomass in Spring than in Summer (Post-hoc LSD tests; $P = 0.049$).  Ambient mesocosms contained 54% less corixid biomass than heated mesocosms over the course of the sampling (Fig. 3.7).  There was a seasonally dependent fish effect with a greater corixid biomass present in fish tanks but only in Autumn (F × S interaction, $P = 0.018$; Fig. 3.8).  A heating × nutrients × season interaction was also present.

**Figure 3.7.** The average biomass of Corixidae found in the sweep sampling of 48 mesocosms across four seasons in ambient and heated mesocosms. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
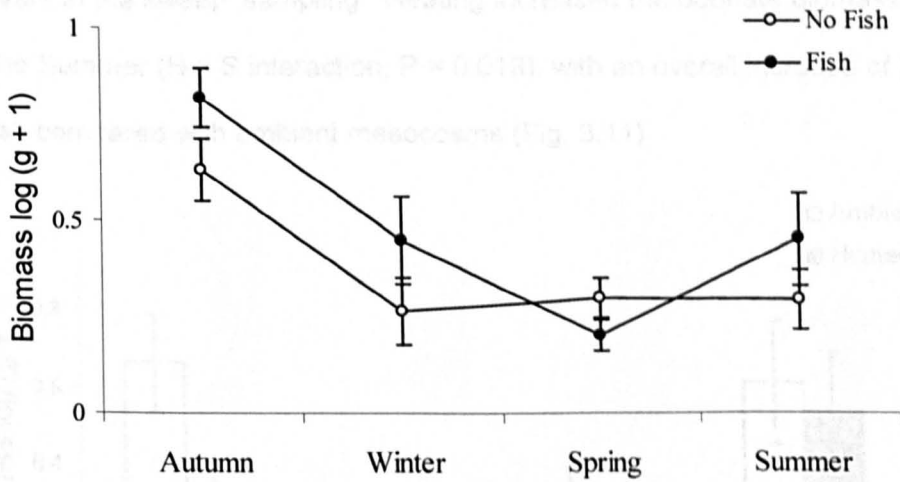


**Figure 3.8**. An interaction plot displaying the effects of fish addition and season on the sweep sampling biomass of Corixidae in 48 mesocosms across a 12 month period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.

Notonectidae

Notonectidae were found primarily in the sweep sampling and biomass was significantly lower in the Winter than all other seasons (Post-hoc LSD tests: Autumn $P < 0.001$; Spring $P = 0.016$ and summer $P < 0.001$; Fig. 3.9). Spring samples had a lower biomass when compared to the Autumn (Post-hoc LSD tests; $P = 0.012$) and Summer sampling (Post-hoc LSD tests; $P = 0.006$; Fig. 3.9). Heating the mesocosms reduced the notonectid biomass by 56% across the experimental period (Fig. 3.9). Mesocosms containing fish had 69% less notonectid biomass though this result was seasonally dependent with the greatest difference between no fish and fish mesocosms being seen in the Spring and Summer seasons (F × S interaction, $P = 0.003$; Fig. 3.10).

**Odonata**

The Odonata consisted of zygopteran and anisopteran nymphs and were mainly present in the sweep sampling. Heating increased the odonate biomass except in the Summer (H × S interaction, $P = 0.019$), with an overall increase of 285% when compared with ambient mesocosms (Fig. 3.11).
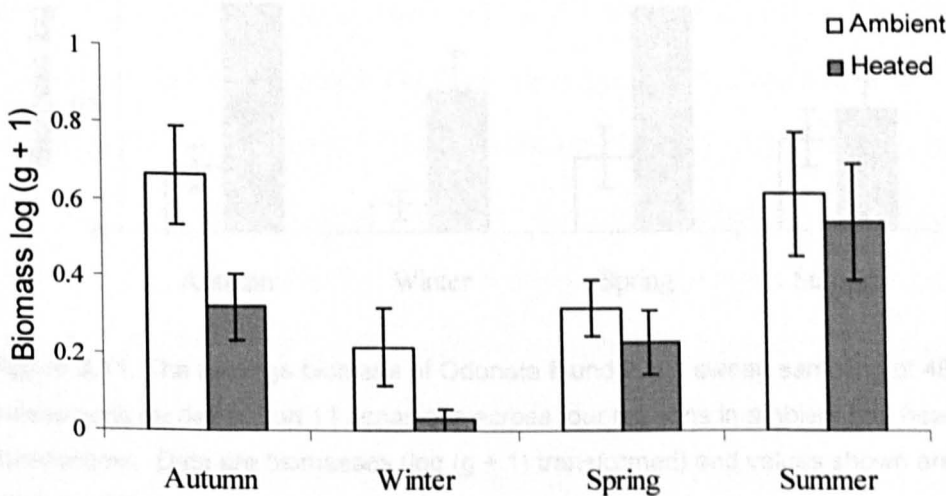


**Figure 3.9.** The average biomass of Notonectidae found in the sweep sampling of 48 mesocosms across four seasons in ambient and heated mesocosms. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
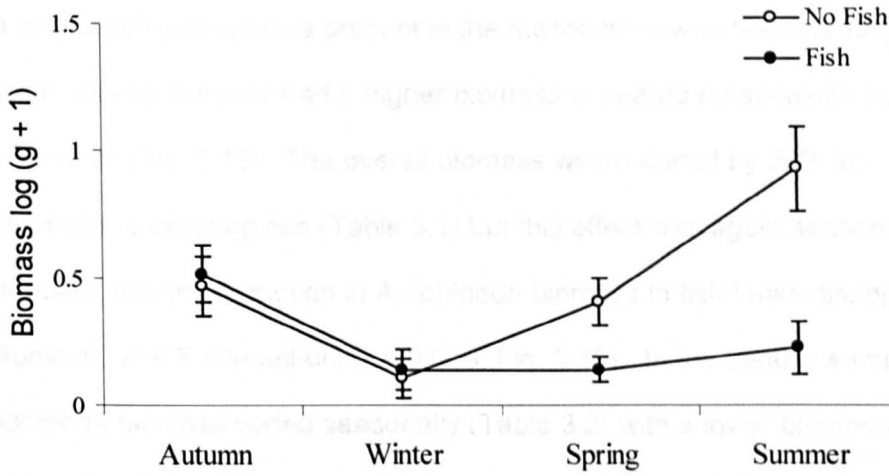
**Figure 3.10**. An interaction plot displaying the effects of fish and season on the sweep sampling biomass of Notonectidae in 48 mesocosms across a 12 month period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
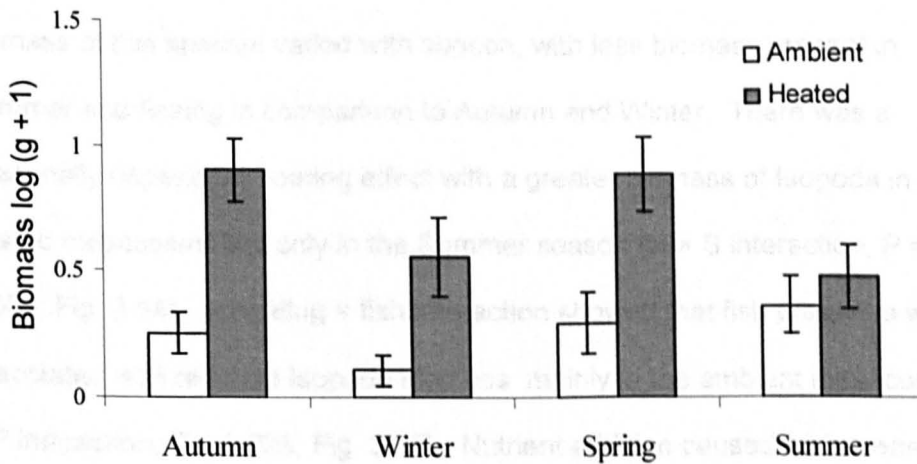


**Figure 3.11**. The average biomass of Odonata found in the sweep sampling of 48 mesocosms carried out on 11 occasions across four seasons in ambient and heated mesocosms. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.

CRUSTACEA

**Amphipoda**

The only amphipod species present in the mesocosms was *Gammarus pulex*, which in sweep samples had a higher biomass in heated mesocosms but only in the Summer (Fig. 3.12). The overall biomass was reduced by 54% in mesocosms containing fish (Table 3.1) but this effect was again seasonally dependent with the reduction in Amphipoda biomass in fish tanks disappearing in Summer (H × S interaction, P = 0.013; Fig. 3.13). In the benthic samples, Amphipoda biomass varied seasonally (Table 3.2) with a lower biomass in summer than in the Winter and Spring seasons (Post-hoc LSD test: P < 0.001 and P = 0.006 respectively). The biomass was also significantly reduced by 16% in mesocosms containing fish (Table 3.2).


**Isopoda**

The species of Isopoda found in the mesocosms was *Asellus aquaticus* and the biomass of this species varied with season, with less biomass present in Summer and Spring in comparison to Autumn and Winter. There was a seasonally dependent heating effect with a greater biomass of Isopoda in heated mesocosms but only in the Summer season (H × S interaction, P = 0.008; Fig. 3.14). A heating × fish interaction showed that fish presence was associated with reduced Isopoda biomass mainly in the ambient mesocosms (H × F interaction, P = 0.025; Fig. 3.15). Nutrient addition caused an increase in Isopoda biomass but this increase was only significant between the no nutrient addition and the N1 (Post-hoc LSD; P = 0.036) and N2 (Post-hoc LSD; P = 0.028) nutrient regimes (Fig. 3.16). A heating × fish × nutrients interaction was also present. Benthic seasonal data also showed a reduction in Isopoda biomass in Summer when compared with other months. Fish mesocosms contained around 47% less biomass of Isopoda than non fish tanks, though

again this effect was temperature dependent with fish effect being greatest in ambient mesocosms (H × F interaction, P = 0.001). A nutrient × season interaction showed biomass to be higher in N2 treatments but only in the winter season. There was also a 3-way heating × fish × nutrients interaction.
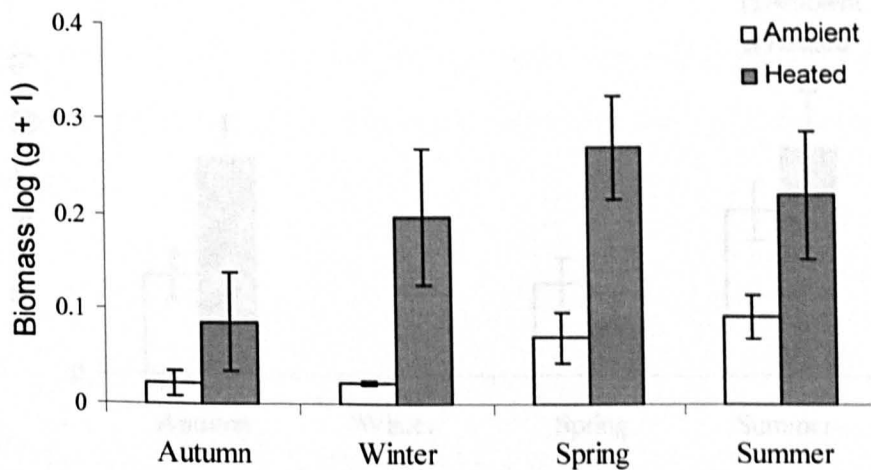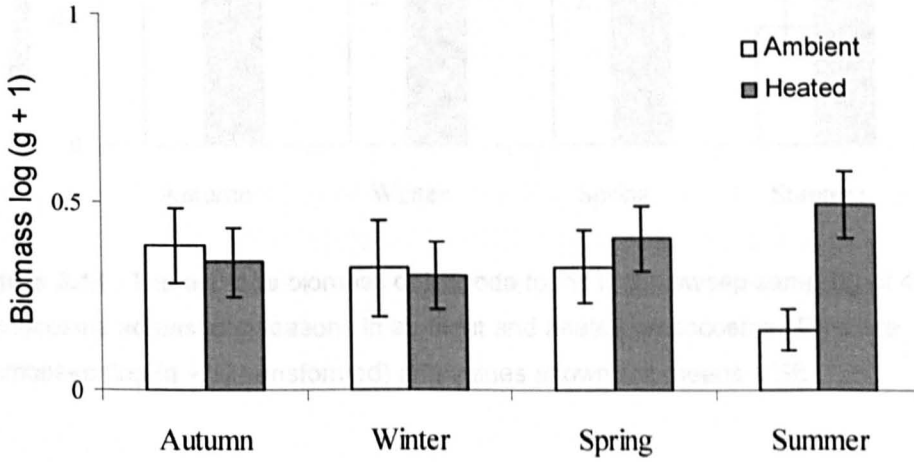


**Figure 3.12**. The average biomass of Amphipoda found in the sweep sampling of 48 mesocosms across four seasons in ambient and heated mesocosms. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
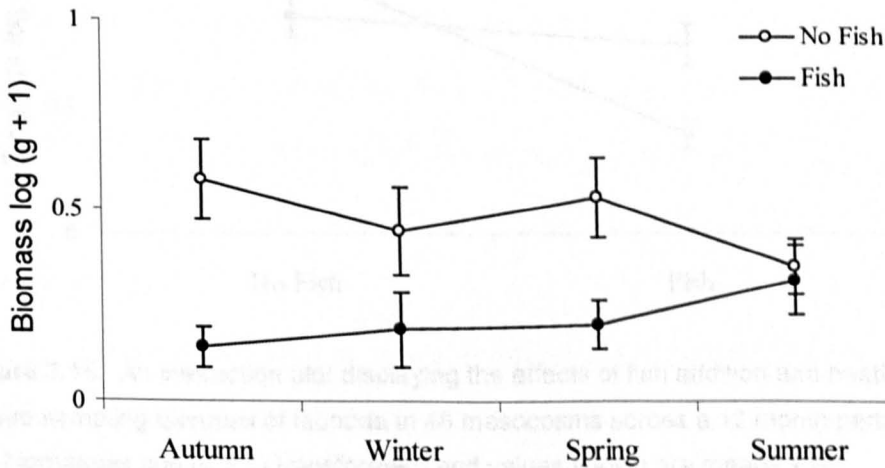


**Figure 3.13**. An interaction plot displaying the effects of fish addition and season on the sweep sampling biomass of Amphipoda in 48 mesocosms across a 12 month period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
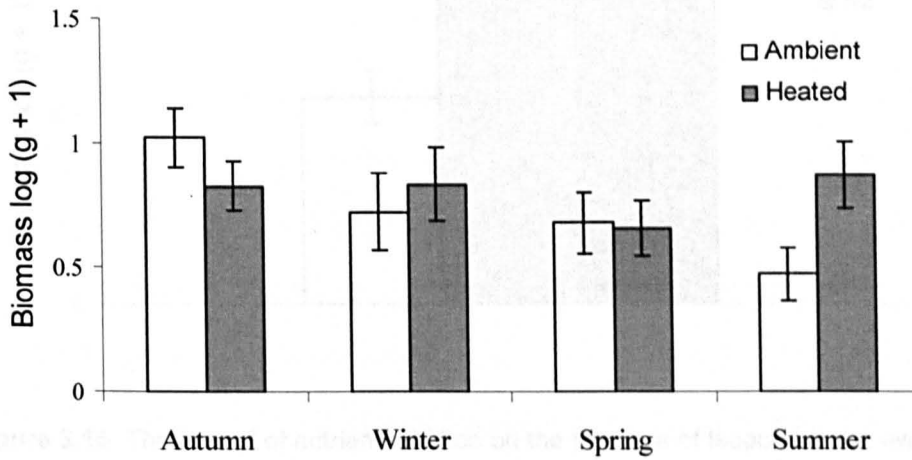
**Figure 3.14**. The average biomass of Isopoda found in the sweep sampling of 48 mesocosms across four seasons in ambient and heated mesocosms. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
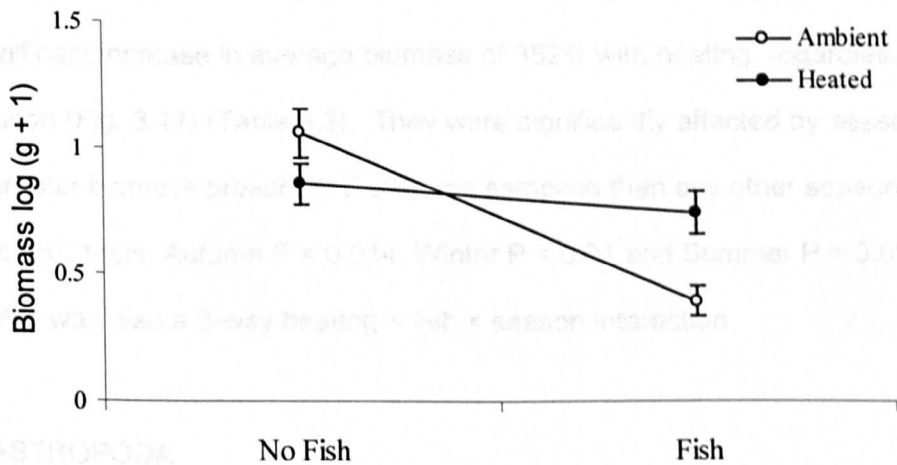


**Figure 3.15**. An interaction plot displaying the effects of fish addition and heating on the sweep sampling biomass of Isopoda in 48 mesocosms across a 12 month period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
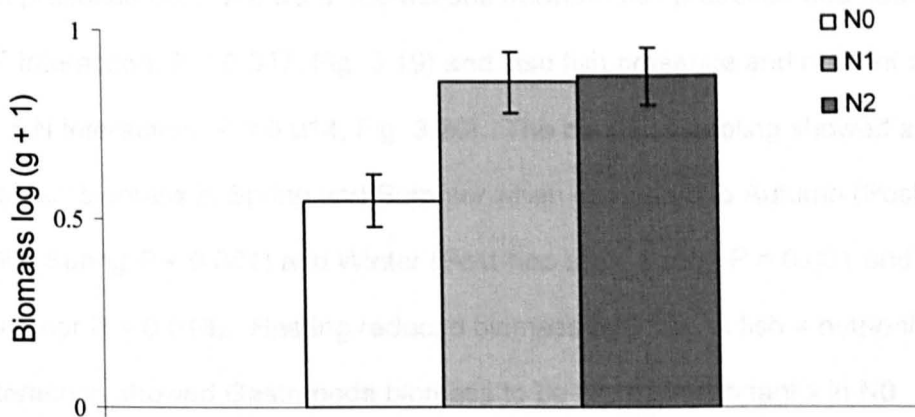
**Figure 3.16**. The impact of nutrient addition on the biomass of Isopoda in the sweep sampling of 48 mesocosms across a 12 month experimental period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.

## Mollusca

### BIVALVIA

Bivalvia were found mainly in the benthic sampling where they showed a significant increase in average biomass of 352% with heating, regardless of season (Fig. 3.17) (Table 3.2). They were significantly affected by season with a greater biomass present in the Spring sampling than any other season (Post-hoc LSD tests; Autumn $P = 0.014$; Winter $P < 0.01$ and Summer $P = 0.002$). There was also a 3-way heating × fish × season interaction.

### GASTROPODA

The Gastropoda present in the sweep sampling varied significantly with season (Table 3.1) with a greater biomass being present in Summer than in any other season (Post-hoc LSD; $P < 0.001$ in all cases). Heating caused an overall 76% decrease in biomass. This effect was seasonally dependent with the greatest decrease in Gastropoda biomass in heated mesocosms during the Winter season (H × S interaction, $P = 0.039$; Fig. 3.18). There was no direct effect of

fish presence but there were interactions between fish presence and heating (H × F interaction, P = 0.037; Fig. 3.19) and also fish presence and nutrient addition (H × N interaction, P = 0.014; Fig. 3.20). The benthic sampling showed a greater biomass in Spring and Summer when compared to Autumn (Post-hoc LSD; Spring P < 0.001) and Winter (Post-hoc LSD; Spring P = 0.001 and Summer P = 0.016). Heating reduced biomass by 78%. A fish × nutrients interaction showed Gastropoda biomass to be higher in fish tanks in N0 treatments but the pattern was reversed in N2 treatments (F × N interaction, P = 0.014; Fig. 3.21). There was also a 3 way heating × fish × season interaction. Details on individual gastropod families/species can be found in Appendix 2.
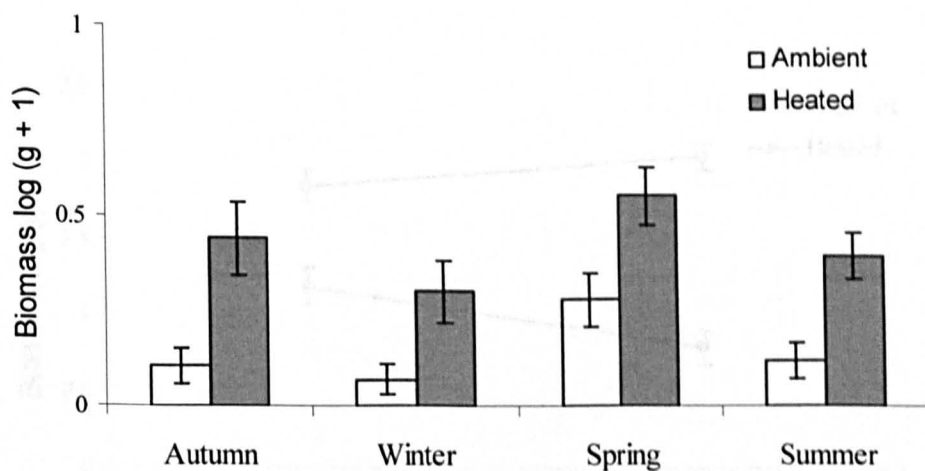


**Figure 3.17**. The average biomass of Bivalvia found in the benthic sampling of 48 mesocosms across four seasons in ambient and heated mesocosms. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
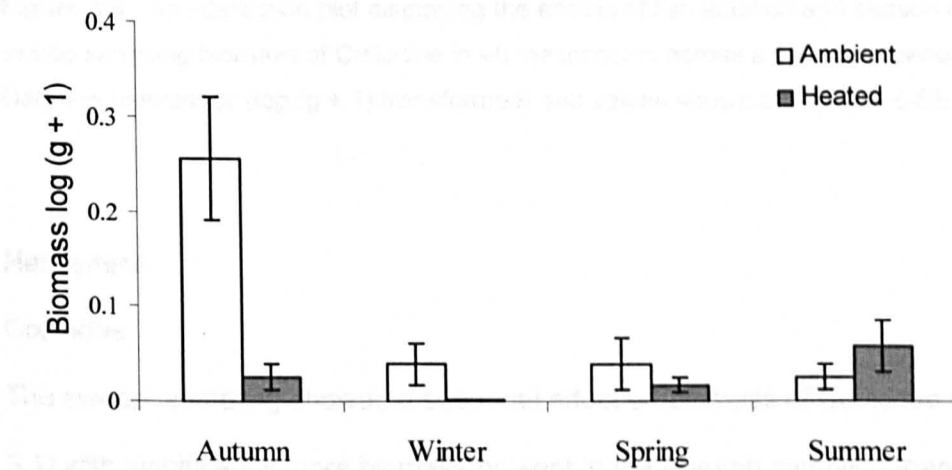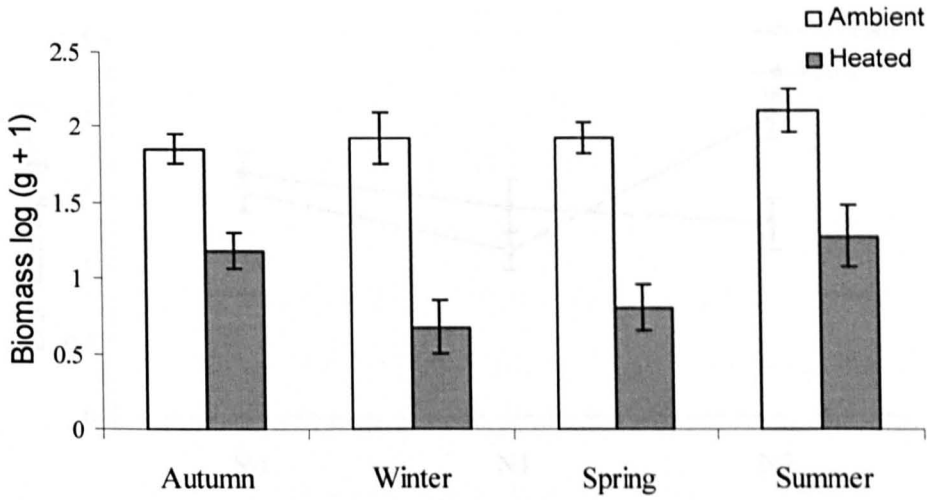
**Figure 3.18.** The average biomass of Gastropoda found in the sweep sampling of 48 mesocosms across four seasons in ambient and heated mesocosms. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
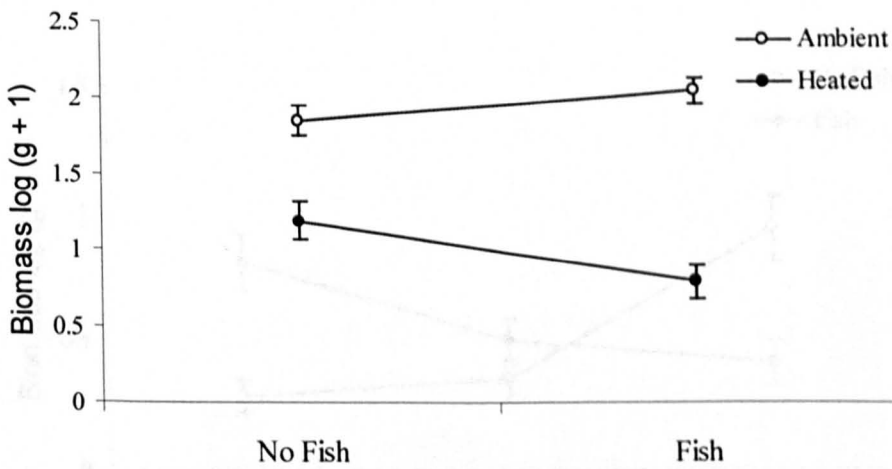


**Figure 3.19.** An interaction plot displaying the effects of heating and fish on the biomass of Gastropoda in the sweep sampling of 48 mesocosms across a 12 month period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
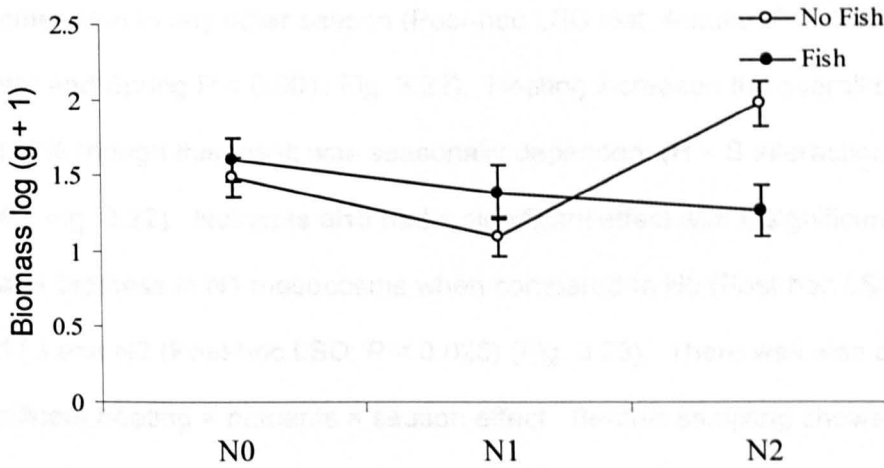
**Figure 3.20.** An interaction plot displaying the effects of nutrient addition and fish on the biomass of Gastropoda in the sweep sampling of 48 mesocosms across a 12 month period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
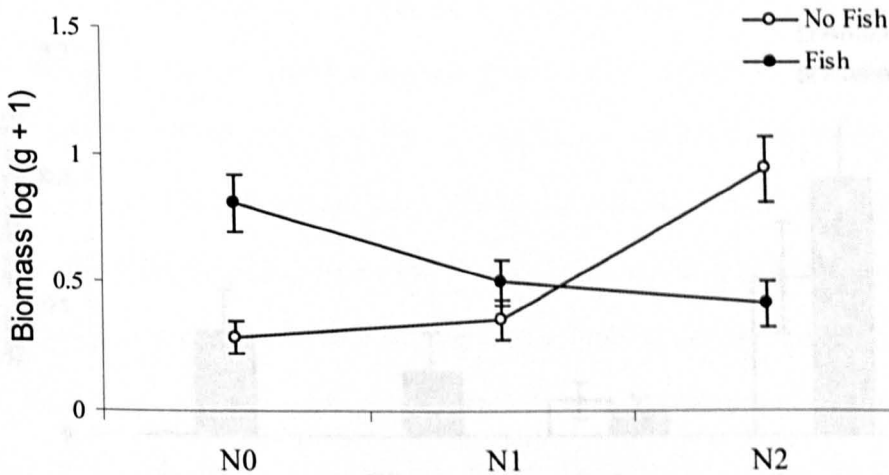
**Figure 3.21.** An interaction plot displaying the effects of nutrient addition and fish on the biomass of Gastropoda in the benthic sampling of 48 mesocosms across a 12 month period. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.

## PLATYHELMINTHES

In the sweep sampling there was a greater biomass of Platyhelminthes in Summer than in any other season (Post-hoc LSD test; Autumn P = 0.001; Winter and Spring P < 0.001; Fig. 3.22). Heating increased the overall biomass by 130% though this result was seasonally dependent (H × S interaction, P = 0.049; Fig. 3.22). Nutrients also had a significant effect with a significantly greater biomass in N1 mesocosms when compared to N0 (Post-hoc LSD; P = 0.011 ) and N2 (Post-hoc LSD; P = 0.025) (Fig. 3.23). There was also a significant heating × nutrients × season effect. Benthic sampling showed the same pattern of increased biomass in summer and in N1 mesocosms (Table 3.2). Fish significantly reduced the biomass of Platyhelminthes in the benthic sampling by 66% (Table 3.2).
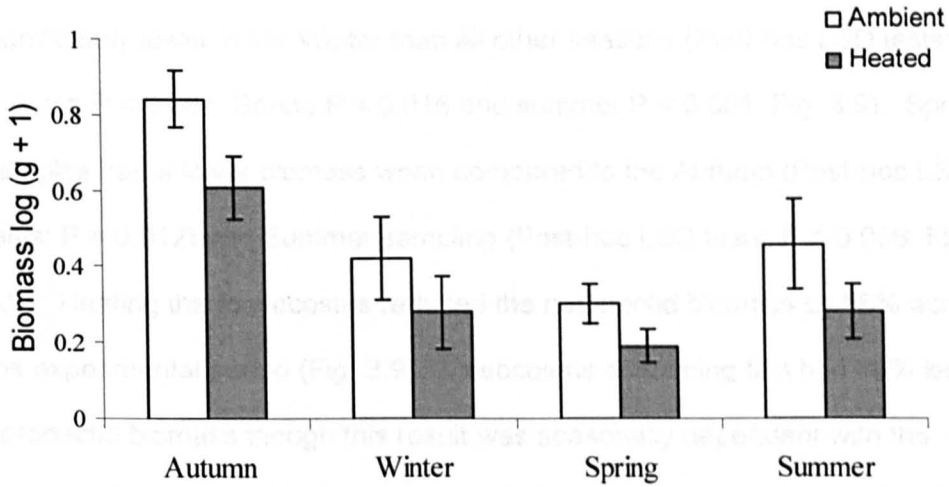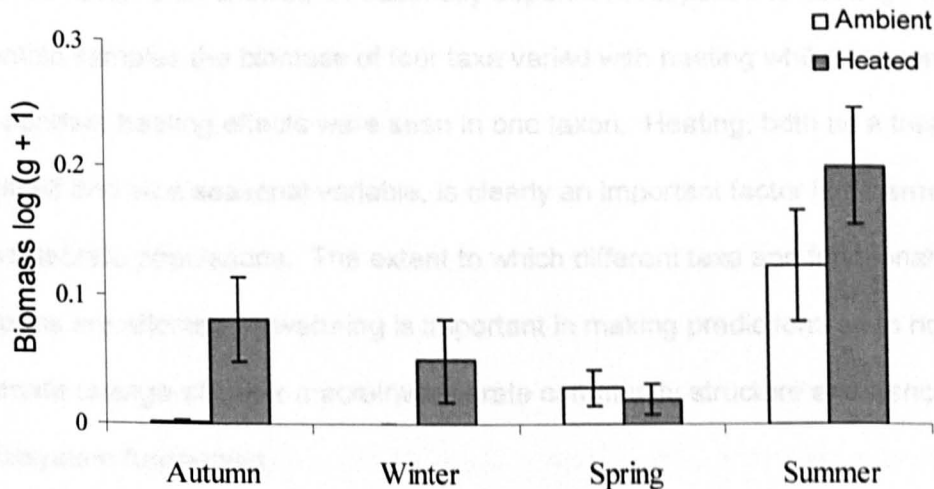


**Figure 3.22.** The average biomass of Platyhelminthes found in the sweep sampling of 48 mesocosms across four seasons in ambient and heated mesocosms. Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.
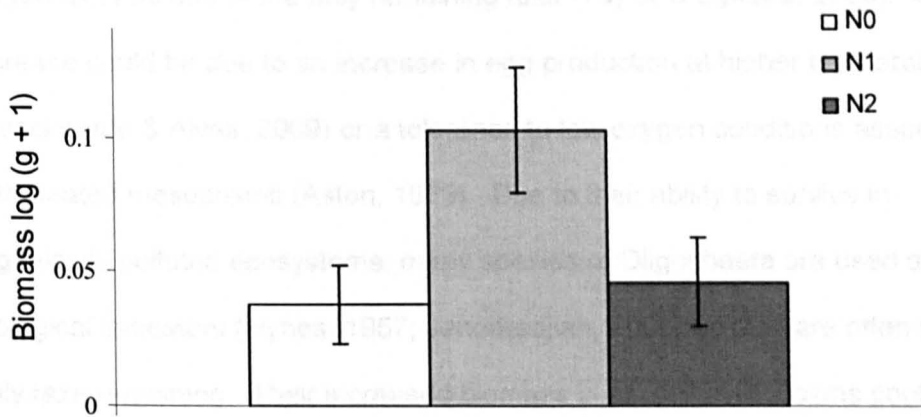
**Figure 3.23**. The impact of nutrient addition on the biomass of Platyhelminthes in the sweep sampling of 48 mesocosms across a 12 month experimental period.  Data are biomasses (log (g + 1) transformed) and values shown are means ± SE.

## Discussion

In the sweep samples four taxa showed a biomass response to heating whilst a further seven taxa showed a seasonally dependent response to heating.  In the benthic samples the biomass of four taxa varied with heating whilst seasonally dependent heating effects were seen in one taxon.  Heating, both as a treatment in itself and as a seasonal variable, is clearly an important factor in determining invertebrate populations.  The extent to which different taxa and functional groups are affected by warming is important in making predictions as to how climate change will alter macroinvertebrate community structure and hence ecosystem functioning.

Oligochaeta increased with temperature both in the sweep and benthic sampling (Fig. 3.2).  The result is consistent with a previous study on the macroinvertebrate assemblage in a Canadian stream heated by a hot spring which showed a shift in community structure to a sparse community with

Oligochaeta as one of the only remaining taxa (Taylor & Dykstra, 2005). This increase could be due to an increase in egg production at higher temperatures (Nascimento & Alves, 2009) or a tolerance to low oxygen conditions associated with heated mesocosms (Aston, 1973). Due to their ability to survive in organically polluted ecosystems, many species of Oligochaeta are used as biological indicators (Hynes, 1957; Jenderedjian, 2005) as they are often the only taxa remaining. Their increased biomass in heated mesocosms could therefore be an indicator of an increasingly stressed system with characteristics of organically polluted water bodies e.g. low oxygen. However, their presence in mesocosms was patchy and they accounted for less than 1% of the invertebrate biomass in ambient mesocosms and approximately 2% and 3% of the total biomass of invertebrates in the heated sweep and benthic community respectively.

Hydracarina biomass increased with heating though in a seasonally dependent manner (Fig. 3.3) with the main effect being seen in Autumn. Although relatively sparsely distributed in the mesocosms (less than 1% of the sweep sampling biomass), water mites have a complex life cycle including parasitic and predatory stages which have been shown to alter the structure of invertebrate communities (Di Sabatino et. al., 2000). Therefore, increases of biomass with temperature change could have consequences for the prey items and the aerial insects parasitized by the water mites (Davids, 1990). Mechanisms behind an increase in mite biomass with temperature could include increases in prey encounter rate at higher temperature leading to higher survival rates or could be an artefact of an increase in aerial host numbers present in heated mesocosms (Fig. 3.11).

The seasonal data on dipteran biomass must be interpreted with caution as summarising the data into seasons may disguise important temperature induced shifts in timing of emergence which could explain seasonal differences in biomass. For example the chironomids showed a significant reduction in biomass in heated mesocosms though, in the benthic sampling, this response was mainly seen in autumn (Fig. 3.5). The reduction in biomass in heated mesocosms in autumn could result from earlier emergence of adults, probably due to accelerated development (Frouz et. al., 2002), thus leaving a higher chironomid biomass present in the ambient mesocosms. Such shifts in life history have already been shown for a number of freshwater invertebrates in natural communities (Hogg & Williams, 1996; Hassall et al., 2007; Tixier et.al. 2009). The possible early emergence of these invertebrates thus removes a potentially important food source for fish and other invertebrates at a time when their metabolic rates are also higher due to increased temperature. Being gatherers, they may also play an important role in capturing FPOM from the water column and transferring it to the substrate. Their bioturbation activities are also important in the mixing of sediment particles (Fukuhara & Sakamoto, 1987; Svensson et.al., 1999). On a large scale, the earlier emergence of Chironomidae and other arthropods is predicted to remove an important 'burst' of food required by migratory birds in the Arctic unless they too undergo a similar shift in life history (Tulp & Schekkerman, 2008).

Biomasses of two families of the order Hemiptera, the Corixidae and Notonectidae, were both significantly reduced with heating by 54% and 56% respectively regardless of season (Fig. 3.7 & Fig. 3.9) (Table 3.1). Both are extremely active swimmers and their reduction could be attributed to the low dissolved oxygen conditions associated with the heated mesocosms

(Chapter 2). Notonectids predate on other invertebrates and can play a role in the regulation of larval populations of mosquitoes (Papacek, 2001; Saha *et. al.*, 2007). More generally, their reduced abundance with increased temperature could potentially remove the control of an important disease vector. Notonectids also form part of the diet of a number of insectivorous fish (Macan, 1965; Cook & Streams, 1984; Schilling *et. al.*, 2009). Corixidae are included in the diet of some fish, birds and even humans (Papacek, 2001) so their sensitivity to increased temperature could be of economic importance.

Odonata are important predators in freshwater ecosystems and showed a consistent significant increase with heating in our mesocosms, except in summer (Fig. 3.11). The non-significant result in summer was possibly due to earlier emergence of adults with heating resulting in no difference in biomass between heated and ambient mesocosms. The mechanisms behind the general pattern of increased biomass of odonates in heated mesocosms are unclear but could be due to adult preference for heated mesocosms or increased survival of juveniles, neither of which have been documented in the literature. The consequences of increased biomass of Odonata may become important if they manage to reduce zooplankton populations enough to reduce grazing pressure on phytoplankton. As in fish-dominated aquatic systems this can result in the loss of clear water and the switch to turbid algal-dominated systems (Zagarese, 1990; Liu & Herzig, 1996; Chappaz *et. al.*, 1998).

The Crustacea were one of the most abundant arthropod classes, comprising up to 77% and 61% of the total sweep sampling and benthic community biomass. Both representatives of the class, Amphipoda and Ispoda, showed a seasonally dependent increase of biomass in sweep samples with heating in the summer season (Fig. 3.12 & Fig. 3.14). This increase could be due to a decrease in the

high predation rates of these invertebrates (Table 3.1 and Table 3.2) by

sticklebacks during the summer when stickleback populations in most heated

mesocosms were lost (Chapter 2). This hypothesis is further supported by the

interaction between heating and fish showing that fish presence reduced isopod

biomass but only in ambient mesocosms (Fig. 3.15). Both species are

detritivores and thus play nutrient cycling roles in the ecosystem (Graça, 2001).

*Gammarus pulex*, found in our mesocosms, has also been shown to predate on

other invertebrates including some species of Ephemeroptera (MacNeil *et. al.*,

1997; Kelly *et. al.*, 2002). Despite some references indicating that *Asellus*

*aquaticus* are relatively inferior shredders (Bjelke & Herrmann, 2005) their sheer

abundance in many freshwater ecosystems makes them important and a

reduction due to fish predation could have consequences for nutrient cycling in

freshwaters.

One of the most consistent decreases in biomass with heating was seen in the

Gastropoda with a 74% reduction in sweep sampling biomass, with the largest

difference seen in winter (Fig. 3.18), and 76% reduction in benthic biomass and

is in contrast to previous mesocosm studies (McKee *et. al.*, 2003). The

mechanisms behind this decrease are again unclear. The presence of

gastropods can be associated with the stabilization of macrophyte dominated

systems via the grazing of periphyton as shown in Underwood's (1991)

experiment where the removal of epiphyton by grazing snails significantly

prolonged the life of individual *Ceratophyllum* leaves. Therefore, the decline of

gastropods with increasing temperature could lead to an increase in algae and a

loss of macrophytes (Jones *et. al.*, 1999). The other Molluscan class

represented in this study, the Bivalvia, showed the opposite trend and increased

with heating independently of season (Fig. 3.17). Freshwater bivalves have a

well-documented nutrient cycling role in freshwater ecosystems. They are filter

feeders and their rate of food uptake is directly related to their metabolic rate, thus meaning bivalves in heated mesocosms have a higher rate of food uptake than those in ambient mesocosms. During their feeding, they enhance the benthic-pelagic nutrient exchange by the intake of organic matter as food and the subsequent transfer of this matter to the sediment as faeces and pseudofaeces. As a result of this they directly affect the macroinvertebrate assemblage, specifically increasing the number of amphipods in one experiment (Greenwood et. al., 2001) and increasing the biomass of common invertebrates in another (Sylvester et. al., 2007).

It would appear that in contrast to previous studies (Baulch et al. 2005) heating does have an impact on macroinvertebrate populations and that this impact is sometimes seasonally dependent. The extent of the impact varied greatly with taxon both in magnitude and the direction of the effect (increasing or decreasing). Of the seasonally dependent heating impacts, differences in populations were due to a number of factors and not just due to heating causing differential mortality dependent on the season (e.g. possible temperature dependent shift in chironomid and odonate emergence patterns; reduced fish predation on amphipods and isopods in heated mesocosms in summer; increased numbers of platyhelminthes in summer due to populations increasing with time). Only gastropods appeared to show a seasonally dependent heating effect with populations being reduced in heated mesocosms especially in the winter. Heating also had complex interactions with both nutrient addition and fish presence.

Impacts on an individual invertebrate species, or on larger taxonomic or functional groups, either through changes in population sizes or life history, have the potential to interfere with trophic interactions and hence ecosystem

functioning. The potential for climate change to alter freshwater ecosystems is therefore great and more research is needed into the response of individual species and functional groups under a wide range of conditions in order to predict future impacts and devise management schemes. The next chapter will concentrate on the mechanisms behind the major population changes present at the end of the experiment.

# Chapter 4

Differential impacts of warming on macroinvertebrates in shallow eutrophic freshwaters

## 4.1 Introduction

Aquatic ectotherms are vulnerable to changes in environmental temperature due

to the dependence of their internal rates and processes on external

temperatures. With global temperatures predicted to increase by 3 to 5°C in

temperate regions over the next century (IPCC, 2007) there may be major

changes in invertebrate diversity and related ecosystem functioning. However,

the literature available on the impact of warming on freshwater

macroinvertebrates is sparse and sometimes contradictory.

Diverse impacts of warming are expected, dependent upon the magnitude of

change and its combination with other environmental stressors such as

anthropogenic nutrient loading. These impacts can be direct, either through

effects on rates, including reproduction, or through stress effects at thermal

limits (Ward & Stanford, 1982). They can also be indirect through a vast number

of processes including alterations to food and habitat availability and quality,

cycling of elements such as phosphorus, alteration of predator/competitor/

parasite numbers and activity, or through altered oxygen availability (Davids,

1990; Di Sabatino et. al., 2000). Dissolved oxygen (DO) has been shown to

decrease with warming (Chapter 2; Moran et. al., in press) through a number of

potential mechanisms: 1) the physical effect of temperature on the ability of

water to hold DO; 2) increase in net respiration relative to that of photosynthesis

leading to a heterotrophic system; 3) increasing plant growth and hence decay

leading to high levels of decomposition (Feuchtmayr et al., 2009; Moran et al. in

press). This decrease is set against the increasing metabolic rates and

therefore DO requirements of aquatic ectotherms with warming, creating a

potential mismatch between supply and demand of oxygen (Pörtner et. al.,

2006). Species that are generally more active and hence have a higher

metabolic rate during routine activity may therefore be more susceptible to

temperature-related decreases in DO concentrations. This may mean that particular feeding guilds are more susceptible than others. For example, slow-moving feeders such as some benthic detritivores may be able to withstand lower DO concentrations than active predators.

Baulche *et al.* (2005) found little effect of experimental warming by 4.5°C on either the abundance or composition of invertebrate communities in near-shore enclosures of a boreal lake. In contrast, an experiment involving manipulation of the thermal regime of a Canadian stream by 2-3.5°C showed an overall decrease in invertebrate density, particularly Chironomidae (Tixier *et. al.*, 2009; Hogg & Williams, 1996). Another study examining long term data sets of Swedish lakes and rivers has shown that an increase in temperature over 10 or more years caused a reduction in the numbers and places of occurrence of certain species of Gastropoda, Ephemeroptera, Coleoptera and Trichoptera, hence leading to an alteration in invertebrate community composition (Burgmer *et. al.*, 2007), but no overall trends in functional group biomass were reported. A major problem with these studies, however, is that they lack true replication (Carpenter, 1989). There is, therefore, a need for large-scale replicated manipulated ecosystem studies that include realistic communities and desirably also incorporate anthropogenic nutrient loadings because eutrophication remains perhaps the most extensive problem faced by freshwater communities and is likely to persist in the foreseeable future. Mesocosm experiments in which temperature and nutrient loading are manipulated address these problems but previous studies have found little effect of warming on macroinvertebrate abundances or community structures (Ventura *et. al.* 2008) with the exception of an increase in abundance of Gastropoda (Mckee *et al.*, 2003; Feuchtmayr *et al.*, 2007).

Temperature may also alter macroinvertebrate community composition by acting upon body size (Daufresne, 2009). At the community level it may lead to an increase in the number of small-sized species as predicted by Bergmann's rule (Bergman, 1847), which, although it was formulated for endotherms, has been demonstrated for ectotherms (Kaspari, 2005; Pincheira-Donoso et. al., 2007). Warming may act at the individual as well as the community level. According to the temperature-size rule, the individual body size of ectotherms tends to decrease with increasing temperature due to a decrease in size-at-age (Atkinson, 1994b) and there are several explanations for this including limited DO availability at increased temperatures (Atkinson, 1994a). This suggests that climate change could lead to a shift in populations to smaller bodied individuals.

This paper reports the fate of 48 invertebrate communities, sampled in September 2007 following a period of 21 months during which they were subject to temperature (ambient and ambient + 4°C) nutrient (no addition, low addition and high addition of nitrogen against a background high phosphorus concentration) and fish (presence/absence) manipulations. We predicted that:

- Heating would cause a decrease in the total invertebrate biomass due to associated decrease in the DO concentrations

- Decreases would be seen mainly in more active taxa, including predatory species

- Decreases in biomass would not only be due to decreases in numbers but also to decreases in the average size of organisms

- Nutrient addition would increase autotroph productivity, hence invertebrate community biomass

- The presence of fish would reduce total invertebrate population biomass, partly through direct predation, and partly through competition with predatory invertebrates for prey such as zooplankton

## 4.2 Methods

Forty-eight shallow lake mesocosms were established at Ness Botanical Gardens on the Wirral Peninsula, UK (53°16'N, 3°02'W). A detailed description of their establishment is provided in Chapter 2. Twenty-four tanks were heated to a continuous 4°C above the temperature of twenty-four ambient tanks. Temperature in the tanks was continuously monitored by sensors at a depth of 45cm, and oxygen readings were taken between 10.30 and 12.30 hours ("mid-day" samples) at a depth of 35cm every fortnight (monthly in winter) using a YSI Model 85 oxygen meter. Three nutrient regimes, reflecting the range of inputs in intensive agricultural landscapes (James et al., 2005), were applied in a randomised block design superimposed on the warming treatments (N0, no nutrient addition; N1 treatment: 250 $\mu$g L$^{-1}$ N and 50 $\mu$g L$^{-1}$ P; N2 treatment: 2500 $\mu$g L$^{-1}$ N and 50 $\mu$g L$^{-1}$ P, added as Na NO$_3$ and KH$_2$PO$_4$ every two weeks). Invertebrates were collected from local ponds and mixed before being distributed amongst the tanks in autumn 2005. Two to three weeks after the initial inocula were added, invertebrate communities were cross mixed between the tanks using standard net sweeps. In May 2006, when the systems had stabilised and oxygen concentrations were adequate, two adult male and two adult female three-spined sticklebacks (Gasterosteus aculeatus (L.)), obtained from an organic-rich pond less than 500m from the mesocosm site, were added

to half of the tanks in a randomised block design. The randomised block design

consisted of 4 blocks each containing 12 tanks.


*4.2.1 Final harvest*

The final harvest of the mesocosms took place from the 10th September 2007

over two weeks. All plants were removed and shaken in a bucket of water to

remove any invertebrates. Plants were then sorted and had their fresh weight

determined following spin-drying for a standard 5 minute period to remove

surplus water. Invertebrates remaining in the mesocosm were collected with a

standard 10 minute net sweep. Benthic invertebrates were sampled by taking a

25 by 25cm core of the sediment from the emptied mesocosms. All

invertebrates were preserved in 70% ethanol before being identified, usually to

family or species level if practicable and counted in the laboratory. The sizes of

the first ten randomly encountered individuals of each species were recorded in

5mm length classes excluding appendages e.g. tail filaments on Odonata and

Ephemeroptera or antennae in Isopoda. Estimates of biomass of invertebrates

were made using literature-obtained parameters of mass-length relationships in

the form:


$$ln\ M = ln\ a + b \cdot ln\ L$$


Where *M* is mass, *L* is Length and *a* and *b* are fitted regression constants

(Benke *et. al.* 1999; Sabo *et. al.* 2002; Baumgartner &, Rothhaupt, 2003; Stoffels

*et. al.* 2003; Chimney *et. al.* 2997; Miyasaka *et. al.* 2008). The mid-point of

each length class was substituted into the equation to produce an average mass

for that length class. This estimate was then multiplied by the proportion of the

individuals in the population in that length class to produce an estimate of

biomass for each size class and from that, for each invertebrate taxon. The

mass of Mollusca was calculated based on regression relationships including the shell mass (Benke *et. al.*, 1999; Baumgartner & Rothhaupt, 2003,).   As no literature mass-length relationship for Hirudinea could be found, one was derived using preserved specimens.  As these had been preserved in 70% ethanol a correction factor was applied to allow for the degradation of the specimens by preservation (Leuven *et. al.*, 1985).  For the analysis of major trophic categories, invertebrates were assigned to particular feeding groups dependent upon their main mode of feeding (Appendix 3).  Although the gastrpods were identified to family (Physidae and Planorbidae) or species (*Lymnaea peregra* and *Lymnaea stagnalis*) they were combined as one single group for analysis.  For analysis of the separate groups see Appendix 4.


### 4.2.2 Statistics

Invertebrate data were tested for normality (Kolmogorov-Smirnov test) then log (n +1) transformed.  After transformation some data remained non-normal but were included in the balanced ANOVA across the 48 mesocosms owing to the robustness of ANOVA against departures from its assumptions.  Block effect was also tested for.  Post-hoc Tukey tests allowed comparison between pairs of nutrient treatments and pairs of other treatment combinations when significant interactive effects were detected.  Regression analyses were carried out between the log (n +1) transformed invertebrate biomass data and the log (n +1) transformed mean DO concentration from the summer prior to final harvest (6 samples).  All analysis was carried out using the Minitab 15 statistical package.

## 4.3 Results

Sixteen groups of invertebrates were identified from the final harvest samples.

Of these, Arthropoda was the most abundant Order (51%) followed by Mollusca

(46%) which were almost entirely gastropods (Fig. 4.1.a). The Arthropoda

consisted mainly of Isopoda (41%) and Amphipoda (28%). (Fig. 4.1.a ii).

Although some invertebrate taxa such as Odonata and Notonectidae made up a

small amount of the total abundance of the Arthropoda (3% and 0.7%) they

accounted for 21% and 13% of the total biomass respectively (Fig. 4.1.b ii).

There was a significant 25% reduction in the total biomass of invertebrates at

final harvest with heating (Table 4.1; Fig. 4.2.a) but this disappeared when

gastropods were removed from the data set. Moreover, the impact on individual

groups was variable. Detritivores showed an increase in total biomass (by 61%)

with heating whilst predators decreased (by 46%) (Table 4.1; Fig 4.2.a). The

total biomass of Amphipoda and Oligochaeta increased with heating whilst

Corixidae, Chaoboridae, Ephemeroptera, Notonectidae and Gastropoda all

decreased (Table 4.1; Fig. 4.2.a). The changes in invertebrate biomass can be

split into either changes in total numbers (Fig. 4.2.b) or average individual body

mass (Fig. 4.2.c). Warming the mesocosms by 4° C led to a 38% decrease in

the average individual invertebrate body mass (Fig. 4.2.c). Again, this result

was group-dependent with warming leading to increases in average individual

body mass of Isopoda, Oligochaeta and Hirudinea and decreases in Corixidae,

Ephemeroptera and Notonectidae.
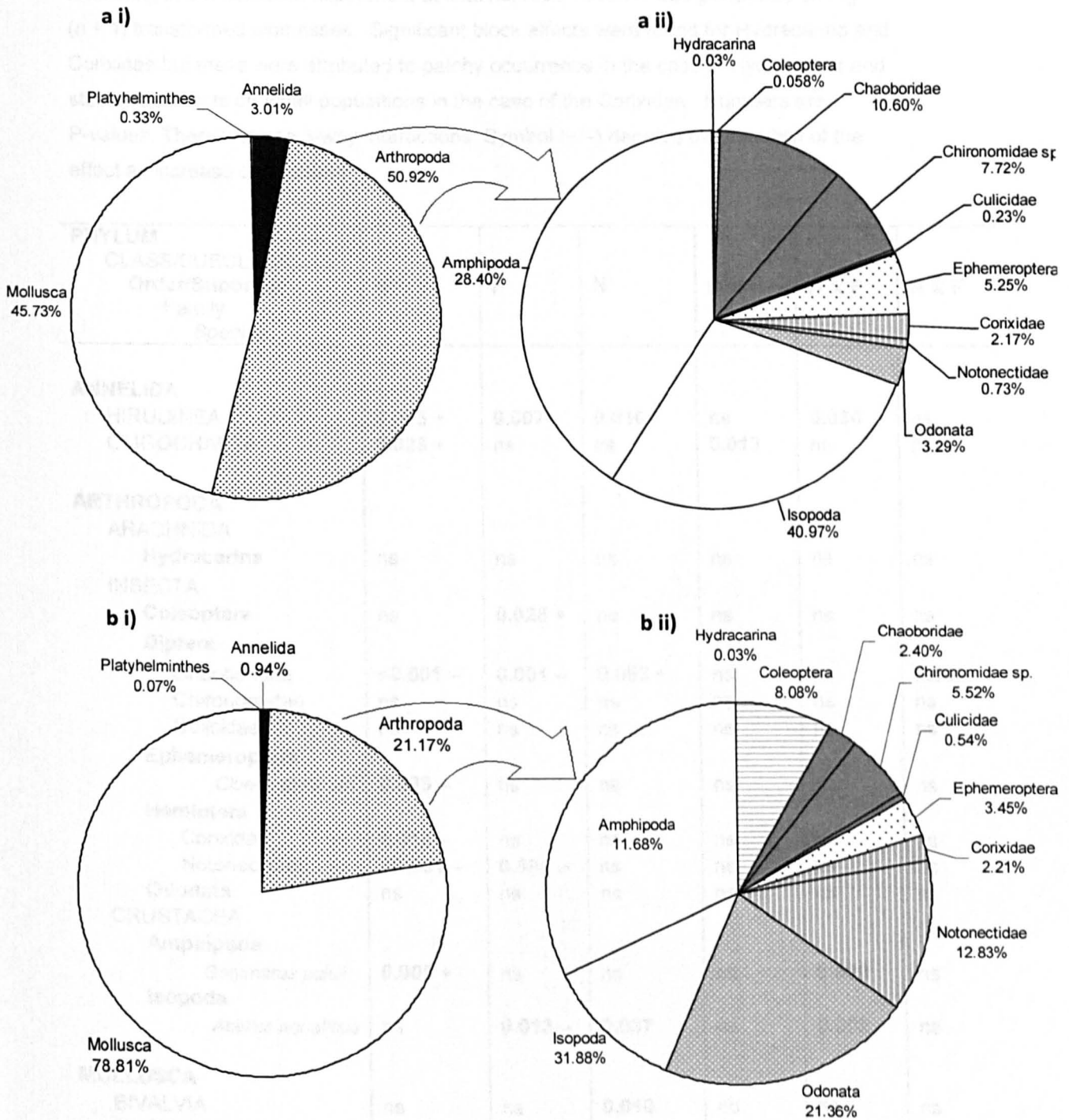
**Figure 4.1**. The proportion of the a i) numbers and b i) biomass made up by the 4 phyla of invertebrates remaining in the mesocosms at final harvest. Of the Mollusca, Gastropoda make up 99.38% of the biomass and 97.55% of the numbers, with Bivalvia making up the remainder. Annelida are split roughly evenly between Hirudinea and Oligochaeta. The Arthropoda are further divided into a ii) numbers and b ii) biomass and are grouped in their major Orders.

**Table 4.1.** Effects of temperature, fish and nutrients on the biomass of invertebrates remaining in a mesocosm experiment at final harvest. ANOVA was performed on log (n + 1) transformed biomasses. Significant block effects were found for Hydracarina and Corixidae but these were attributed to patchy occurrence in the case of Hydracarina and stochastic effects on small populations in the case of the Corixidae. Numbers are P-values. There were no 3-way interactions. Symbol (+/–) denotes the direction of the effect as increase or decrease.

| PHYLUM<br>  CLASS/SUBCLASS<br>    Order/Suborder<br>      Family<br>        *Species* | H | F | N | H x N | H x F | N x F |
|---|---|---|---|---|---|---|
| **ANNELIDA** | | | | | | |
|   HIRUDINEA | 0.055 + | 0.007– | 0.016 | ns | 0.030 | ns |
|   OLIGOCHAETA | 0.026 + | ns | ns | 0.013 | ns | ns |
| | | | | | | |
| **ARTHROPODA** | | | | | | |
|   ARACHNIDA | | | | | | |
|     **Hydracarina** | ns | ns | ns | ns | ns | ns |
|   INSECTA | | | | | | |
|     **Coleoptera** | ns | 0.026 + | ns | ns | ns | ns |
|     **Diptera** | | | | | | |
|       Chaoboridae | <0.001 – | 0.001 – | 0.055 + | ns | ns | 0.002 |
|       Chironomidae | ns | ns | ns | ns | ns | ns |
|       Culicidae | ns | ns | ns | ns | ns | ns |
|     **Ephemeroptera** | | | | | | |
|       *Cloeon dipterum* | 0.003 – | ns | ns | ns | ns | ns |
|     **Hemiptera** | | | | | | |
|       Corixidae | 0.014 – | ns | ns | ns | ns | ns |
|       Notonectidae | <0.001 – | 0.005 – | ns | ns | ns | ns |
|     **Odonata** | ns | ns | ns | ns | ns | ns |
|   CRUSTACEA | | | | | | |
|     **Amphipoda** | | | | | | |
|       *Gammarus pulex* | 0.005 + | ns | ns | ns | 0.029 | ns |
|     **Isopoda** | | | | | | |
|       *Asellus aquaticus* | ns | 0.013 – | 0.037 | ns | 0.002 | ns |
| | | | | | | |
| **MOLLUSCA** | | | | | | |
|   BIVALVIA | ns | ns | 0.010 | ns | ns | ns |
|   GASTROPODA | 0.004 – | ns | ns | ns | ns | ns |
| | | | | | | |
| **PLATYHELMINTHES** | ns | ns | ns | ns | ns | ns |
| **Total** | 0.030 – | ns | 0.013 + | ns | ns | ns |
| **Total (-snails)** | ns | 0.020 – | ns | ns | ns | 0.004 |
| Detritivores | 0.028 + | 0.051 – | ns | ns | 0.004 | ns |
| Predators | 0.036 – | 0.043 – | ns | ns | ns | ns |
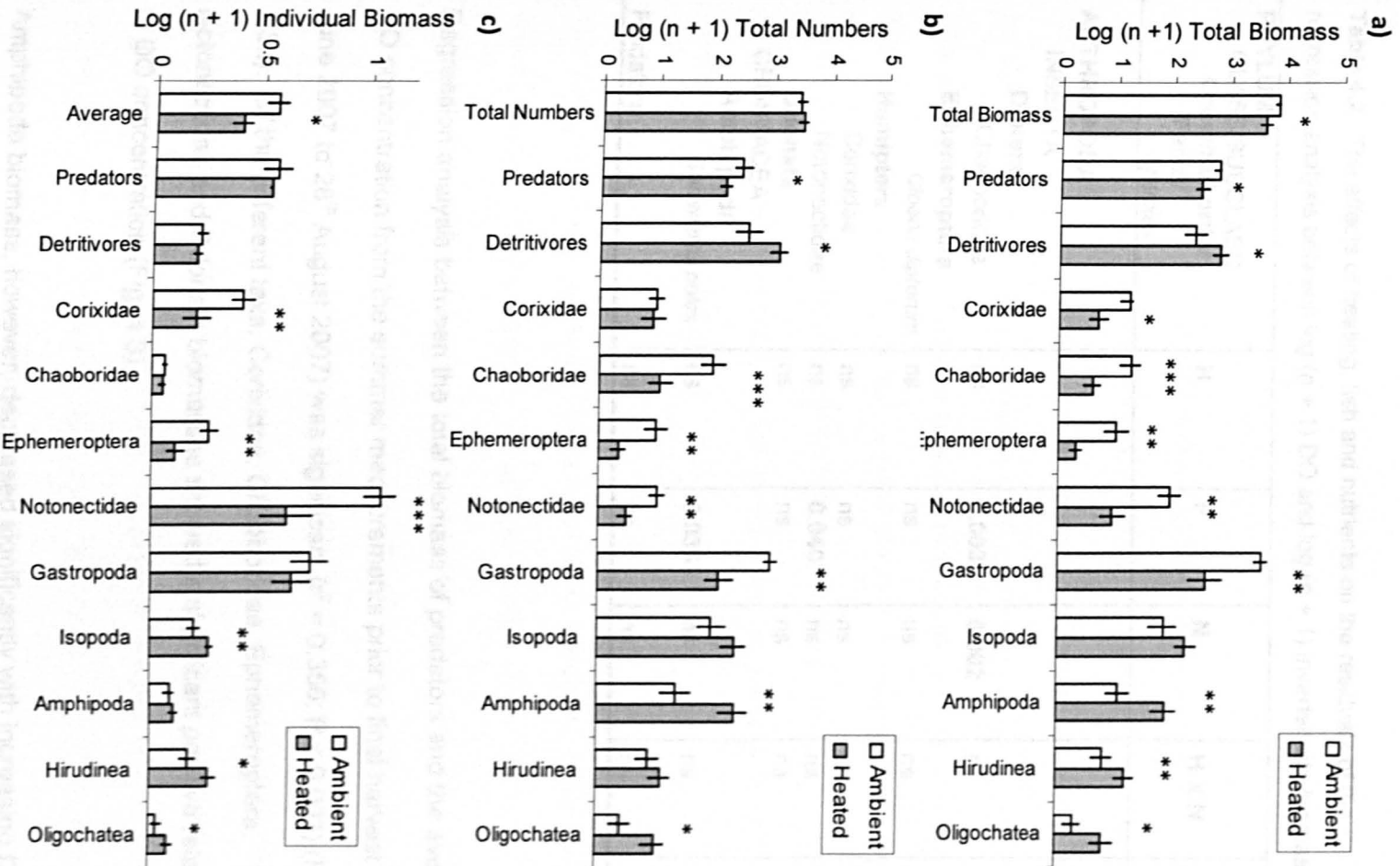
**Figure 4.2.** The impact of heating on the a) total biomass, b) total numbers and c) individual biomasses of the invertebrates remaining in the mesocosms at final harvest. Balanced ANOVA was performed on log (n + 1) transformed data and values displayed are means ± SE. Levels of significance are displayed as; *(0.050>P>0.010); **(0.010>P>0.001) and ***(P<0.001).

**Table 4.2.** The effects of heating, fish and nutrients on the residuals of the regression analysis between log (n + 1) DO and log (n + 1) invertebrate biomass

| PHYLUM CLASS/SUBCLASS Order/Suborder Family Species | H | F | N | H X N | H X F | N X F |
|---|---|---|---|---|---|---|
| **ARTHROPODA** | | | | | | |
| **INSECTA** | | | | | | |
| **Diptera** | | | | | | |
| Chaoboridae | ns | **0.003** | **0.002** | ns | **0.004** | **0.015** |
| **Ephemeroptera** | | | | | | |
| *Cloeon dipterum* | ns | ns | ns | ns | ns | ns |
| **Hemiptera** | | | | | | |
| Corixidae | ns | ns | ns | ns | ns | ns |
| Notonectidae | ns | **0.040** | ns | ns | ns | ns |
| **Odonata** | ns | ns | ns | ns | ns | ns |
| **CRUSTACEA** | | | | | | |
| **Amphipoda** | | | | | | |
| *Gammarus pulex* | ns | **0.036** | ns | ns | ns | **0.013** |
| Predators | ns | ns | ns | ns | ns | ns |

Regression analysis between the total biomass of predators and the average DO concentration from the summer measurements prior to final harvest (5th June 2007 to 28th August 2007) was significant ($r^2$ = 0.356; P < 0.001) (Fig. 4.3.f). Of the different taxa, Corixidae, Chaoboridae, Ephemeroptera, Notonectidae and Odonata biomasses showed a significant positive relationship to DO concentration (Fig. 4.3).

Amphipoda biomass, however, decreased significantly with increasing DO concentrations ($r^2$ = 0.178; P = 0.003). Analysis of the residuals of these regression relationships (Table 4.2) revealed that heating did not account for any of the remaining variation in the invertebrate biomasses after the effects of oxygen were removed. There was also a significant effect of heating and

nutrients on the mean oxygen levels for the summer prior to final harvest (P < 0.001 and P = 0.014 respectively).

Fish had a negative impact on Hirudinea, Chaoboridae, Notonectidae and Isopoda (Table 4.1; Fig. 4.4). They also had an overall negative impact on the total invertebrate biomass minus the snails and on predators. There was a marginally non-significant decrease in the total biomass of detritivores in the fish treatment (P = 0.051). The total biomass of Coleoptera increased by 32% in fish tanks (Table 4.1). There was a heating × fish interaction for Hirudinea, Amphipoda and Isopoda with the biomass of these taxa increasing in heated fish tanks but decreasing in unheated fish tanks when compared with their fishless counterparts (Table 4.1; Fig. 4.5).

Compared with heating and fish, there were fewer effects of nutrient addition on the biomass of individual groups, though the addition of high nutrient concentrations (N2) did cause an overall increase in total invertebrate biomass when compared with mesocosms with no nutrient addition (N0) (Table 4.1; Fig. 4.3). Of only three taxa responding to nutrient addition, Hirudinea and Isopoda showed an increase between N0 and N1 treatments, whilst Bivalvia biomass decreased between N1 and N2 treatments (Fig. 4.3). There was a heating × nutrients interaction for Oligochaeta biomass with nutrient loading causing an increase in biomass at ambient temperatures, but a decrease in heated mesocosms (Fig. 4.5d).
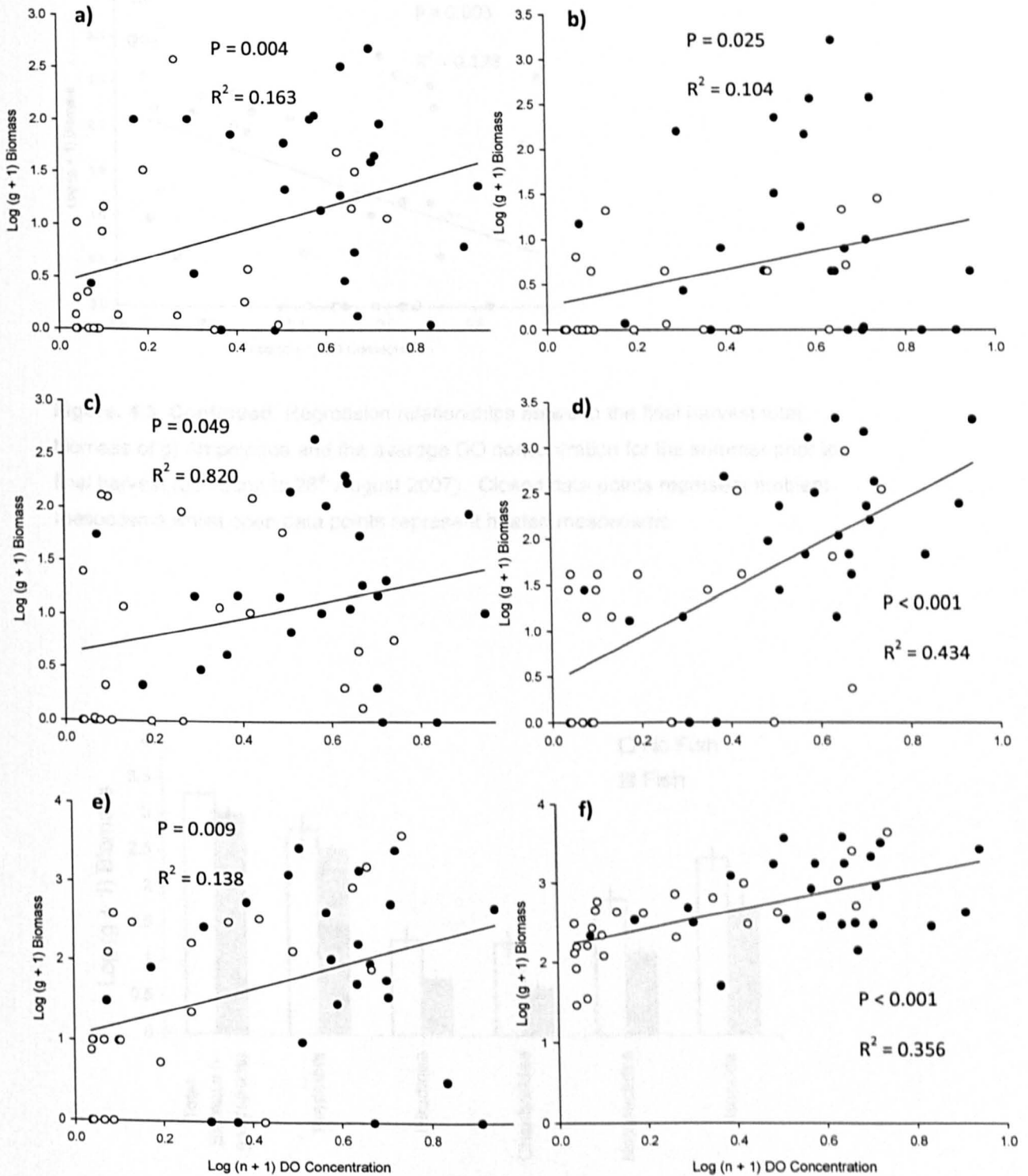
**Figure. 4.3.** Regression relationships between the final harvest total biomass of a) Chaoboridae; b) Ephemeroptera; c) Corixidae; d) Notonectidae; e) Odonata and f) Predators and the average DO concentration for the summer prior to final harvest (05th June to 28th August 2007). Closed data points represent ambient mesocosms whilst open data points represent heated mesocosms.
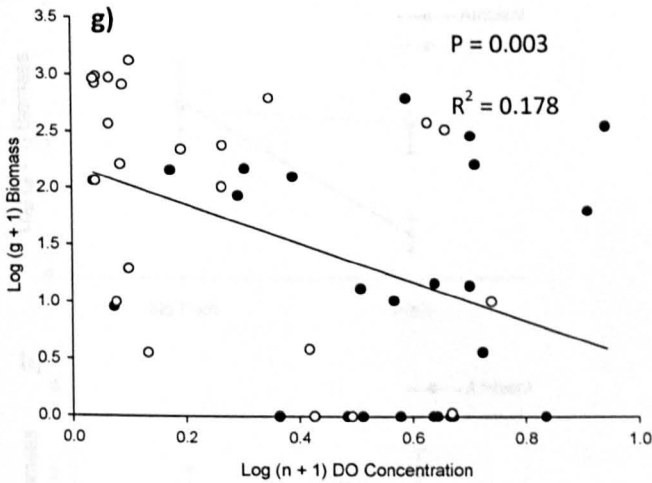
**Figure. 4.3**. **Continued**: Regression relationships between the final harvest total biomass of g) Amphipoda and the average DO concentration for the summer prior to final harvest (05th June to 28th August 2007). Closed data points represent ambient mesocosms whilst open data points represent heated mesocosms.
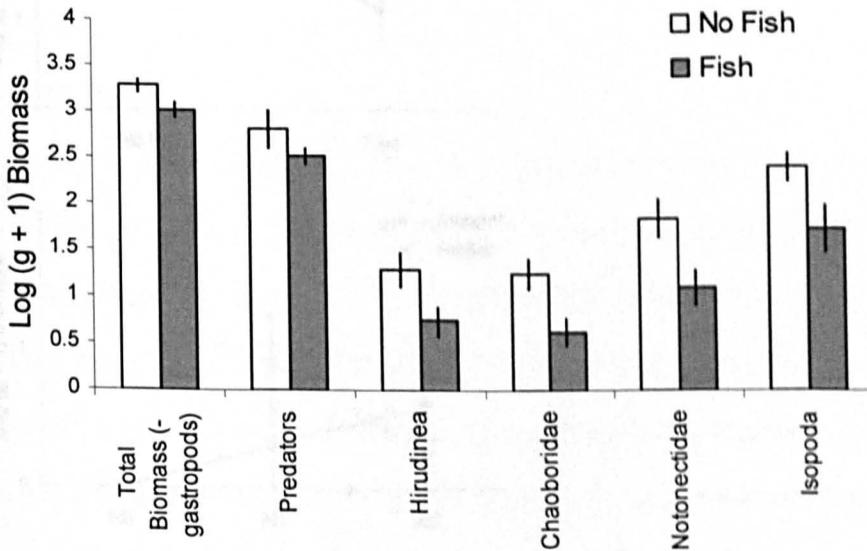


**Figure. 4.4**. The biomass of invertebrates remaining in fish and fishless tanks at final harvest in a mesocosm experiment. Data have been log n +1 transformed and values displayed are means (g) ± SE. ANOVA results are displayed in Table 4.1.
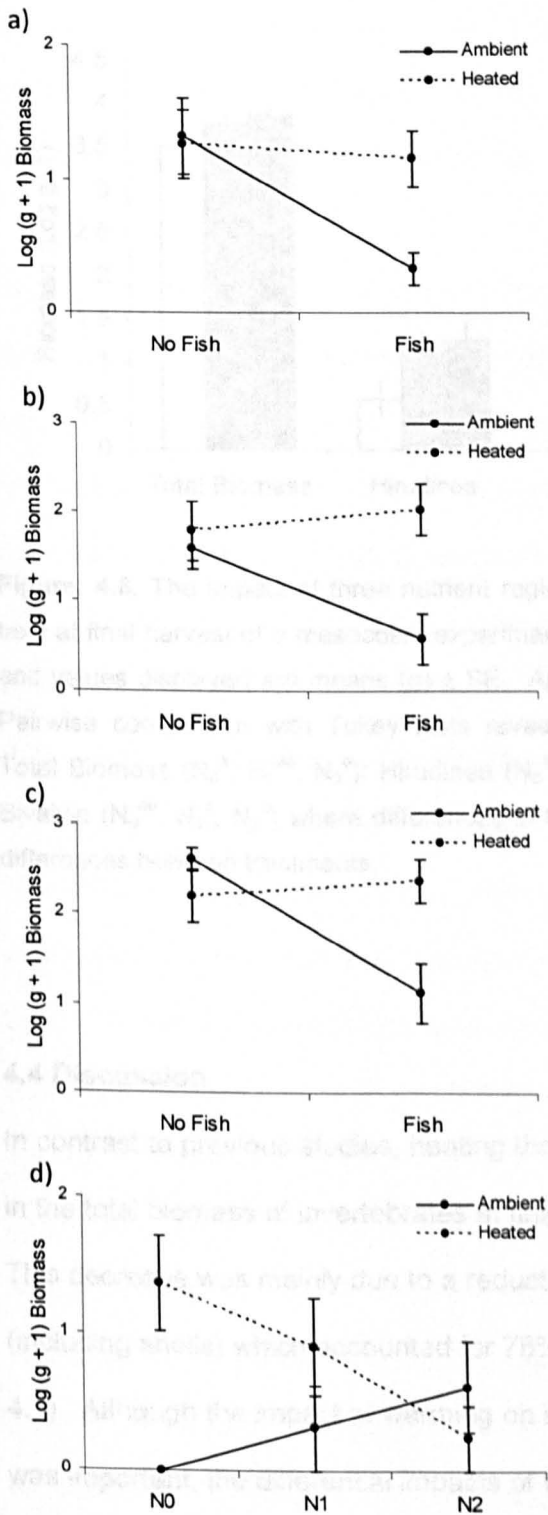
**Figure. 4.5.** Interaction plots displaying the effects of heating and fish on a) Hirudinea, b) Amphipoda and c) Isopoda and the effects of heating and nutrients on d) Oligochaeta. Values are means that have been log (g + 1) transformed.
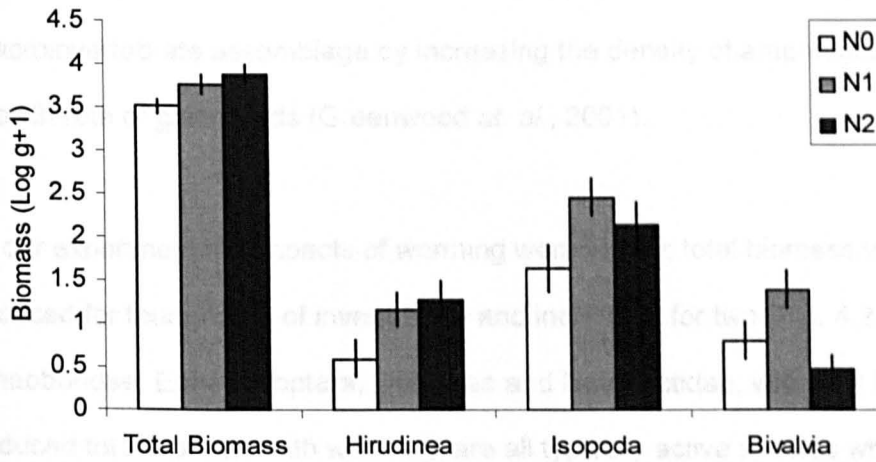
**Figure. 4.6**. The impact of three nutrient regimes on the total biomass of invertebrate taxa at final harvest of a mesocosm experiment. Data have been log n +1 transformed and values displayed are means (g) ± SE. ANOVA results are displayed in Table 4.1. Pairwise comparison with Tukey tests revealed the following significant differences; Total Biomass ($N_0^a$, $N_1^{ab}$, $N_2^b$); Hirudinea ($N_0^a$, $N_1^{ab}$, $N_2^b$); Isopoda ($N_0^a$, $N_1^b$, $N_2^{ab}$) and Bivalvia ($N_0^{ab}$, $N_1^a$, $N_2^b$) where differences in the superscript letter represent significant differences between treatments.

## 4.4 Discussion

In contrast to previous studies, heating the mesocosms by 4°C led to a reduction in the total biomass of invertebrates at final harvest of around 25% (Table 4.1). This decrease was mainly due to a reduction in the total biomass of Gastropods (including shells) which accounted for 78% of the final harvest biomass (Fig. 4.1). Although the impact of warming on invertebrate communities as a whole was important, the differential impacts of warming on invertebrate taxa may alter trophic interactions and therefore ecosystem functioning. For example, freshwater bivalves such as zebra mussels (*Dreissena polymorpha*) play a nutrient cycling role in the freshwater ecosystem. They are efficient filter feeders and enhance the benthic-pelagic nutrient exchange by the intake of organic matter as food and the subsequent transfer of this matter to the sediment as

faeces and pseudofaeces. As a result of this they can directly affect the macroinvertebrate assemblage by increasing the density of amphipods and growth rate of gastropods (Greenwood *et. al.*, 2001).

In our experiment the impacts of warming were varied; total biomass was reduced for four groups of invertebrate and increased for two (Fig. 4.2). Chaoboridae, Ephemeroptera, Corixidae and Notonectidae, which all had reduced total biomass with warming, are all typically active species which we expected would show greater susceptibility to temperature-related decreases in DO. These heated mesocosms have already been shown to hold less DO than ambient mesocosms (Feuchtmayr *et. al.*, 2009; Moran *et. al.*, 2010). Regression analysis showed the biomass of these taxa to be positively related to the summer average DO concentration in the mesocosms (Fig. 4.3) with heating accounting for none of the remaining residual variation (Table 4.2). In contrast to previous experiments using the same mesocosms (McKee *et. al.*, 2003; Feuchtmayr *et. al.*, 2007), Gastropoda decreased in abundance and total biomass with warming. Gastropoda are less active than the other taxa that showed a decrease with warming in this experiment, and, as expected for a more sedentary group, their decrease was not related to decreasing DO concentrations. All families of gastropods found in the mesocosms were pulmonates and therefore also had access to atmospheric oxygen. The mechanisms behind this decrease cannot easily be explained. We found no relationship between the biomass of gastropods and available plant biomass suggesting that the available surface area for periphyton food wasn't a limiting factor.

Oligochaeta and Amphipoda showed an increased biomass with heating

(Fig. 4.2). For Oligochaeta, whose occurrence was very patchily distributed

across the mesocosms, this increase was not general but occurred only in the

absence of nutrient addition (Fig 4.5.d). The increase in Amphipoda biomass in

heated mesocosms may be related to a decrease in predation due to a

temperature-related reduction in stickleback populations (Moran *et. al.*, in press;

Chapter 2). The seasonal data strongly suggests amphipods to be a preferred

food of sticklebacks (Chapter 3). Hirudinea, Amphipoda and Isopoda showed a

decrease in total biomass with fish presence but only in the absence of heating

(Table 4.1), demonstrating that warming can act indirectly on invertebrate

populations, probably by reducing the numbers of an important

predator/competitor (Fig. 4.5; Chapter 2).


Invertebrates are commonly split into functional feeding groups dependent on

their main mode of feeding (Cummins & Klug, 1979). Analysis of invertebrate

biomass according to their feeding group (Appendix 3) showed that total

biomass of detritivores increased with warming whilst that of predators

decreased. Predators are generally more active feeders and their biomass was

shown to be directly correlated with the mean DO concentration measured

during the summer prior to final harvest (Fig. 4.3.f). Detritivore biomass may

have therefore increased due to a decrease in fish predation pressure with

heating (Chapter 2), and in the absence of a high sensitivity to low DO.


The change in total biomass of invertebrates was due to a change in

abundance, mean individual biomass, or a mixture of the two. The decrease in

total biomass of Corixidae, for example, appears to be entirely due to decreases

in individual body mass (Fig. 4.2). A number of theories hypothesize that

increased temperature may cause communities to consist of smaller-bodied

species or smaller individuals within a species (Bergman, 1847; Atkinson, 1994b). However, we cannot determine the extent to which the shift in mean body size was due to smaller bodied species, or reduced mean size within species.

Predation by fish is important in freshwater ecosystems as they can reduce invertebrate numbers and alter trophic interactions (Diehl, 1992; Marklund *et. al.*, 2002; Persson & Svensson, 2006) Mesocosms containing sticklebacks had less total invertebrate biomass than fishless mesocosms (Table 4.1; Fig. 4.4) (snails were excluded from this analysis as fish will have been 'gape limited' and therefore not included gastropods in their diet). This was presumably due either to predation (e.g. of Isopoda; Wootton, 1976) or to direct competition with another predator (e.g. the predominantly predatory Hirudinea, Chaoboridae and Notonectidae). The total biomass of Coleoptera increased in mesocosms containing fish, which contrasts with a previous study on ponds, which showed a decline in Coleoptera in the presence of fish (Fairchild, Faulds & Matta, 2000). The sheer diversity of Coleopteran sizes, diets and niches, together with their patchy occurrence among mesocosms impedes further inference.

Nutrient addition had less of an impact on the macroinverterates than heating or fish addition. It caused an increase in total invertebrate biomass between N0 and N2 mesocosms, and specifically in Hirudinea and Isopoda, possibly due to an increase in primary productivity (Fig. 4.6). In Bivalvia the total biomass increased in N1 then decreased in N2 mesocosms. Although this result was only significant between N1 and N2 mesocosms it is consistent with high-level nutrient loadings giving intensified eutrophication problems such as decreased oxygen levels. The invertebrate groups that increased in biomass with nutrient loading are benthic so it is possible that they benefited from an increase in

organic matter production due to an increase in plant growth and hence decay, and possibly from improved food quality associated with likely improved C:N ratios (Cross *et. al.*, 2006).

In conclusion, it would appear that warming was the most important factor affecting macroinvertebrate populations in our mesocosms. More specifically, increasing temperature had a greater impact on the total biomass of active species such as predators, probably through its effects on DO concentrations. It also altered non-predator invertebrate communities probably through alteration of predator/competitor populations. Changes in the macroinvertebrate community composition are likely to lead to changes in trophic interactions and alterations to ecosystem functioning.

# Chapter 5

The impact of temperature
on the functional responses of the
three-spined stickleback feeding on
various invertebrate prey species

## 5.1 Introduction

Teleosts are important predators of freshwater and marine systems. They can demonstrate a high degree of selectivity in their diet which can alter the structure of prey communities and in turn have a cascading effect on the whole ecosystem (Zagarese, 1990; Cowan & Houde, 1992; Zhengwen & Herzig, 1996; Mookerji et. al., 1997; Chappaz et. al., 1998; Jones & Sayer, 2003). Three-spined sticklebacks (Gasterosteus aculeatus) are no exception to this: these small voracious predators, which inhabit a wide range of environments and hunt primarily by sight (Wootton, 1976), can reduce and then suppress invertebrate densities both in microcosms (McKee et. al., 2003) and in freshwater lakes (Hynes, 1950 & Campbell, 1991). However, despite the ability of the three-spined stickleback to alter freshwater invertebrate communities, little work has attempted to understand the processes that lead to these changes, including factors affecting feeding decisions.

A useful approach to exploring the underlying behavioural mechanisms of predator- prey dynamics is to examine the functional response demonstrated by a predator. The functional response can be defined as the change in response of food consumption by a predator to varying prey densities and is based on the early cornerstone work of Solomon (1949) and Holling (1966). All functional responses have zero consumption at low prey densities and saturation at high density. These responses can be classified into three general forms depending on the rate at which they approach saturation (Rindorf & Gislason, 2005). A Type I functional response (Fig. 5.1) involves a linear approach to saturation level: examples of these are somewhat rare in nature and are usually displayed in some herbivore-plant interactions or in filter feeders such as bivalves (Jeschke et. al., 2004). The more common Type II response demonstrates a decelerating approach, whilst Type III approaches saturation in a sigmoidal

manner (Fig. 5.1). The type and intensity of a functional response are important when considering the ability of a predator to regulate prey populations and hence community structure. For example, in a Type III response, as prey density decreases, the rate of change in predation (hence prey mortality) slows down, which can help stabilize the prey population. Eggleston (1990) found that large juvenile oysters had a partial prey refuge below a density of 16.7 oyster $m^{-2}$ due to female blue crabs (*Callinectes sapidus*) exhibiting a Type III response, thereby allowing juvenile oysters to persist. In contrast, a Type II response results in proportionally higher mortality at low prey densities and therefore has the ability to push small populations to extinction (Fig. 5.1) (Lipcius & Hines, 1986; Eggleston, 1990).

Several theories account for the so-called 'stabilizing' Type III response. Akre & Johnson (1979) proposed that the sigmoidal nature of the functional response of the *Anomalagrion hastatum* damselfly naiads was caused by this predator using two alternative search modes which altered its encounter frequency with the two prey species. However, sigmoidal functional responses can occur toward a single prey species where switching to an alternative prey is not possible. Type III responses to a single prey species can be attributed to aggregative behaviour (Hassell & May, 1974), prey refuges in heterogeneous habitat (Hildrew & Townsend, 1977), or declining search rate at low encounter frequencies (Hassell et. al., 1976). Previously, Holling (1966) suggested that associative learning is a necessary component of potentially regulatory sigmoidal functional responses to prey density. However, as with many theories there is the criticism that functional responses demonstrated in the laboratory do not bear much relation to those existing in the field, as many of the costs associated with foraging e.g. risk of predation, are not present in the laboratory(Abrams,1982). Experiments

are required that relate laboratory-derived functional responses to 'wild'

populations.

An important feature of predator-prey interactions in natural environments is

their responses to varying abiotic factors such as temperature. Abiotic factors

have been described as the least appreciated mechanisms in community

ecology (Dunson & Travis, 1991) when in fact fluctuating abiotic factors have
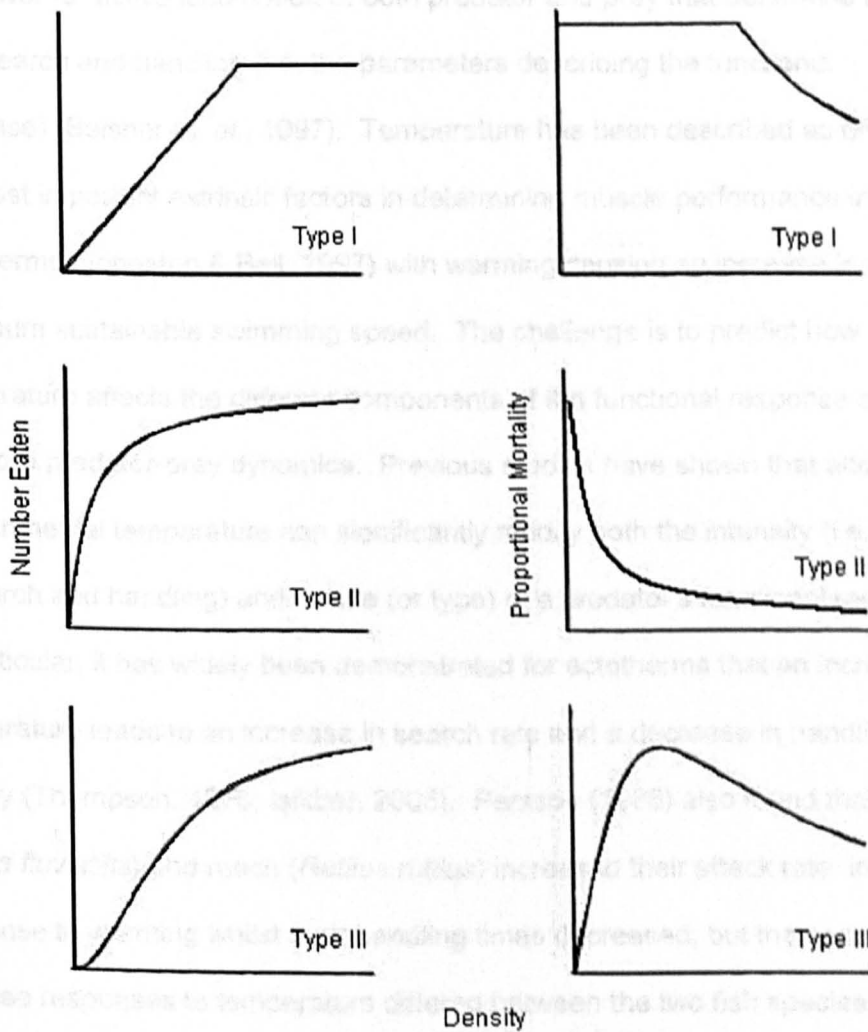


**Figure 5.1.** The three types of functional response with their corresponding proportional mortality curve (Juliano, 2001).

the potential to structure communities by altering biotic interactions. Temperature variation has a particularly important influence on fish as their body temperature lies within a few fractions of a degree of their environment (Wood & McDonald, 1997). With climate change predictions indicating an increase in mean temperature of 3 – 5°C over the next century (IPCC, 2007) there is a lack of material examining how changes in temperature will alter predator-prey interactions (Anderson et. al., 2001).

The temperature of an ectotherm's environment alters metabolic rates, hence the power for active locomotion of both predator and prey that determine rates of food search and handling (i.e. the parameters describing the functional response) (Beisner et. al., 1997). Temperature has been described as one of the most important extrinsic factors in determining muscle performance in ectotherms (Johnston & Ball, 1997) with warming causing an increase in maximum sustainable swimming speed. The challenge is to predict how temperature affects the different components of the functional response and therefore predator-prey dynamics. Previous studies have shown that altering environmental temperature can significantly modify both the intensity (i.e. rates of search and handling) and nature (or type) of a predator's functional response. In particular, it has widely been demonstrated for ectotherms that an increase in temperature leads to an increase in search rate and a decrease in handling time of prey (Thompson, 1978; Işikber, 2005). Persson (1986) also found that perch (Perca fluviatilis) and roach (Rutilus rutilus) increased their attack rate in response to warming whilst their handling times decreased, but the sensitivities of these responses to temperature differed between the two fish species, leading to a shift in species with the highest competitive ability. By altering the functional response of a predator, increasing temperature has been shown to have the ability to de-stabilise a prey population (Beisner et. al., 1997).

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There has been less documentation of instances where temperature change causes a switch in type of functional response but the implications of such a change are quite important. Switching to a Type II functional response with increasing temperature has been shown for a number of ectothermic invertebrate species and has important implications for prey populations (Eggleston, 1990a; Mohaghegh et. al., 2001; Taylor & Collie, 2003). The sand shrimp, Crangon septemspinosa, has been shown to alter its response to its juvenile flounder prey, Pseudopleuronectes americanus, from Type III to Type II with a 6 °C rise in temperature (Taylor & Collie, 2003). These results highlight the increased ability of the shrimps to drive the flounder to local extinction during warmer conditions due to this alteration of functional response. Similarly, Eggleston (1990a) demonstrated that the functional response of the blue crab, Callinectes sapidus, varied significantly in type and intensity as a function of temperature. Fewer studies, however, have been carried out on vertebrate ectotherms.

The components of a predator's functional response that are more thermally sensitive may depend on the nature of the predatory strategy. Manatunge & Asaeda (1999) describe three main predatory strategies in fish predators. Ambush foragers search for prey whilst stationary, whereas cruise predators move more or less continuously and scan for prey while swimming. An intermediate search strategy called 'saltatory search', described for several fish species, is defined as a series of movements or 'runs' interspaced with pauses during which the predator actively searches for its prey. For the ambush predator who does not search but employs a sit-and-wait tactic, handling time may be the more thermally sensitive element of the predator's functional response. By contrast, actively searching predators may have a higher thermal

sensitivity for search rate as this is likely to be strongly determined by thermal sensitivity of the metabolic rates of the predator.

A useful tool for examining the thermal sensitivity of rates (e.g. search rate) is to calculate the $Q_{10}$. This is a useful descriptor of the effect of temperature on the rate of a reaction and is defined as the factor by which the rate of the function being studied increases with a 10°C rise in temperature:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2-T_1}\right)}$$

where $R_1$ = initial rate, $T_1$ = initial temperature, $R_2$ = second rate and $T_2$ = second temperature.

If the rate of a reaction is completely temperature independent then the $Q_{10}$ will be equal to 1. If the rate of reaction increases with temperature then $Q_{10}$ will be greater than 1. Processes that rely on underlying enzymatic processes usually have a $Q_{10}$ in the range 2-3 (i.e. doubling or tripling of rate for every 10°C rise in temperature). However, $Q_{10}$ values can be specific to the temperature interval examined so must be quoted for a specific temperature range. For example, the Colorado potato beetle has a $Q_{10}$ value for oxygen consumption of 2.17 for the temperature interval 7 - 30°C. However, when calculated for more specific temperature intervals the $Q_{10}$ is shown to remain constant at 2.5 up until 20°C above which the value falls. So although the use of $Q_{10}$ values may be illuminating they must be treated with caution (Schmidt-Nielsen, 1970).

Investigations into the impact temperature change will have on species and community interactions are somewhat limited (Dunson & Travis, 1991, Anderson et. al., 2001). The major goal of this piece of work is to investigate the nature of three-spined stickleback predation on members of the macroinvertebrate

community by examining functional responses when preying on a variety of prey species over a range of summer temperatures. Specifically, the work will focus on:

- The type of functional response demonstrated by sticklebacks

- The impact of increasing temperature on the type and parameters of the functional response, i.e. search rate and handling time

- How functional response varies with prey type

## 5.2 Methods

### 5.2.1 Experimental set up

Functional response experiments were carried out in the University of Liverpool's aquarium. Fish were collected from ponds at Ness gardens (53°16'N, 3°02'W) and Thurstaston Visitor Centre pond (53°20'N, 3°08'W) and held in quarantine for a week in the University's aquarium at approximately 15 °C. They were then transferred to stock tanks containing a filter, heater, gravel substrate and plastic plants. The aquarium was lit by overhead fluorescent bulbs on a 16L:8D regime. Fish were fed once daily on frozen blood worm (Chironomidae). They were also fed on live invertebrates once a week (also collected from Ness) to ensure the fish were not naïve to the prey used in experimental trials (although three-spined sticklebacks have been show to have a relatively long memory window of >25 days in freshwater species, Mackney & Hughes,1995). The water was gradually heated over the course of a week to either 18°C, 22°C or 24°C. Four stock tanks for each temperature were used to ensure rotation of the fish used in trials. Summer temperatures were chosen as this is when the impacts of climate change are expected to be more prominent. The lower temperatures, 18°C and 22°C, represent the summer average

temperatures in the ambient (approx 18.8°C) and heated mesocosms (approx 22.6°C) at Ness that fish were exposed to. The relevance of using temperatures similar to the Ness mesocosms will be discussed in the next chapter where the theory of optimal diet will be tested using data on summer shifts in invertebrate community composition in the mesocosms and prey profitabilities calculated in this chapter.

The experimental tanks consisted of (295mm (w) × 595mm (l) × 300mm (h)) glass aquaria filled with a total of 40.40L of treated mains water. All tanks also contained an in-tank heater, a sponge filter and a sand/gravel substrate which was uniform across the tanks to prevent differences in foraging preferences (Webster & Hart, 2004). Three sides of the tank were covered with white plastic to ensure minimal disturbance to the fish during the feeding trials. The remaining side was left free for observations. All tanks had thermometers for regular temperature monitoring.

### 5.2.2 Experimental procedure

For the procedure of the feeding trial, individuals were removed from their stock tank and weighed using an electronic balance (to the nearest 0.01g) and measured (standard length to the nearest 0.01mm) using digital Vernier callipers. They were then transferred to an experimental tank of equivalent temperature to their stock tank. Only fish measuring 40 – 50 mm (standard length) were used. Once in the experimental tank, the fish were fed to satiation on an excess mixture of bloodworm and the invertebrate to be used in the trial (starved sticklebacks require the equivalent of at least 70 *Daphnia* or 40 *Cloeon* before they are satiated; Visser, 1982), then starved for 24hours to ensure an approximately uniform level of hunger. The invertebrates to be used in the trial were measured in advance (5 ± 1 mm as these should not be too large so as to

cause gape size of the fish to be a limiting factor and isopods of this size have been shown to be the most favoured by sticklebacks of 45mm length; Hart & Ison, 1991; Gill & Hart, 1998) using a low-power microscope and stored in containers at 18 and 22 °C. Invertebrates used were Chironomidae sp., the amphipod *Gammarus pulex*, the mayfly *Cloeon dipterum* and the isopod *Asellus aquaticus,* and were selected because they were abundant in the Ness mesocosms, and were known to be part of the three-spined stickleback diet (Hynes, 1950; Wootton, 1976). For the trial, the fish were restricted to one end of the tank via a mesh screen. Prey were then introduced into the larger portion of the tank and allowed to disperse and settle for 1 minute. After this, the screen was removed and the fish allowed to feed for 5 minutes. During this time the observer sat motionless just to one side of the tank and recorded the number of prey eaten. Prey were not replaced. The experimental tanks were lit with a bulb (100 W) at a distance of 2 metres to ensure light intensity across all experimental tanks was approximately equal. At the end of the trial the fish was returned to its stock tank and all remaining prey were removed. Appropriate densities of prey used for each invertebrate species were determined during practice trials.

It was not practicable to replace prey during the experiment, therefore the data were best described by Rogers' random predator equation (1972), which was devised to account for diminishing prey abundance:

$$N_e = N \{1 - exp [-a (T - T_h N_e)]\}$$

Where $N_e$ is the number of prey eaten, $N$ is the number of prey offered, $T$ is the total time available for the predator, $a$ is the attack rate and $T_h$ is the handling time.

Estimates of $a$ and $T_h$ were made using non-linear least squares regression in SPSS.

The experimental procedure for obtaining a visual estimate of search rate was the same as for the functional response trials. Fish were allowed to feed for 5 minutes on an initial density of 10 *Cloeon dipterum* whilst being watched by the observer. A search was defined as the stickleback halting and orientating (tilting approximately 25°) towards the substrate (Hart & Ison, 1991)

### 5.2.3 Tests of functional response type

Proportional mortality data was analysed using a General Linear Model on Arcsine transformed proportions with temperature and prey density as fixed factors. Where the differences were significant, post-hoc Tukey tests were used to distinguish between treatments. Type II responses show a significantly higher proportional mortality at low densities whilst Type III responses show significantly lower proportional mortality at low densities. In cases, where distinguishing between responses with this method was not obvious, analysis of the general functional response model (Real, 1977; Real, 1979; Lipcius & Hines, 1986; Taylor & Collie, 2003) was performed;

$$N_a = \frac{K \times N_t^{\beta}}{X + N_t^{\beta}}$$

Where $N_a$ = number eaten, $K$ = the maximum feeding rate (estimated from data) $N_t$ = initial prey density, $X$ = the density of prey when $N_a$ = 0.5$K$ and $\beta$ = the parameter associated with the form of the functional response such that when $\beta$ = 0 the functional response is Type I, when $\beta$ = 1 the functional response is Type II and when $\beta$ > 1 the functional response is Type III.

The slope parameter, $\beta$, is estimated by regression analysis on the log-transformed data (log ($N_a / K - N_a$) against $X$). A t-test on the departure of $\beta$ from the set values of 0, 1 or 2 can therefore indicate the type of functional response (Chatterjee & Hadi, 2006). Statistics were carried out using Minitab 15 and SPSS 16.0.

## 5.3 Results

The number of prey items consumed during the feeding trials increased with density for all prey types and at all temperatures. Rogers' random predator equation showed a good fit to the data ($R^2 > 0.80$ in all cases except *Asellus* at 18°C; $R^2 = -0.64$ and *Asellus* at 22°C; $R^2 = 0.50$). In cases where $R^2$ was low (Fig. 5.1a), Rogers' equation still showed a good visual fit to the data. Visual inspection of the graphs (Fig. 5.2a – 5.5a) indicate that sticklebacks demonstrated a Type II functional response in all trials.

All prey types showed significantly higher proportional mortality at low compared to high densities at all temperatures (Fig 5.2b – 5.5b; $P < 0.001$ in all cases; for post-hoc results see figure legends) which further corroborate the fit of the Type II, rather than the Type III, model to the data (Taylor & Collie, 2003) (c.f. Fig. 5.1). Temperature had a significant impact on the proportional mortality of *Gammarus*, *Asellus* and *Cloeon* ($P < 0.050$ in all cases). There was an interaction effect between temperature and prey density for *Gammarus* *proportional mortality* due to there being significantly higher proportional mortality at the lowest density (5 *Gammarus*) at 22 (84% mortality) and 24°C (96%) when compared with 18°C (44%) (temperature × density interaction; $P = 0.001$). This can be suggestive of a switch in functional response from Type III to Type II with increasing temperature. Further analysis of the data using the general functional response model (Real, 1977) showed that the slope parameter, $\beta$, did not differ significantly from 1 at 18, 22 and 24°C, which indicates a Type II response (t-test; $P > 0.050$ in all cases). *Asellus* showed an overall increase in proportional mortality with increasing temperature ($P = 0.042$) but post-hoc tests showed this to be marginally non-significant between 18 – 24°C ($P = 0.095$) and 22 – 24°C ($P = 0.057$). The increase in proportional mortality with temperature for *Cloeon* was only significant between 18 and 24°C

(P = 0.027) where the overall proportional mortality increase was from 38% to 56%.

The search rate and handling time varied with prey type and temperature though not in a uniform manner (Table 5.1). A GLM carried out on the search rate and handling time of all four prey types showed no significant response to increasing temperature (P > 0.05). However, even small changes in search and handling time can produce changes in the optimal diet of a predator (to be discussed in detail in Chapter 6) so trends in search and handling time with temperature will still be described. The search rate for chironomids increased between 18 and 22°C but decreased between 22 and 24°C. The search rate for Cloeon dipterum decreased slightly at 22°C but otherwise varied very little. The search rate for Gammarus pulex followed a similar pattern to Chironomidae sp. with an increase between 18 and 22°C but a decrease between 22 and 24°C. Finally, the search rate for Asellus aquaticus showed a marked decrease at 22°C but a similar search rate between 18 and 24°C. Handling time varied between species with Asellus aquaticus having the longest handling time of the prey items. Handling time showed little variation with changing temperature.

Visual estimates of search rate (number of searches over a 5 minute period) for mayfly, Cloeon dipterum, showed a significant increase in the number of searches between 18 and 22°C (Fig. 5.6 (Students t-test; P = 0.013). The $Q_{10}$ for the number of observed searches per unit time was 3.32.

**Table 5.1.** Estimates of instantaneous search rate (*a*; per tank per minute) and handling time (*T_h*; minutes) produced using the random predator equation for three-spined sticklebacks preying on various invertebrates at 18, 22 and 24°C.

| Temp | Prey Type | *a* | SE | *T_h* | SE |
|------|-----------|-----|-----|-----|-----|
| **18°C** | Chironomidae sp. | 3.709 | 0.921 | 0.020 | 0.002 |
| | *Cloeon dipterum* | 0.611 | 0.141 | 0.034 | 0.007 |
| | *Gammarus pulex* | 1.152 | 0.315 | 0.038 | 0.009 |
| | *Asellus aquaticus* | 3.003 | 1.466 | 0.169 | 0.006 |
| **22°C** | Chironomidae sp. | 4.126 | 1.577 | 0.020 | 0.002 |
| | *Cloeon dipterum* | 0.55 | 0.116 | 0.018 | 0.007 |
| | *Gammarus pulex* | 4.676 | 1.816 | 0.065 | 0.003 |
| | *Asellus aquaticus* | 0.934 | 0.302 | 0.123 | 0.012 |
| **24°C** | Chironomidae Sp. | 2.475 | 0.845 | 0.022 | 0.003 |
| | *Cloeon dipterum* | 0.648 | 0.157 | 0.020 | 0.007 |
| | *Gammarus pulex* | 2.347 | 0.325 | 0.048 | 0.002 |
| | *Asellus aquaticus* | 3.826 | 0.452 | 0.128 | 0.001 |
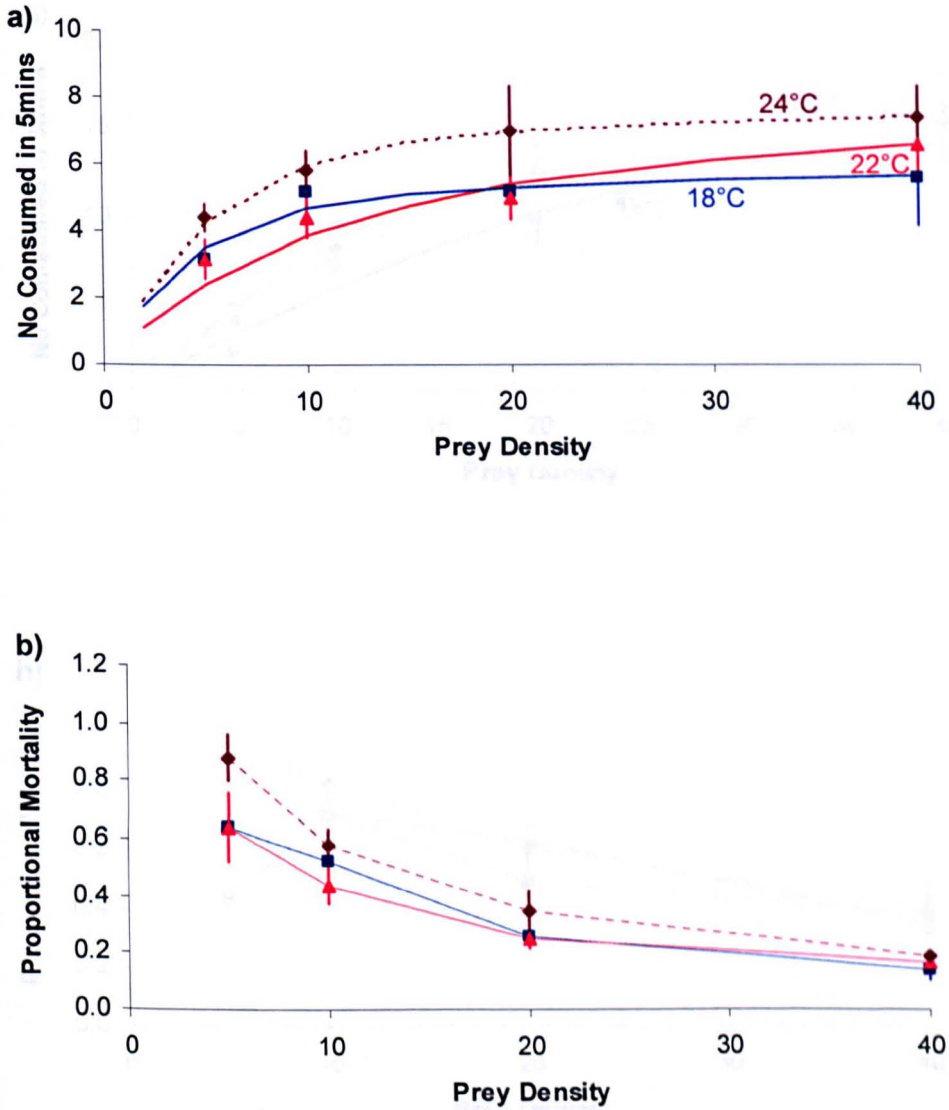
**Figure. 5.2.** The functional response (a) and mortality rate (b) of the three-spined stickleback preying on *Asellus aquaticus* (n=5) at 18, 22 and 24°C. Values are means ± SE. (b) GLM performed on Arcsine transformed proportions with post-hoc Tukey tests showed significantly higher proportional mortalities ($P < 0.05$) at low densities (Density; $5^{10,20,40}$, $10^{5,20,40}$, $20^{5,10}$ & $40^{5,10}$) and increased temperatures (18°C 22°C & 24°C). Superscript numbers represents pair-wise comparison of the densities that differ significantly. There was no interaction between temperature and density.

**a)**



**b)**
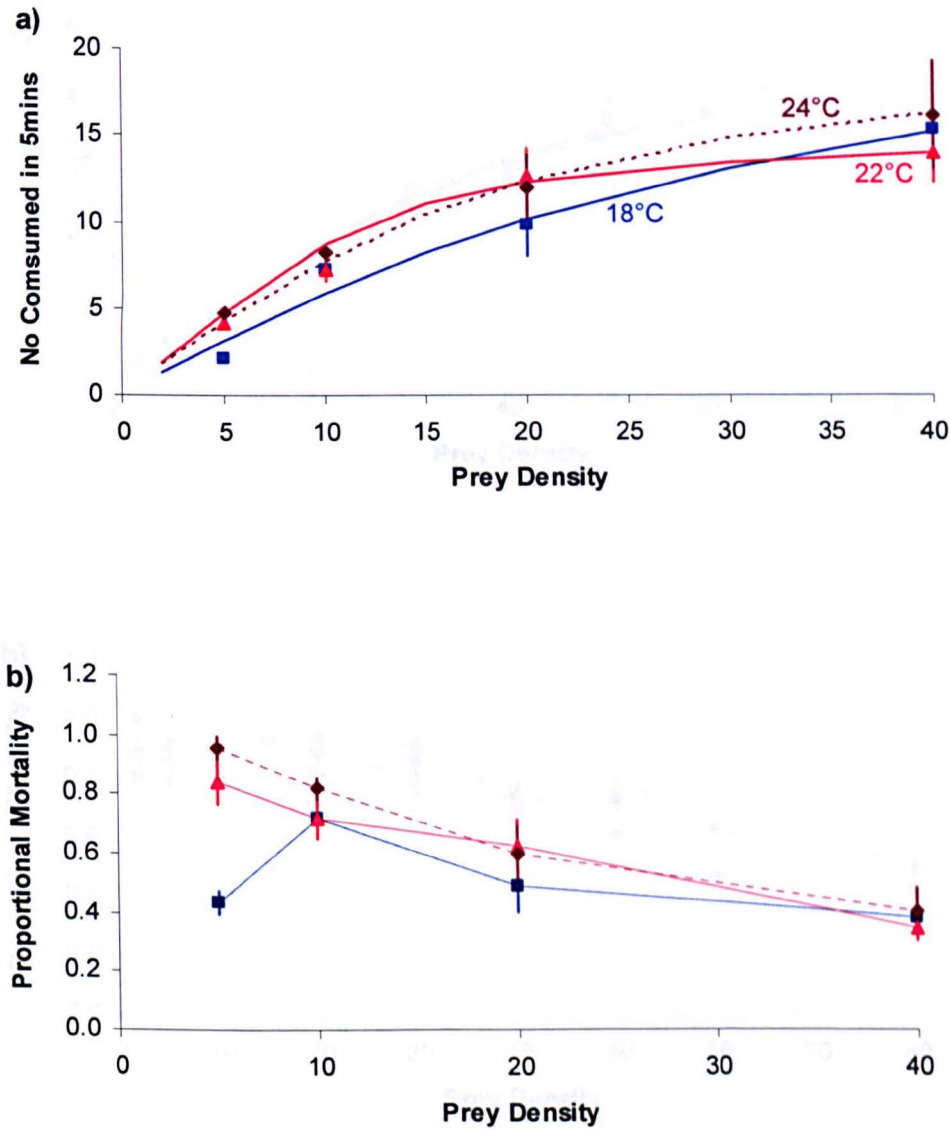


**Figure. 5.3**. The functional response (a) and prey mortality rate (b) for the three-spined stickleback preying on *Gammarus pulex* (n=5) at 18, 22 and 24°C. Values are means ± SE. (b) GLM performed on Arcsine transformed proportions with post-hoc Tukey tests showed significantly higher proportional mortalities ($P < 0.05$) at low densities (Density; 5[20,40], 10[20,40], 20[5,10,40] & 40[5,10,20]) and increased temperatures (18°C[22,24], 22°C[18] & 24°C[18]). Superscript numbers represents pair-wise comparison of the densities that differ significantly. There was no interaction between temperature and density.
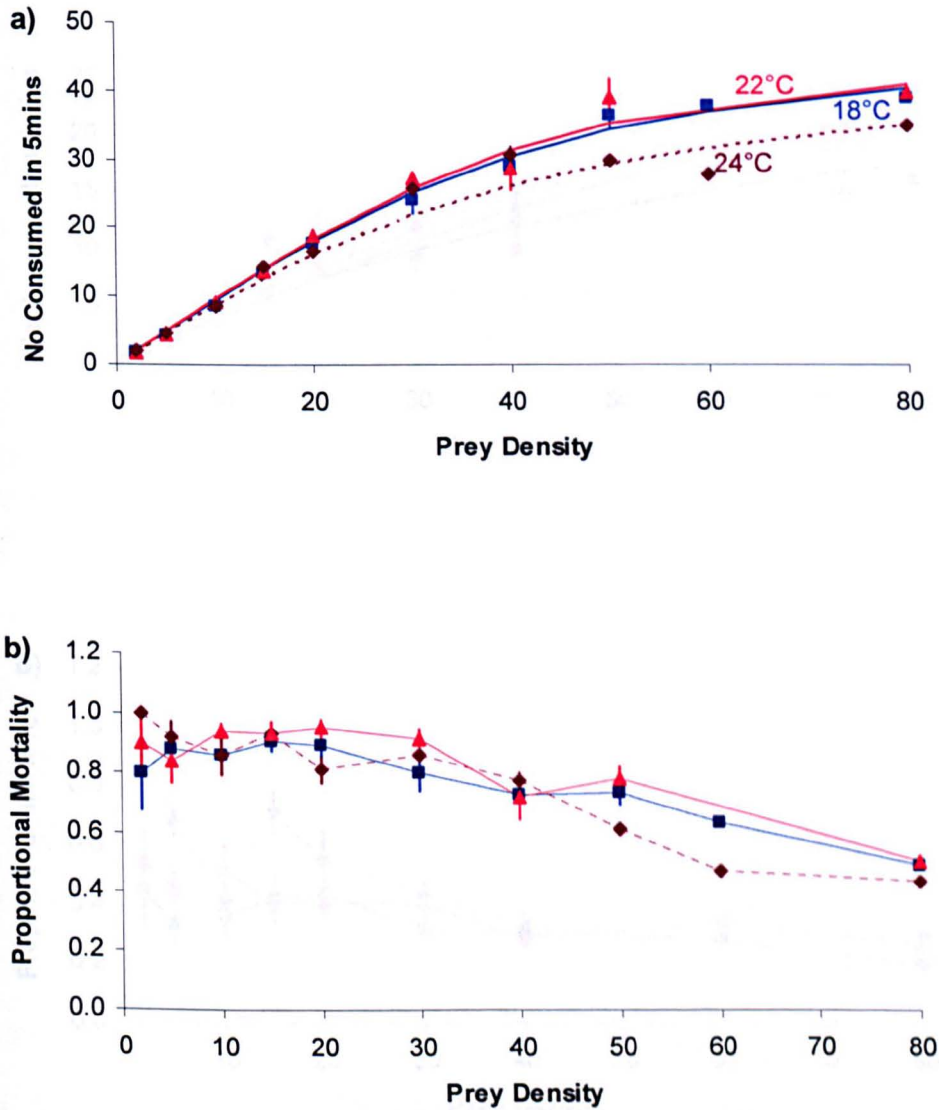
**Figure. 5.4.** The functional response (a) and mortality rate (b) of the three-spined stickleback preying on *Chironomidae sp.* (n=5) at 18, 22 and 24°C. Values are means ± SE. (b) GLM performed on Arcsine transformed proportions with post-hoc Tukey tests showed significantly higher proportional mortalities ($P < 0.05$) at low densities (Density; $2^{30,40,50,80}$, $5^{40,50,80}$, $10^{40,50,80}$, $15^{40,50,80}$, $20^{50,80}$, $30^2$, $40^{2,5,10,15}$, $50^{2,5,10,15,20}$, 60 & $80^{2,5,10,15,20}$). Superscript numbers represents pair-wise comparison of the densities that differ significantly. There was no significant effect of temperature or interaction between temperature and density.
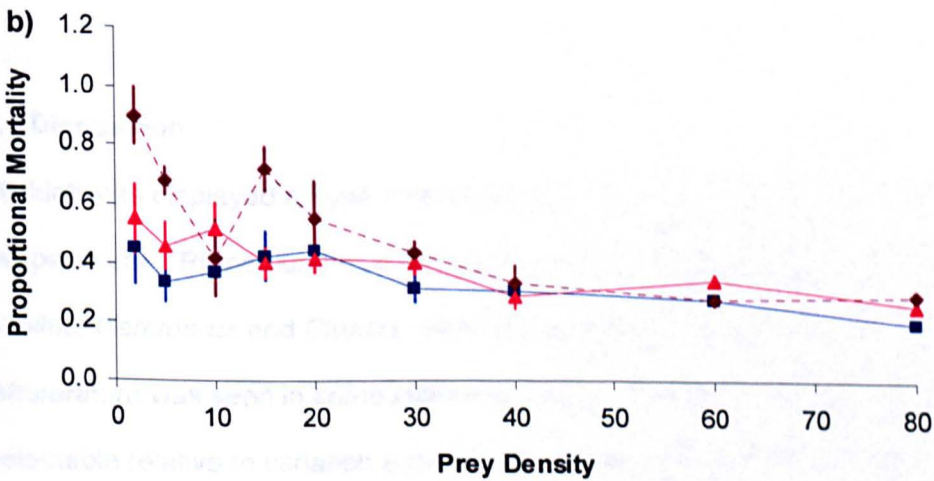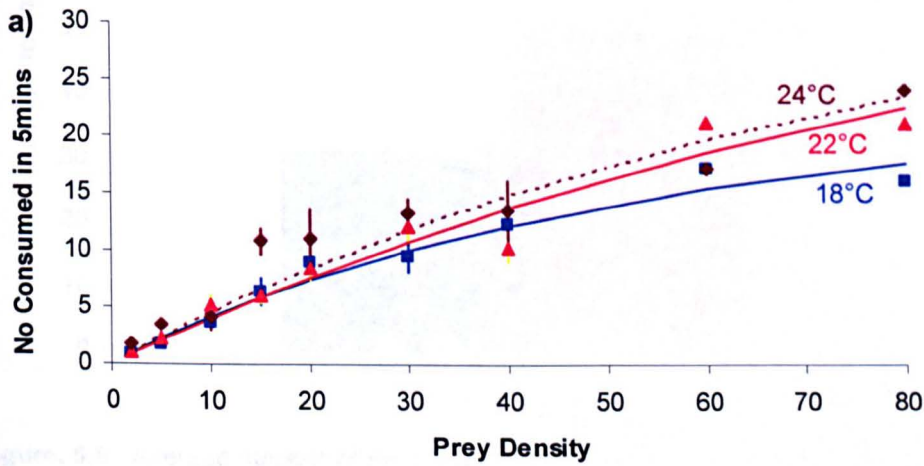
**Figure. 5.5.** The functional response (a) and mortality rate (b) of the three-spined stickleback preying on *Cloeon dipterum* (n=5) at 18, 22 and 24°C. Values are means ± SE. (b) GLM performed on Arcsine transformed proportions with post-hoc Tukey tests showed significantly higher proportional mortalities ($P < 0.05$) at low densities (Density; $2^{5,10,15,20,30,40,80}$, $5^2$, $10^2$, $15^2$, $20^2$, $30^2$, $40^{2.}$, 50 , 60 & $80^2$ and increased temperatures ($18°C^{24}$, 22°C & $24°C^{18}$). Superscript numbers represents pair-wise comparison of the densities that differ significantly. There was no interaction between temperature and density.
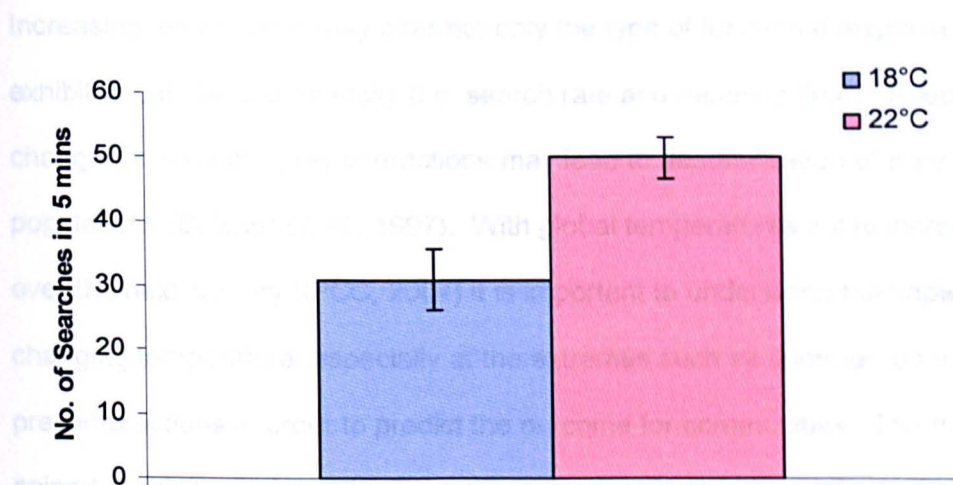
**Figure. 5.6**. Average number of searches over a 5 minute period for sticklebacks preying on 5-6mm mayfly nymphs at an initial density of ten per tank at 18 and 22 °C (n = 5). Values are means ± SE. Students t-test; P = 0.013.

## 5.4 Discussion

Sticklebacks displayed a Type II response to all prey types and across all temperatures. Proportional mortality increased significantly with temperature for *Asellus*, *Gammarus* and *Cloeon*. Although an increase in search rate with temperature was seen in some instances, strong effects of temperature were not detectable relative to variation between replicates. Despite this, a visual estimation of search rate produced a $Q_{10}$ of 3.32 which suggests search rate is thermally sensitive. Handling time appeared relatively robust to changes in temperature across all prey types although the longest handling time was at 18°C for *Asellus aquaticus*. The implications of the alterations in predator-prey interactions will be discussed with reference to the potential impact of climate change on freshwater communities.

Studies of predator-prey interactions in ectotherms need to include temperature as an important abiotic factor in dictating the nature of the interaction.

Increasing temperature may alter not only the type of functional response exhibited but also the intensity (i.e. search rate and handling time). Resulting changes in predator-prey interactions may lead to destabilisation of prey populations (Beisner *et. al.*, 1997). With global temperatures set to increase over the next century (IPCC, 2007) it is important to understand the impacts of changing temperature, especially at the extremes such as summer, on predator-prey interactions in order to predict the outcome for communities. The three-spined stickleback showed a Type II functional response regardless of prey type or temperature. Type II responses are generally considered to be 'de-stabilizing' with an increasing risk of prey mortality with decreasing prey density (Fig. 5.1). Aside from visual inspection of the fitted curves the type of functional response was assessed with proportional mortality plots (Fig. 5.2b – 5.5b) with GLM's and post-hoc Tukey tests on Arcsine transformed proportional mortality data. Proportional mortalities of all prey were significantly higher at the lower densities when compared with the highest densities across all the temperatures (Fig. 5.2b – 5.5b; See figure for P - values). This indicates a Type II response in all cases and, along with $R^2$ values, a good fit of the random predator equation to the data.

Proportional mortality increased with temperature for three prey types, *Asellus*, *Gammarus* and *Cloeon*. This effect was particularly pronounced at the lowest density of *Gammarus* with an increase in mortality from 44% at 18°C to 84% and 96% at 22 and 24°C respectively. This increase in proportional mortality with temperature could therefore lead to the increased risk of local extinction of small populations of *Gammarus* with climate change. Although a change in type of functional response with temperature was not seen in this case, this could be due to the experimental conditions preventing the processes that can produce a Type III response i.e. learning (Sticklebacks were well familiarised with prey)

(Holling, 1966), prey refuge (substrate was uniform in all experiments with no plants or additional structures to provide refuge for prey) (Hildrew & Townsend, 1977) or switching to another prey type (Sticklebacks were only offered one prey type) (Akre & Johnson 1979). Therefore, the results obtained here under tightly controlled and simplified conditions do not preclude the possibility of a Type III response occurring in more complex, natural, systems.

Although sticklebacks showed no evidence of a change in functional response the effects of temperature on its components are still potentially important in determining how prey profitability changes with temperature. Many previous studies have highlighted temperature as an important factor in ectotherm predator-prey interactions in the study of biological control agents such as parasitoids (Mohaghegh et. al., 2001; Flinn & Hagstrum, 2002; Menon et. al., 2002; Jones et. al., 2003; Garcia-Martin et. al., 2008). With current climate change predictions this recognition of temperature as an important factor needs to extend to all ectotherm predator-prey interactions. Sticklebacks showed relatively small changes in search rate with increasing temperature (Table 5.1) when compared with previous studies on ectotherms (Thompson, 1978; Murdoch et. al., 1984). These changes were also variable across prey types. The search rate of sticklebacks preying on mayfly was the lowest of all prey types and showed the least variation across the temperature range (Table 5.1). This could be due to mayfly being the most cryptic of the four prey types, remaining motionless and quite camouflaged against the sand substrate (pers. obs.). Search rate is a measure of the success of encounters between predator and prey (Lipcius & Hines, 1986). If overall encounters between predator and prey are low due to the prey being difficult to spot then the estimation of search rate will be low. A further experiment to elicit the thermal sensitivity of the search rate of sticklebacks preying on mayfly produced a $Q_{10}$ of 3.32 (Fig. 5.6).

This indicates that the search rate of sticklebacks for mayfly nymphs has a thermal sensitivity to temperature that is consistent with encounter being determined by primarily by metabolic processes, which have similar $Q_{10}s$ (Schmidt-Nielsen, 1970). Note that this finding would not be expected if much of the encounter relied on relatively passive encounter, which would be somewhat insensitive to temperature ($Q_{10}$ near to 1).

The search rate for sticklebacks preying on Asellus showed a slight increase between 18 and 24°C but was markedly decreased at 22°C. There is no obvious biological explanation for the decrease at 22°C and the most likely cause is experimental error in the estimation of search rate. The search rate for Gammarus and chironomids increased with a 4°C increase in temperature but then decreased at 24°C. The highest increase was for Gammarus between 18 and 22°C indicating that the number of successful encounters where the prey were captured and consumed increased. Amphipods are a common prey of sticklebacks (Wootton, 1976) and increases in search rate with temperature could lead to reductions in amphipod populations. The impact of an increase in temperature to 24°C on search rate was variable which could be due to sticklebacks being close to their upper limit, and experiencing a certain level of thermal stress. Indeed, sticklebacks kept at 24°C were noticeably thinner than sticklebacks kept at lower temperatures (pers. obs.).

Handling time was relatively insensitive to increases in temperature. Of the prey types, Asellus required the longest handling time (Table 5.1). Its long appendages and ability to curl into a ball when attacked make it difficult for the predator to swallow thus increasing the time taken to handle it. Both the thermal insensitivity of handling time and variable response of search rate to increasing

temperature are in contrast to a number of previous studies on functional responses in ectotherms. They tend to show a sigmoidal or exponential change in search rate with increasing temperature and an exponential decay in handling time (Thompson, 1978; Murdoch et. al., 1984; Taylor & Collie, 2003). However, for a species of damselfly, Ischnura elegans, which inhabits the same thermal range as British three-spined sticklebacks the greatest change in handling time is between 5 and 16°C (Thompson, 1978). Above this the handling time shows little response to increasing temperature. The same is true for search rate; the change in rate is reduced above 20°C. If we examine the data further (Fig. 5.7) we find that the $Q_{10}$ for search rate is highest between 8 and 12°C and declines after this (Table 5.2). Summer temperatures were used in the stickleback experiments as it was expected that changes in predator-prey interactions would be at their greatest when pushed to their thermal limits. However, examining the data for Ischnura elegans indicates that increasing temperature may have its greatest effects on functional responses over the mid-range of their thermal niche. Similarly, data on the handling time of two freshwater fish species perch, Perca fluviatilis, and roach, Rutilus rutilus, show the greatest change in handling time between 12 – 15°C and 15 – 18°C respectively (Table 5.3) (Persson, 1986). The experiments in this study, which focused on summer temperatures, may therefore have missed the large alterations seen in functional response at lower temperatures. It also suggests that the impacts of temperature on predator-prey interactions could be greatest in the months of spring and autumn when temperatures are slightly lower.

In conclusion, sticklebacks exhibited a Type II functional response which can potentially destabilise prey populations. Estimates of search rate and handling time from fitting the random predator equation showed little change across a 6 °C range of summer temperatures, although a direct visual estimate of search

rate produced a $Q_{10}$ of 3.32. However, based on other studies, we may expect

thermal sensitivity of these components of the functional response, and hence

effects on prey populations, to be more prominent at lower temperatures. Also,

slight alterations to search rate and handling time might alter the 'profitability' of

a prey item by altering the amount of energy expended for the amount of energy

gained by including the prey item in the diet. This theme will be discussed in the

next chapter when parameters of search and handling calculated here will be

used to test whether diet breadth of the stickleback is altered by increasing

temperature.
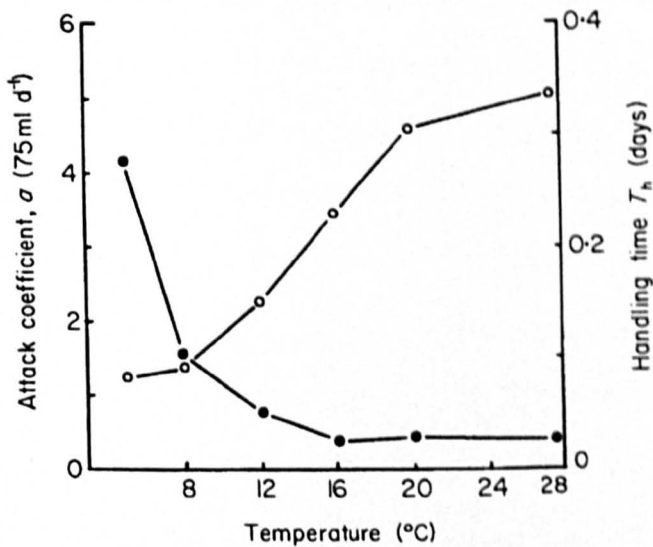


**Figure 5.7**. The effect of temperature on the search rate (○) and handling time (●) of
*Ischnura elegans*. Replicated with kind permission from Thompson (1978).

| Temperature Range (C°) | $Q_{10}$ |
|---|---|
| 5 - 8 | 1.29 |
| 8 - 12 | 3.59 |
| 12 - 16 | 2.70 |
| 16 - 18 | 2.58 |
| 18 - 20 | 1.69 |
| 20 - 22 | 1.24 |
| 22 - 27.5 | 1.16 |

**Table 5.2.** Estimates of $Q_{10}$ for the search rate of *Ischnura elegans* across a range of temperatures. Values were calculated from a graph in Thompson (1978) replicated here in Figure 5.7.

| Temperature Range (C°) | Perch | Roach |
|---|---|---|
| 12 - 15 | -25.5 | -13.1 |
| 15 - 18 | 4.9 | -38.8 |
| 18 - 21 | -15.9 | -22.6 |

**Table 5.3.** The percentage change in handling time of perch, *Perca fluviatilis*, and roach, *Rutilus rutilus*, calculated from data in Persson (1986).

# Chapter 6

Impacts of increasing summer temperatures on the optimal diet of the three-spined stickleback

## 6.1 Introduction

Teleost fishes are important predators in freshwater and marine ecosystems. They can be highly selective predators with prey selection being defined as a diet that is different from the random assortment of available prey in the predator's environment (Ivlev, 1961). Preference for a particular prey type has been shown to alter the structure of prey communities and in turn have a cascading effect on the ecosystem (Zagarese, 1990; Cowan & Houde, 1992; Zhengwen & Herzig, 1996; Chappaz et al., 1999). For example, in lakes selective predation of large daphnids can be intense and result in local extinction. This relieves grazing pressure on phytoplankton leading to an increase in biomass with associated turbidity and loss of macrophytes (Meijer et. al.. 1994). Even small changes in zooplankton size structure have been shown to lead to significant alterations in the phytoplankton community (Vanni, 1987). Fish can even indirectly alter something as seemingly unrelated as the precipitation of calcite through their selective predation of zooplankton (Hanson et. al., 1990). Much work has investigated the nature of prey selection by fish with a number of theories being proposed to explain diet choice.

One theory for the selective predation in fish is that they are 'gape limited' and simply choose the largest prey they can possibly consume (Anders & Brönmark, 2000). However, experiments have shown that fish actually choose prey that is approximately half (0.6) of their gape size (Bence & Murdoch, 1986; Wanzenbock, 1995). Many planktivorous fish, including sticklebacks, are visual predators and subsequently base their feeding decisions on visual observations (Wootton, 1976). O'Brien et. al. (1976) proposed the Apparent Size Model (ASM) which predicted that fish were choosing their prey by selecting the item that appeared the largest in the visual field. This model predicted that large prey items would appear larger in the visual field more often so would be chosen

more frequently and therefore lead to adaptive shifts in foraging behaviour. The model also predicted that small items at close proximity would appear largest in the fish's field of vision some of the time so would therefore be consumed, and that if visibility was reduced so that only one prey item was visible then the fish would demonstrate no selectivity. An experiment by Gardner (1981) demonstrated that bluegill sunfish feeding under extremely turbid conditions where only one prey item could be visible at a time showed exactly the same selectivity as their counterparts under normal clear water conditions. This, he attributed to the fishes decision to actively ignore smaller prey items and not to the differential encounter rate of prey due to a visual bias for larger items as suggested by O'Brien. Walton *et. al.* (1992) showed that even small sunfish with their visual constraints (the relationship between increasing fish size and visual resolution has been well researched; Hairston & Li, 1982; Li *et. al.*, 1985) could measure some form of absolute prey size and that their feeding habits were better described by optimal diet theory.

Optimal diet theory (ODT) is one of the more successful theories for correctly predicting diet selection. A 1986 evaluation of 60 studies showed that only 18% of these studies directly contradicted the theory's basic predictions (Stephens & Krebs, 1986). The main prediction of ODT is that a predator should choose the prey for which it gains maximum energy ($E$) per unit handling time ($Th$) (MacArthur & Pianka, 1966). It does this by specialising on only the more profitable prey ($E/Th$) above a critical threshold density or encounter rate and then generalising to less profitable prey below this (Werner & Hall, 1974; Charnov, 1976; Harvey & White, 1990). In other words, prey of lower value should be ignored irrespective of their abundance: instead, the decision to add a lower value prey to the diet is based entirely on the abundance of the more profitable prey (Krebs, 1977).

This is described in the following inequality from Harvey & White (1990):

$$\frac{a_1 N_1 E_1}{1 + a_1 N_1 T_{h1}} > \frac{a_1 N_1 E_1 + a_2 N_2 E_2}{1 + a_1 N_1 T_{h1} + a_2 N_2 T_{h2}}$$

Equation 6.1

Where $a_1$, $a_2$ = search rate for prey types 1 (most profitable) and 2; $T_{h1}$, $T_{h2}$ = handling time of prey types 1 and 2; $E_1$, $E_2$ = energy content of prey types 1 and 2; $N_1$, $N_2$ = initial densities of prey types 1 and 2.

If the rate of energy gained by taking both prey types (right hand side) is less than when the more profitable prey type alone is taken (left hand side) then the lower value prey will be excluded from the diet. This exclusion is independent of the abundance of the less profitable prey type and occurs at a critical density of the more profitable prey type which is given by the following inequality (Harvey & White, 1990):

$$N_1 > \frac{E_2}{a_1 \left( E_1 T_{h2} + E_2 T_{h1} \right)}$$

Therefore, the critical density above which only the more profitable prey type should be taken can be calculated with the following equation:

$$N_1 = \frac{E_2}{a_1 \left( E_1 T_{h2} + E_2 T_{h1} \right)}$$

Equation 6.2

An experiment on great tits (*Parus major*) successfully demonstrated that above the critical threshold encounter rate they would only take the more profitable prey irrespective of the abundance of the lower prey (Krebs *et. al.*, 1977). However, instead of an all-or-nothing response to the lower value prey they found a sigmoidal response which they attributed to the birds sampling in order to assess the availability and profitability of prey types.

Most ODT models are derived from Charnov's (1976) model developed to predict diet breadth in the mantid, *Hierodula crassa*. The main assumptions of the model are that:

1) The predator encounters prey individually; the value of each prey item is measured in calories or grams

2) Handling and searching are mutually exclusive (handling time being defined as from the point when a predator 'decides' to go after a prey item)

3) Prey recognition is instantaneous and no learning is required

4) Prey are not large enough to be a threat to the predator

5) There is no differential risk of mortality to the predator during the different foraging processes

6) The predator does not engage in non-foraging activities during a foraging bout

7) The energetic cost to the predator is the same throughout the foraging process.

Although ODT has had some success, it also has its failings. Sih & Christensen (2001) carried out a review of 164 studies of ODT to examine where and why ODT failed in predicting diet and diet shifts. From this, they discovered that quantitative studies were more likely to fit ODT than qualitative but that the type of study (experimental or non-experimental), place of study (field or laboratory) or the type of forager examined (Invertebrate, vertebrate ectotherm or vertebrate endotherm) did not significantly affect the ability of ODT to predict diet. Of the variables examined, only increased mobility of prey significantly reduced the fit of ODT to observed diet patterns. Mobile prey can exhibit anti-predator behaviour thus producing costs not included in the ODT model such as pursuit

time or the escape ability of the prey. It has been shown for the pike cichlid

(*Crenicichla saxatilis*) preying on large guppies (*Poecilia reticulata*) that pre-

capture costs, such as approach and attack time, may be more important than

post-capture costs, such as handling time, in determining optimal diet

(Johansson, 2004). Also, more active prey are more visible to visual predators

than cryptic prey so may be overrepresented in the diet and produce results that

do not corroborate ODT. The model also assumes that the motivational state of

the animal remains static throughout whereas realistically the level of hunger of

the animal might vary. An experiment on the fifteen-spined stickleback,

*Spinachia spinachia* (L), demonstrated that handling time and therefore optimal

prey choice changed as the gut filled and the fish became satiated (Kislalioglu &

Gibson, 1976) though this is not wholly supported (Gill & Hart, 1994). Other

factors such as learning, competition, vigilance, predator size (Gill & Hart, 1996)

and reproductive state may violate the assumptions of ODT and influence

foraging choices (Gill, 2003).


Temperature is an important consideration when attempting to predict the

foraging behaviour of an ectotherm (Persson, 1986) as it can alter the

components of the optimal diet model, such as search rate and handling time,

and therefore optimal diet. Previous studies on terrestrial and aquatic

ectotherms have shown a decrease in handling time and increase in search rate

with increasing temperature (Thompson, 1978; Murdoch *et. al.*, 1984). Changes

in handling time ($T_h$) will change the profitability of prey items ($E/T_h$) and

therefore the predator's decision to include or exclude them from the diet.

Increases in search rate would cause a decrease in the critical density of the

more profitable prey (Equation 6.2) and therefore result in a more specialised

diet. With mean global temperatures expected to rise over the next century by

3-5°C (IPCC, 2007) changes in diet of predatory fish could have important

consequences for freshwater ecosystems.


To date, no study has attempted to examine the influence of temperature on the

optimal diet of a predator feeding on a number of prey types.   This chapter will

investigate how increasing temperature will alter the predictions of optimal diet

for the three-spined stickleback preying on four of the most common

invertebrate prey taxa in the Ness mesocosms.  It will also provide the first

attempt to test these predictions on real communities by analysing the

population changes of these invertebrates in the mesocosms in response to fish.

It is expected that the more profitable prey types will be taken first and that their

numbers will reduce until below some threshold when the next prey type is

added (Fig. 6.1).   Predictions will be tested in both the pelagic and benthic

zones.

a)



b)
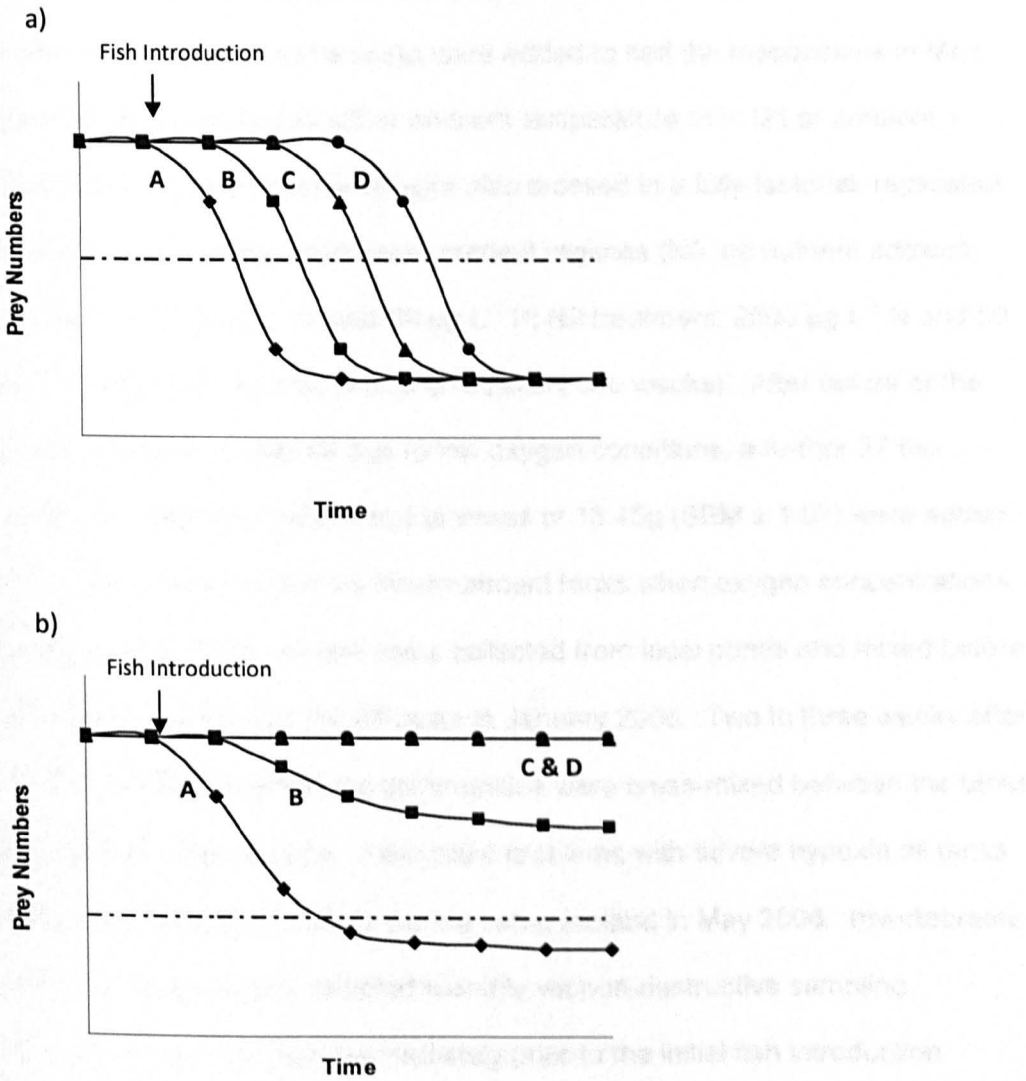


**Figure 6.1.** An example of how prey populations might be exploited in the mesocosms according to ODT and the use of critical densities. For ease of demonstrating the principles, we consider all prey types to have the same critical density, which is represented by the dashed line, and that profitabilities decrease from type A to type D. In example a) when prey type A falls below its critical density, prey type b is added to the diet; this process also applies to the inclusion of type C and finally D. In example b) prey type B is added to the diet, but, because it never drops below the critical threshold density, types C and D are never added to the diet.

## 6.2 Methods

For full description of the set up and establishment of the 48 mesocosms see Chapter 2. Four adult sticklebacks were added to half the mesocosms in May 2006 and were exposed to either ambient temperature (n = 12) or ambient + 4°C (n = 12). These treatments were also crossed in a fully factorial, replicated (x4) experimental design with three nutrient regimes (N0, no nutrient addition; N1 treatment: 250 µg L$^{-1}$ N and 50 µg L$^{-1}$ P; N2 treatment: 2500 µg L$^{-1}$ N and 50 µg L$^{-1}$ P, added as Na NO$_3$ and KH$_2$PO$_4$ every two weeks). After failure of the fish populations to establish due to low oxygen conditions, a further 27 fish, broadly size-matched, with a total biomass of 13.45g (SEM ± 1.07) were added in July 2006 to each of the 24 fish-treatment tanks when oxygen concentrations had increased. Invertebrates were collected from local ponds and mixed before being distributed among the 48 tanks in January 2006. Two to three weeks after the initial inocula, invertebrate communities were cross-mixed between the tanks using standard net sweeps. After initial problems with severe hypoxia all tanks were subsequently re-stocked via the same method in May 2006. Invertebrates in the mesocosms were sampled monthly via non-destructive sampling methods. Sampling began immediately prior to the initial fish introduction. Invertebrates in the water column were sampled using a standard sweep net method as described in Chapter 3 (they will be referred to as pelagic invertebrates in this chapter despite there being a large plant biomass in the pelagic zone). The volume of water sampled was calculated (approximately 440 L$^3$) then used to convert the invertebrates sampled to numbers per L$^3$. Benthic invertebrates were sampled using a standard Eckman grab (14.6 by 14.6cm) then converted to numbers per m$^2$.

Estimates of search rate (a) and handling time (T$_h$) were calculated in the previous chapter for four invertebrate types; Amphipoda (Gammarus pulex),

Chironomidae, Ephemeroptera (*Cloeon dipterum*) and Isopoda (*Asellus aquaticus*), at 18 and 22°C using Rogers' random predator equation (Chapter 5). The temperatures 18 and 22°C were selected as the impacts of climate change are expected to be more pronounced at the extremes of temperatures i.e. summer. These estimates were then used to calculate profitability ($E/T_h$) of the prey items at different temperatures in terms of the amount of energy gained in calories ($E$) per unit handling time ($T_h$) for a particular prey type. Prey were then ranked according to their profitability (Table 6.1). The energy content of individual invertebrates was derived from literature estimates of calorific content (Wissing & Hasler, 1971; Cross & Vohs, 1988; Hart & Gill, 1992) and dry weights of individual invertebrates calculated in the laboratory (ten replicates of five animals). Critical densities were calculated using Equation 6.2 (Harvey & White, 1990) and then converted to numbers per $L^3$ (pelagic) and per $m^2$ (benthic) (Table 6.1). Invertebrates used to estimate prey profitabilites were approximately 5mm in length. This size class was selected as sticklebacks would not be gape limited and it was representative of the size of the chosen invertebrates in the mesocosms (pers. obs.). However, the invertebrates and the fish in the mesocosms will be present in a range of sizes which may alter profitabilities and the rank order of prey in the stickleback's diet. For this experiment it is assumed that fish will select the most profitable size invertebrate (0.6 of gape;(Gill, 2003) and that rank order will therefore remain consistent.

### 6.2.1 Statistics

Effects of treatment and date on each of the four invertebrate prey groups was analysed using repeated-measures mixed-model analysis for the four dates directly after the major fish addition in July (Table 6.2). Heating, nutrients and

fish presence were used as fixed factors with block as a random factor and date as the repeated measure. Statistics were carried out with SPSS 16.0 and Minitab 15.

## 6.3 Results

### 6.3.1 Predictions

ODT predictions for sticklebacks foraging at 18°C indicate that to maximise energy intake ($E/T_h$) they should take prey in the following order of preference: Chironomidae, Amphipoda, Ephemeroptera with Isopoda being the least profitable prey item. They should only switch to the next most profitable prey below the critical densities as shown in Table 6.1. At 22°C, there is a switch in the profitability ranks with the second most profitable prey item changing from Amphipoda to Ephemeroptera. The critical density above which sticklebacks should only take Chironomidae increases with an increase in temperature. Critical densities for Amphipoda and Ephemeroptera decrease with increasing temperature

### 6.3.2 Tests of predictions

In the sweep sampling of the pelagic zone, at the point of the main fish introduction (July 2006), the three most profitable prey types were below the critical densities in both ambient (Fig. 6.2) and heated (Fig. 6.3) mesocosms, and hence non-selectivity was predicted. Chironomids showed no difference in numbers between fish and non-fish treatments but were significantly lower in heated mesocosms (Table 6.2). Numbers of Ephemeroptera were lower in fish treatments but only in ambient mesocosms (Fig. 6.2c) (a 44% reduction). There were significantly lower numbers of Amphipoda in fish tanks compared to non-fish tanks in both ambient (Fig 6.2b; 90% reduction) and heated mesocosms (Fig. 6.3b; 93% reduction) with the effect being seen mainly in N1 and N2

treatments (Table 6.2). Isopoda numbers were lower in fish treatments, with this effect being seen mainly in ambient mesocosms (Fig. 6.2d; 75% reduction).

In the benthic sampling, numbers of chironomids were above the critical density and remained above this until the November sampling in both ambient (Fig. 6.4a) and heated (Fig. 6.5a) mesocosms. However, there was no significant difference in chironomid numbers between fish and non-fish treatments (Table 6.2). Ephemeroptera numbers were mainly above their critical density in ambient mesocosms (Fig. 6.4c) but below in heated mesocosms (Fig. 6.5b) and showed no significant response to fish presence (Table 6.2). Amphipoda numbers were below the critical density in ambient mesocosms (Fig. 4b) but above it in heated mesocosms (Fig. 6.5c) and there were significantly fewer amphipods in fish treatment tanks in both ambient (92%) and heated (82%) mesocosms but this result was again seen mainly in N1 and N2 treatments (Table 6.2). Numbers of Isopoda were significantly lower in fish treatments but again, mainly in ambient mesocosms (89% reduction)

Table 6.1. Estimates of instantaneous search rate ($a$; per tank per minute) and handling time ($T_h$; minutes) of sticklebacks preying on four prey types at 18 and 22°C. Prey are ordered in terms of their profitability ($E/T_h$) (Where $E$ = Energy (Kcal)). Calculations of critical density (see Introduction for details) are included for both benthic and pelagic invertebrate population samples in the mesocosms.

|  | Taxon | $a$ | $T_h$ | $E$ | $E/T_h$ | Critical Density | |
|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  | Pelagic ($L^{-1}$) | Benthic ($m^{-2}$) |
| **18°C** | Chironomidae | 3.7 | 0.2 | 0.006 | 0.060 | 0.037 | 8.463 |
|  | Gammarus pulex | 1.2 | 0.2 | 0.004 | 0.021 | 1.144 | 263.334 |
|  | Cloeon dipterum | 0.6 | 0.2 | 0.003 | 0.019 | 0.046 | 10.604 |
|  | Asellus aquaticus | 3.0 | 0.8 | 0.003 | 0.003 | - | - |
| **22°C** | Chironomidae | 4.1 | 0.1 | 0.006 | 0.060 | 0.094 | 21.657 |
|  | Cloeon dipterum | 0.6 | 0.1 | 0.003 | 0.037 | 0.258 | 59.316 |
|  | Gammarus pulex | 4.7 | 0.3 | 0.004 | 0.012 | 0.009 | 1.988 |
|  | Asellus aquaticus | 0.9 | 0.6 | 0.003 | 0.004 | - | - |

**Table 6.2.** The effects of heating (H), fish (F), nutrients (N) and their main
interactions on the numbers of invertebrates found in the sweep
and benthic sampling of 48 mesocosms in a four-month period following
fish addition in July 2006 (Sample dates (D): August – November). Values
shown are P–values derived from repeated-measures linear mixed
models. Three- and four-way interactions are not included.

| Prey Type | H | F | N | D | H×F | H×N | H×D | F×N |
|---|---|---|---|---|---|---|---|---|
| **Sweep** | | | | | | | | |
| Chironomidae | 0.020 | ns | ns | 0.001 | ns | ns | 0.039 | ns |
| Amphipoda | 0.004 | ns | 0.007 | ns | ns | ns | ns | 0.014 |
| Ephemeroptera | ns | ns | ns | <0.001 | 0.032 | ns | ns | ns |
| Isopoda | ns | 0.003 | ns | 0.006 | 0.010 | ns | ns | 0.005 |
| **Benthic** | | | | | | | | |
| Chironomidae | <0.001 | ns | ns | <0.001 | ns | ns | 0.025 | 0.019 |
| Amphipoda | ns | <0.001 | 0.040 | ns | ns | 0.020 | 0.023 | 0.007 |
| Ephemeroptera | ns | ns | ns | 0.019 | ns | ns | 0.050 | ns |
| Isopoda | ns | <0.001 | ns | 0.031 | <0.001 | ns | <0.001 | ns |
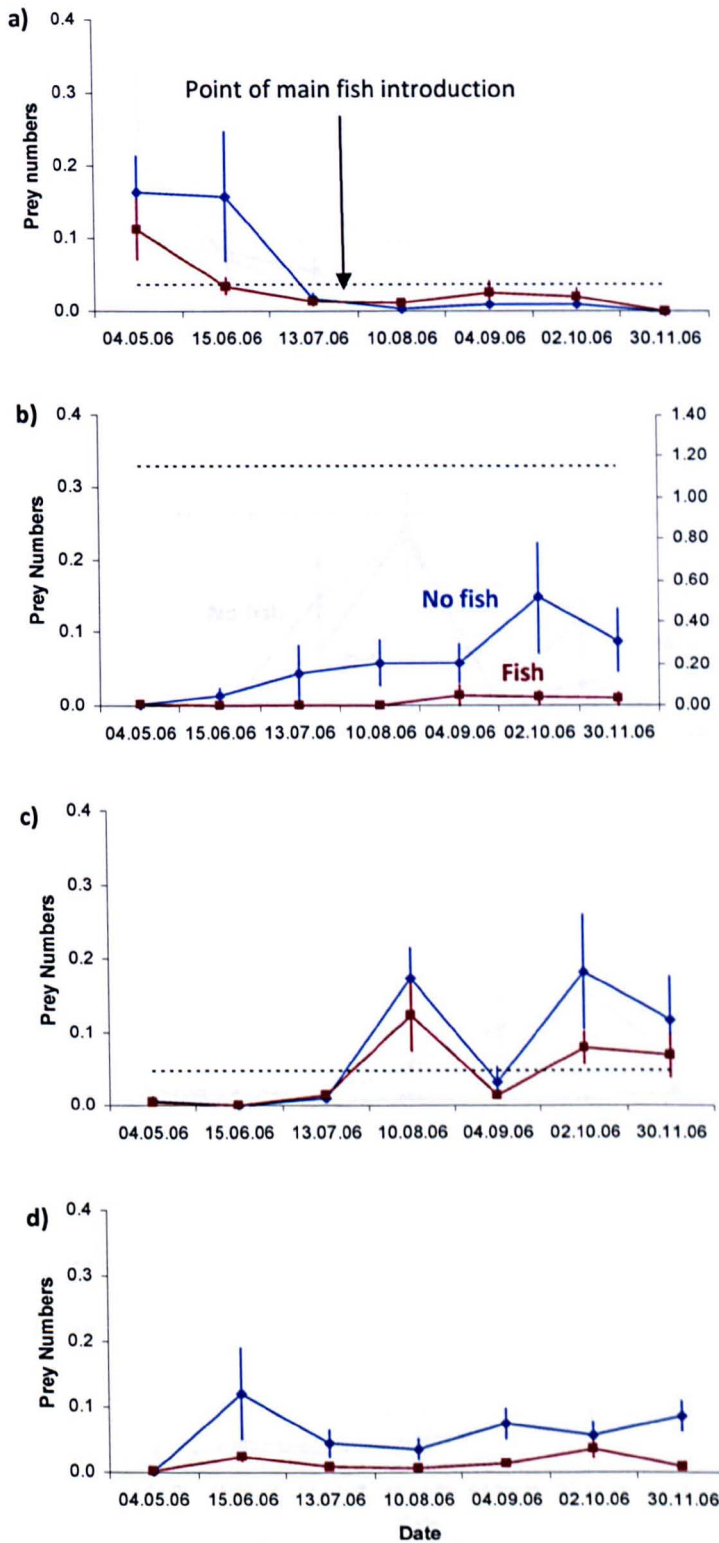
**Figure 6.2. Pelagic Ambient:** Changes in populations of a) Chironomidae; b) Amphipoda; c) Ephemeroptera and d) Isopoda in fish and no fish mesocosms. Graphs are presented in order of decreasing prey profitability and dashed lines represent the critical density below which then next prey type should be added to the diet. Prey numbers are per litre. The second axis in b) represents the prey density.
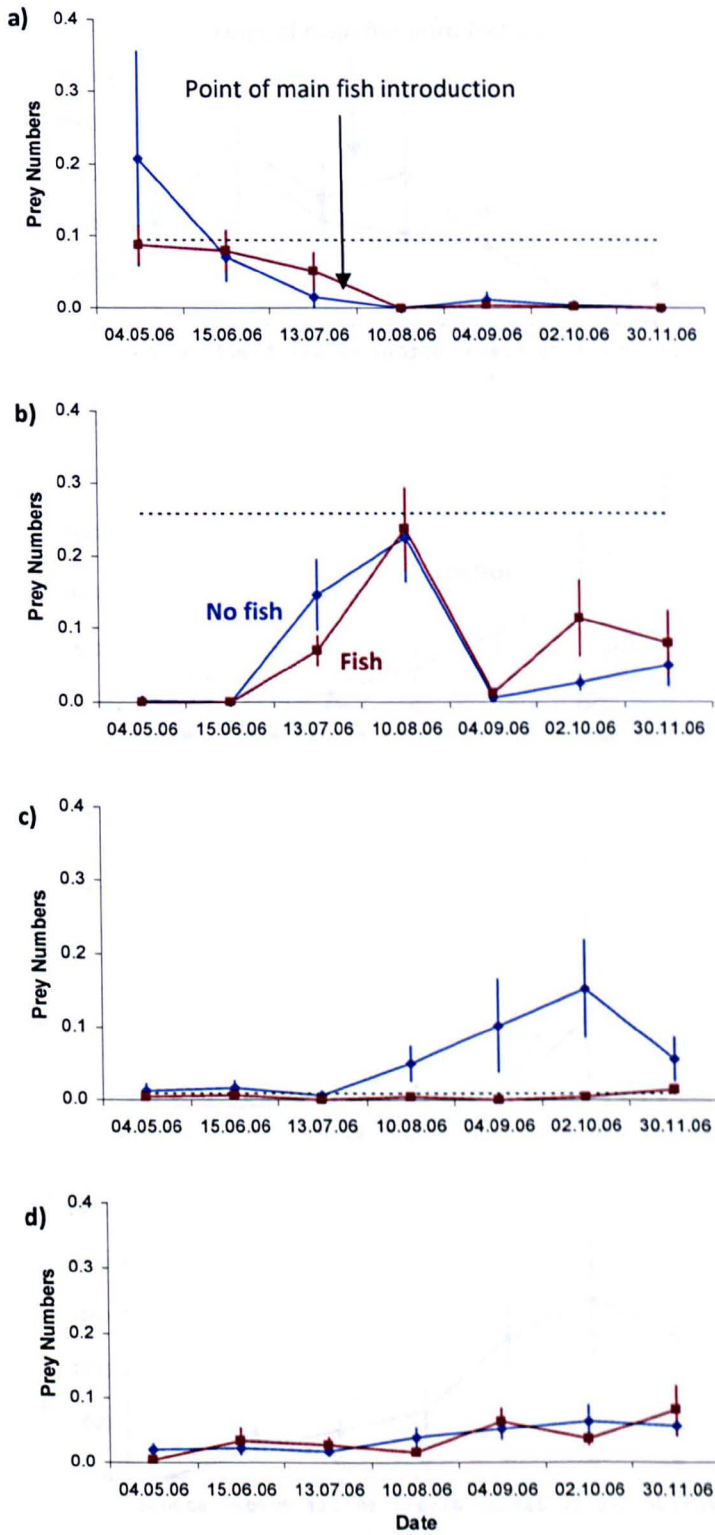
**Figure 6.3. Pelagic Heated:** Changes in populations of a) Chironomidae; b) Ephemeroptera; c) Amphipoda and d) Isopoda in fish and no fish mesocosms. Graphs are presented in order of decreasing prey profitability and dashed lines represent the critical density below which the next prey type should be added to the diet. Prey numbers are per litre.
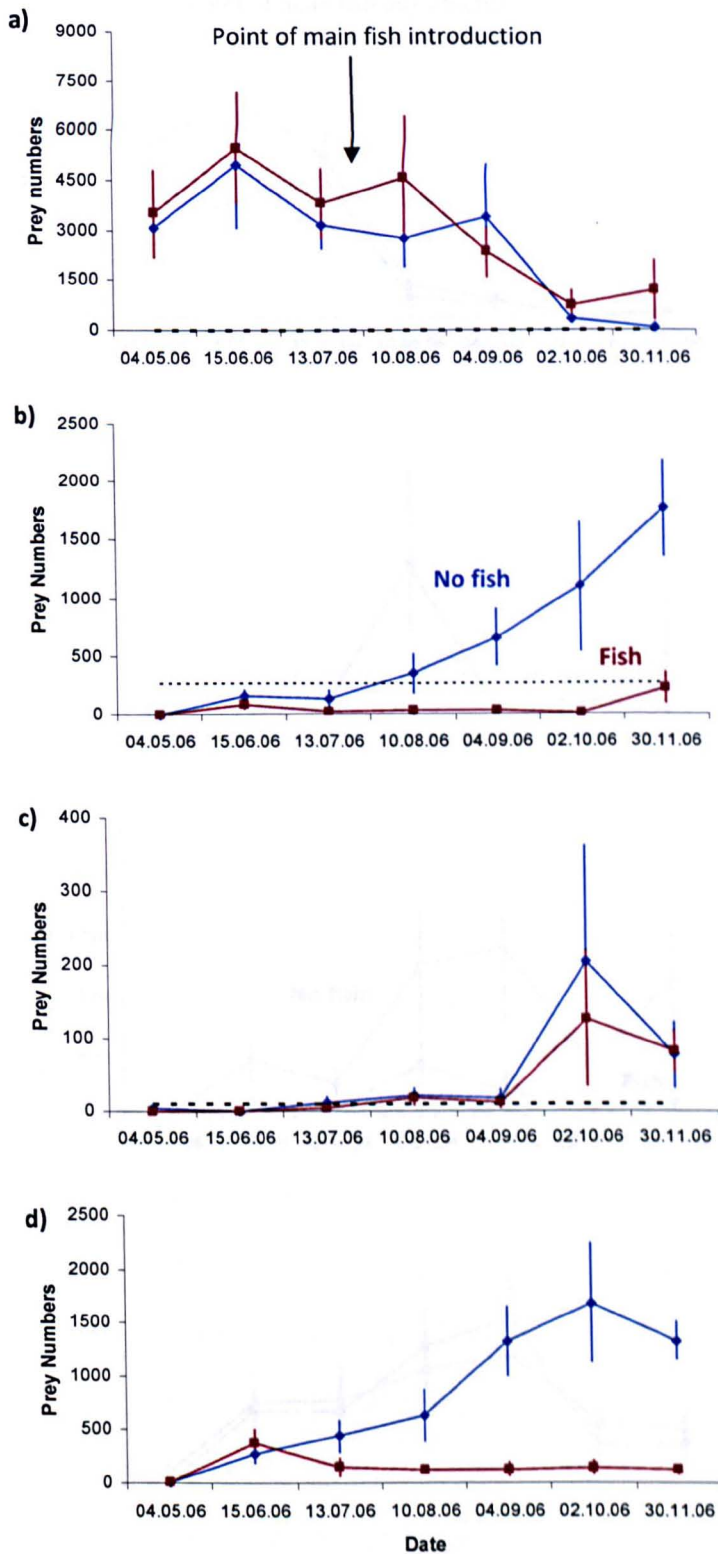
**Figure 6.4. Benthic Ambient:** Changes in populations of a) Chironomidae; b) Amphipoda; c) Ephemeroptera and d) Isopoda in fish and no fish mesocosms. Graphs are presented in order of decreasing prey profitability and dashed lines represent the critical density below which then next prey type should be added to the diet. Prey numbers are per litre.
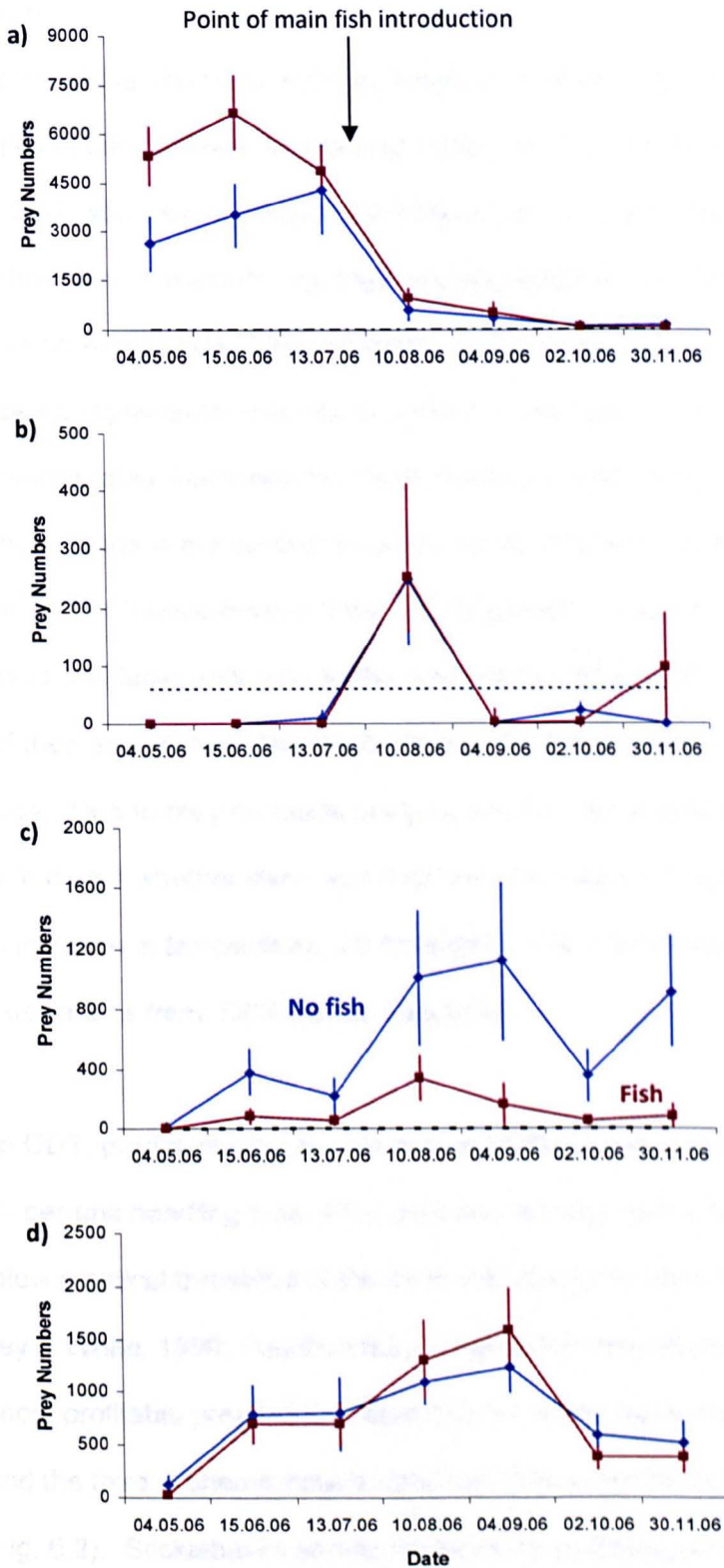
**Figure 6.5. Benthic Heated:** Changes in populations of a) Chironomidae; b) Ephemeroptera; c) Amphipoda and d) Isopoda in fish and no fish mesocosms. Graphs are presented in order of decreasing prey profitability and dashed lines represent the critical density below which then next prey type should be added to the diet. Prey numbers are per litre.

## 6.4 Discussion

In the pelagic sampling, the three most profitable prey types were below their critical densities in both ambient and heated mesocosms and therefore, according to ODT, sticklebacks should have taken all four prey types upon encounter. However, in ambient mesocosms there was only a significant impact of sticklebacks on Amphipoda, Ephemeroptera and Isopoda with only Amphipoda being significantly reduced in heated mesocosms. The impacts of fish on the invertebrates was therefore not completely predicted by ODT. The number of chironomids in the benthic sampling never dropped below the critical density in ambient or heated mesocosms. Sticklebacks should therefore have fed on this most profitable prey type alone, ignoring the other three prey types regardless of their abundance. However, there was no significant impact of fish on chironomids. Due to prey numbers being below the critical thresholds it was not possible to detect whether there was a switch to the second most profitable prey with an increase in temperature, as predicted. The impact of temperature on, and the deviations from, ODT will be discussed.

According to ODT, predators should rank prey in terms of their profitability (energy gain per unit handling time; $E/T_h$) and only add the next prey type to their diet below a critical threshold of the more valuable prey item (Charnov, 1976, Harvey & White, 1990; Equation 6.2). In ambient mesocosms, the density of the two most profitable prey types (Table 6.1) remained below the critical threshold and the third, Ephemeroptera, dropped below the threshold on a one occasion (Fig. 6.2). Sticklebacks should therefore have displayed a generalist diet preying upon the first three prey types and taking Isopoda only when Ephemeroptera numbers dropped below their critical threshold. This was largely the case with a significant reduction (Table 6.2) in numbers of Amphipoda (92%), Ephemeroptera (44%) and Isopoda (74%) but not Chironomidae in

mesocosms containing fish (Fig. 6.2). Isopoda showed a reduction in numbers (Fig. 6.2d) in spite of Ephemeroptera density being above the critical density on for the majority of the experimental period (Fig. 6.2c). However, Ephemeroptera dropped then rose above the critical density in a 'peak' (Fig. 6.2c) due to the emergence of adults followed by the laying of eggs. The large numbers of Ephemeroptera were therefore made up of newly hatched small-bodied individuals which will have had an impact on the profitability of the prey and therefore the decision to add isopods to the diet. The lack of significant fish effect on chironomids is not explicable by their being less abundant than the other prey types, as at the time of fish addition the abundances of all four prey types were similar (Fig 6.2 & 6.3). Many species of chironomid are tube-dwelling invertebrates and as such are hidden from visual predators. The ODT predictions were derived from laboratory experiments (Chapter 5) where the chironomids were allowed to disperse but obviously lacked sufficient time to construct a tube in the bottom substrate. Experimental tanks were also well lit and the substrate more uniform than in a natural system making the bright red chironomids easy to spot and capture. Persson & Greenberg (1990) suggested habitat-dependent search rates may have caused observed diet to differ from ODT predictions in perch (*Perca fluviatilis*) feeding on invertebrates. These factors will have produced higher estimates of search rate in the laboratory than would occur naturally for this predator-prey scenario by not allowing for anti-predator mechanisms and therefore caused discrepancies between ODT predictions and observed diet (Christensen, 1996; Sih & Christensen, 2001).

With heating by 4°C it was expected that sticklebacks would switch to Ephemeroptera as the second most profitable prey type (Table 6.1). However, as numbers of all prey types in the heated pelagic samples were below the critical density it was impossible to detect if such a switch occurred (Fig. 6.3).

Again, sticklebacks were predicted to employ a generalist diet strategy feeding on all prey types upon encounter. However, only Amphipoda showed significantly lower numbers in fish treatments (Fig. 6.3c; 93% reduction). As discussed earlier, anti-predator behaviour may cause inaccuracies in optimal diet predictions. Sticklebacks are visual predators and are attracted by the movement of prey (Wootton, 1976). Ephemeroptera tend to remain fairly motionless and cryptic against the background in the presence of fish and employ rapid bursts of speed in order to escape predation (pers.obs.). This, coupled with periodic emergence of mayfly, reduced their encounter rate with fish and therefore possibly their presence in the stickleback's diet. Isopoda, however, are fairly active but were not significantly reduced by fish despite amphipods falling below their critical density (Fig. 6.3d). A different explanation is therefore required. Amphipod numbers in mesocosms containing fish were only slightly below their critical density (Fig. 6.3c). Predators generally show a gradual addition of lower value prey to their diet rather than an 'all-or-nothing' response (Krebs et. al., 1977; Stephens & Krebs, 1986). Sticklebacks may therefore be 'sampling' the isopods in order to assess whether or not to add them to their diet based on their abundance and profitability. Also, ODT models do not take into account learning and assume all prey are instantaneously recognised on encounter. If prey are being introduced to the diet for the first time this may not be realistic and predators may take time to 'learn' how to handle prey, thus making laboratory derived predictions of handling time in non-naïve fish inaccurate.

In the benthic sampling, the number of the most profitable prey type, Chironomidae, never dropped below the critical density. Sticklebacks were therefore predicted to specialise taking this prey type alone. However, there was no impact of fish on chironomid numbers which steadily declined in both

ambient (Fig. 6.4a) and heated (Fig. 6.5a) mesocosms. Again, the ability of chironomids to display anti-predator behaviour may lead to them being underrepresented in a diet based on laboratory predictions where prey were unable to hide. Also, chironomids periodically emerge, which influences encounter rates. In ambient mesocosms, Amphipoda, the next most profitable prey, remained below the critical density and were therefore expected to be predated upon by fish (92% reduction) whenever encountered along with the next prey type, mayfly. There is, however, no significant impact of fish on mayfly numbers whose abundance rises above the critical density on a number of occasions. Despite mayfly being above the critical density, the next most profitable prey type, Isopoda, are reduced in fish treatments. Explanations for the lack of fish effect on mayfly again include anti-predator mechanisms and periodic emergence of adults leading to the encounter rate between predator and prey being low. Isopoda are therefore added to the diet based on the lack of encounter with and the reduced profitability of mayfly. Heated mesocosms again showed a lack of fish impact on the two most profitable prey types, Chironomidae (Fig. 6.5a) and Ephemeroptera (Fig. 6.5b). The numbers of the third most profitable prey item, Amphipoda, remained above the critical density for the duration of the sampling (Fig. 6.5c). There was a significant effect of sticklebacks on Amphipoda (82 % reduction) but not Isopoda (Fig. 6.5d), in agreement with ODT predictions.

Overall, the causes of failure of ODT to predict the impact of sticklebacks on the invertebrate community was due to the inclusion of chironomids and mayflies, which may have complicated and confounded predictions because of their emergence patterns, radical size shifts, and reduced visibility between laboratory trials and field conditions. However, ODT succeeded when predicting critical densities for amphipods below which isopods should be

impacted on by fish. Testing the predictions of ODT on the diet breadth of a predator preying upon multiple invertebrates in a 'real' community is demanding of time and expertise and has never to my knowledge been attempted before. Although this study focused on four of the most abundant macroinvertebrates in the mesocosms, sticklebacks will also have been feeding on other prey types and also a range of invertebrate sizes which will have varied in their calorific content. Zooplankton, for example, are a well known food source of sticklebacks occupying up to 70% of a sticklebacks diet in summer months (Hynes, 1950). Although this experiment did not provide data on the profitability of various zooplankters to the sticklebacks, if these exceeded the profitabilities of our chosen experimental invertebrates then this will also have impacted on the fit of ODT predictions to observed prey abundances.


A particularly novel element of this study was not only predicting optimal diet in sticklebacks but examining whether an increase in summer temperature, such as changes expected with climate change, had any impact on optimal diet. Laboratory-derived predictions indicated a switch to include the second most profitable prey type in the diet with increasing temperature. This, however, proved difficult to test as prey abundances were always below critical densities, irrespective of heating treatment. According to the literature, the search rate of actively foraging ectotherms tends to increase with temperature (Persson, 1986). This leads us to predict that predators will become more specialised at higher temperatures due to increased search rates lowering the critical densities of prey (Equation 6.2) and prey will therefore be at higher risk of local extinction. However, the effect of temperature on a predator may depend upon the mode of predation. For example, for sit-and-wait predators whose rate of predation depends upon food floating past, search rate will be relatively insensitive to increasing temperature and the sensitivity of handling time will be more

important. In the laboratory experiments, only sticklebacks preying on

Chironomidae and Amphipoda showed an increase in search rate with

temperature resulting in fairly small changes in critical densities. As discussed in

the previous chapter, the summer temperatures used may not have included the

range of temperatures where changes in search and handling were greatest.

When examining the profitability of a species of phantom midge larvae,

*Chaoborus obscuripes*, preyed upon by roach (*Rutilus rutilus*) and perch (*Perca*

*fluviatilis*) Persson (1986) found the greatest changes in profitability at lower

temperatures (Table 6.3).


In conclusion, optimal diet theory partially predicted the impact of sticklebacks

preying on four invertebrate types in replicated realistic outdoor ecosystems.

Anti-predator mechanisms such as crypsis are possibly better at explaining

discrepancies from ODT predictions of diet than prey mobility as previously

suggested. Whilst increased temperature altered prey profitabilities and

therefore predicted rankings of prey in the stickleback's diet it was not possible

to test these predictions. Further experiments should include a range of

temperatures as the impacts of climate change may be more important at mid-

range rather than extremes of temperature.


| Temperature Range (C°) | Perch | Roach |
|---|---|---|
| 12 - 15 | + 34.3% | + 15.1% |
| 15 - 18 | − 4.7% | + 63.4% |
| 18 - 21 | +18. 9% | + 29.2% |

Table 6.3. Percentage changes in the profitability of *Chaoborus obscuripes* ($E/T_h$) being preyed upon by two species of fish with 3°C warming over three temperature ranges. Values were calculated from estimates of handling time and dry mass of prey in Persson, (1986).

# Chapter 7

## Discussion and Conclusion

## 7.1 Discussion

The key aim of this study was to examine the response of three-spined stickleback and macroinvertebrate populations, and their interactions, to warming. The experiment utilised large scale replicated mesocosms systems which incorporated realistic nutrient loadings. Results showed a detrimental effect of warming on already nutrient loaded systems with the loss of fish populations and reduction in some macroinvertebrate taxa.

### 7.1.1 Impacts on fish

Heating is thought to impact on fish populations through effects on physiology, behaviour, reproduction and trophic interactions (Rijnsdorp et. al., 2009). Although it was not possible to examine all aspects of the sticklebacks' response to increasing temperature, there was evidence that increasing temperature resulted in increased mortality associated with a reduction in other resources, in this case, oxygen (Chapter 2). There was no statistically significant effect of warming on breeding or successful reproduction. There was no significant evidence that sticklebacks were more prone to the impacts of warming at the extremes (i.e. summer) though oxygen did vary significantly with season (Chapter 2). Sticklebacks were used in this experiment as they are hardy native species that can withstand low oxygen and high temperatures. We expected changes in populations due to warming but the complete loss of populations from heated nutrient rich mesocosms was quite unexpected: it suggests that warming will exacerbate eutrophication. Sticklebacks are of low economic value in themselves, being taken occasionally in Scandinavia for fishmeal and oil (www.Fishbase.org). However, their importance as a functional cog in an ecosystem cannot be overlooked. They provide food for piscivores such as Perch (*Perca fluviatilis*) which is a popular recreational species and is also eaten in some parts of the world. They are important predators in their own right and

have been shown to reduce macroinvertebrate (Chapter 4) and zooplankton (Jakobsen *et. al.*, 2004) abundance. Previous studies have shown that predation by fish can have a cascading effect on the ecosystem (Jones & Sayer, 2003; Moss *et. al.*, 2003). By reducing numbers of algal grazing zooplankton, they can induce turbid states in lakes with associated loss of macrophytes. Removal of planktivorous fish has been shown to cause a switch to the clear water phase (Leah *et. al.* 1980; Shapiro & Wright, 1984; Potthoff *et. al.*, 2008; Tatrai *et. al.*, 2009) and therefore, loss of fish populations such as sticklebacks with warming may help to buffer warming exacerbated eutrophic effects by producing conditions that favour the growth of oxygenating macrophytes.

### 7.1.2 Impacts on macroinvertebrates

At present, there is only a small amount of literature available on freshwater macroinvertebrate responses to climate change. In this study, warming was more important than nutrient addition or fish predation in altering macroinvertebrate populations. Although temperature effects varied seasonally, there was not the expected increased impact of warming on populations at the extreme of summer. The results highlight the sheer diversity of responses across the various taxa, and the pressing need for more research. On a broad scale, the study attempted to examine how several important functional feeding groups were affected by warming. Warming caused the biomass and abundance of detritivores to increase and of predatory taxa to decrease. Such broad responses will inevitably have consequences for ecosystem functioning. As with the sticklebacks, the impact of temperature on macroinvertebrates was associated particularly with a reduction in dissolved oxygen. This impact was not universal and was seen mainly in active species whose metabolic demand for oxygen is higher. Some more sedentary, low-oxygen tolerant taxa such as the oligochaetes showed an increase in biomass with warming. There were

unexplained responses in gastropods, which showed a decrease in total biomass, and predatory odonates which increased with warming. When considering the sheer diversity of responses and complexity of interactions the extent to which warming induced changes will impact on ecosystems are extremely difficult to predict.

### 7.1.3 Impacts on interactions

Climate change can alter trophic interactions in a number of ways. Changes to population sizes at one trophic level will impact on the next. For example, in this study the loss of stickleback populations in heated mesocosms led to an increase in amphipod and isopod biomass. Warming-induced shifts in phenology will also affect how organisms at different trophic levels interact. Shifts in zooplankton peak production have already been shown in this (Feuchtmayr et. al., unpublished data) and other studies (Scheffer et. al., 2001; De Senerpont Domis, Mooij et al. 2007). A similar shift in breeding season was not seen in stickleback populations (Chapter 2), leading to a mismatch between predator and prey reproductive peaks. Chironomids possibly emerged earlier in this study and have been shown to emerge earlier in other studies giving rise to a potential mismatch between airborne dipterans and avian predators. Warming can also act directly upon the trophic relationship, for example, by altering the interaction between predator and prey. Part of this study attempted to examine the predatory response of sticklebacks to four invertebrate prey types at different summer temperatures. Although laboratory predictions showed only slight changes in predatory response of sticklebacks to invertebrates, even small changes can lead to alterations in optimal diet. However, predicted shifts in optimal diet could not be observed in this study due to prey populations being below critical switch densities. This does not diminish the importance of such changes as shifts in optimal diet of ectothermic predators will have

consequences for the rest of the ecosystem. In this case, the predicted shift

was from preference for *Gammarus pulex* to *Cloeon dipterum* with warming by

4°C. Theoretically we would expect that with climate change sticklebacks would

consume more mayfly and fewer amphipods. The multitude of interactions

between these prey and their ecosystem are complex and therefore the

consequences of such alterations in predator preference are difficult to predict.

However, it may be that predator-prey interactions are robust to climate change

thus reducing the impact on ecosystems.

### 7.1.4 Applicability and future work

The impacts of climate change are already visible in many ecosystems with

changes in phenology, loss of species at range edges and changes in

population dynamics. Future work on climate impacts, especially in aquatic

systems, needs to incorporate, as in this experiment, realistic environmental

variables such as nutrients, although to perform such experiments on large-

scale replicated basis is costly. However, even in such expensive set ups the

applicability of any results to the 'real world' must still be considered.

Our mesocosms were relatively small, contained ecosystems (with a surface are

of 3.14m²) and could never wholly represent large shallow lakes which are

dynamically interacting with the surrounding land. Varying land and recreational

use mean that in real systems nutrient inputs and levels of physical disturbance

are constantly changing and this may alter the impact climate change will have.

The relatively small populations contained in the mesocosms were also more

vulnerable to stochastic events than would necessarily be true in larger systems.

For example, a major result of our study was that decreased oxygen levels with

heating and nutrient addition led to a reduction in stickleback and invertebrate

populations. However, in real lake systems there is likely to be pockets of more

favourable conditions where populations can better persist. Also, the use of a single fish species such as sticklebacks cannot indicate the outcome for all other British fish species. It does however suggest that species less tolerant to extremes of oxygen and temperature will be negatively impacted by the conditions that warming already eutrophic waters will produce.

A previous mesocosm experiment (McKee *et. al.* 2003) did not find strong effects of temperature and nutrients on the fish and invertebrate populations. A likely cause of the differences seen in this study was the use of higher, more realistic nutrient loadings as well as the addition of organic matter to the initial sediment. Although the increased organic content of the sediment resulted in initial poor conditions that may have caused a divergence between the two experiments, the resulting sediment at the end of the experiment was similar in organic content to natural lake sediment. However, the lengthy settling period of the mesocosms resulted in a reduced period of data recording (one year instead of two) which may have also resulted in some differences. Despite differences in major findings, a common conclusion from both experiments was that warming will exacerbate the eutrophic conditions already present in our nutrient rich lakes. It can do this through mechanisms such as alterations to water chemistry (e.g. increased phosphorus concentrations; McKee *et. al.* 2003), increased floating plant cover resulting in reduced light penetration for submerged photosynthesising macroophytes or by increasing the respiration of heterotrophs above and beyond that of autotrophs. Whatever the mechanism, the message is clear. Reducing the nutrient input into our lakes will potentially alleviate some of the future impacts of climate change.

A possible consideration for future studies is that they should focus on mid-range temperatures as previous studies have found the greatest changes in

things such as predator-prey interactions occur here (Thompson, 1978) rather than at the seasonal extremes (i.e. summer) as expected. This study also concentrated on the effects of temperature on broad changes in functional invertebrate groups rather than individual species. It was hoped that by doing this, important changes in invertebrate groups that held broadly similar positions in the ecosystem (e.g. detritivores) would be detected and that these broad changes would be more important than detecting alterations in individual species. However, it is always useful to have species level information where possible and a future improvement to this study would be an increase the taxonomic resolution.

## 7.2 Conclusions

Climate change is a very real phenomenon and likely to occur at a rapid rate over the next century. Impacts of warming in this study were severe in the presence of nutrients, and also complex when considering the vast array of interactions in any given ecosystem. The impacts of climate change are likely to be particularly severe in aquatic ecosystems such as small ponds and shallow lakes where thermal refuge or range shifts are not possible for the resident organisms. Along with moves to reduce carbon emissions, steps need to be taken to reduce nutrient loadings of aquatic systems in order to reduce the impact of future warming.

# Appendix

# Appendix 1

# Global warming and eutrophication: effects on water chemistry and autotrophic communities in experimental hypertrophic shallow lake mesocosms

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## Summary

1. Shallow lakes are important components of the biosphere, but they are also highly vulnerable to damage from human activities in their catchments, such as nutrient pollution. They may also be particularly vulnerable to current warming trends.

2. Forty-eight tanks were used to create 3-m³ mesocosms of shallow lake communities, in which the effects of warming by 4 °C and regular nutrient loading at two levels relevant to current degrees of eutrophication were studied in the presence and absence of fish.

3. Warming changed concentrations of soluble phosphate, total nitrogen and conductivity, increased total plant biomass and decreased the amount of phytoplankton through shading by floating plants. Nutrient additions decreased total plant biomass but increased floating plant biomass. Nitrogen increase and warming increased floating plant biomass and decreased plant species richness. The plant community remained intact and did not switch to the turbid-water, phytoplankton-dominated community often predicted to be a consequence of global warming and eutrophication.

4. *Synthesis and applications.* Likely future temperature increase will exacerbate some, but not all symptoms of eutrophication in shallow lakes. Alone it will not cause a switch from plant-dominated to algal-dominated systems, but may result in nuisance growths of floating lemnids. Currently underplayed, nitrogen loading should be taken more seriously in the management of European freshwaters.

**Key words:** macrophytes, nitrogen, climate change, shallow lake, floating plants, temperature, *Lemna*

## Introduction

Shallow lakes and wetlands dominate inland waters (Downing *et al.* 2006), and are particularly important in carbon storage (Downing *et al.* 2008). They are also prone to environmental change because of their large surface-to-volume ratios, and to switch between states of high biodiversity and plant domination to waters made turbid by suspended sediment or phytoplankton (Scheffer *et al.* 1993). Such switches are influenced by nutrient loading, but driven by deliberate weed cutting, overgrazing by birds or cyprinid fish, herbicide and other biocide run-off, change in water level or salinity (Moss, Madgwick & Phillips 1996). Water level and salinity may be indirectly affected by

climate, but there are suggestions that direct effects of increasing temperature may also act as a driver (Mooij *et al.* 2005).

Global temperatures are rising, bringing with them changes in seasonality and precipitation (IPCC 2007). Current predictions are that temperatures over the coming century may rise up to 10 °C with uncertainty dependent on what mitigation measures are taken and how rapidly these are adopted (IPCC 2007). Current policy assumes that a rise of 2–5 °C is likely (IPCC 2007; Monbiot 2007).

Of several approaches for predicting ecological effects of such change, experimentation at the ecosystem level is the most direct, yet rarest because of its expense. Two such experiments have hitherto been conducted in controlled, heated mesocosms: in the UK (McKee *et al.* 2000, 2003; Feuchtmayr *et al.* 2007), and in Denmark (Liboriussen *et al.* 2005). The UK experiment

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found that a 3 °C temperature increase accelerated flowering of *Potamogeton natans* and increased the predominance of *Lagarosiphon major*, a South African exotic. Macrophyte dominance was maintained even with increased nutrient loading and warming had comparatively little effect on phytoplankton composition or biomass (Moss *et al.* 2003). The additional nutrient treatment was modest, however, compared with the current state of many shallow lakes. The Danish experiment is still underway.

Meanwhile, comparisons across latitudinal gradients suggest substantial change in shallow lakes as temperatures increase. Warmer lakes have longer growing seasons and greater diversities of highly fecund fish that exert increased predation, reduce zooplankton grazing and lead to greater phytoplankton crops and development of floating plant communities (Gyllström *et al.* 2005; Jeppesen *et al.* 2005; Meerhoff *et al.* 2007). The influences of temperature as such, and biogeographical history, however, cannot easily be separated in such comparisons. Experimental studies in lakes across Europe, using parallel mesocosm systems that eliminated much of the biogeographical effect also showed a stronger tendency for phytoplankton dominance with decreasing latitude (Moss *et al.* 2004) owing to greater fish predation on zooplankton grazer communities. Nonetheless, there is still much uncertainty as to how future temperatures will influence shallow lake structure and function. The continuation of many other pressures adds further complications.

Eutrophication, for example, remains a major problem for both lakes and society. Increasingly both phosphorus and nitrogen are implicated (Elser *et al.* 2007), not predominantly just phosphorus, as was widely believed in the past. In summer, abundant phosphorus release from sediment stores, and uptake and denitrification of available nitrogen compounds may lead to nitrogen scarcity. Increased nitrate loading leads to reduced diversity of submerged plant communities (James *et al.* 2005; Barker *et al.* 2008). Submerged plants derive much of their nutrient supply from sediments (Barko, Gunnison & Carpenter 1991), but their growth is inhibited by availability of light (Falkowski & Raven 2007) that may be denied by growth of phytoplankton, periphyton and floating plants, all of which must take their nutrients from the water. Floating plant communities appear to be favoured by increased nutrient loading (Scheffer *et al.* 2003). Adding to the problem of eutrophication. Adding to the problem of eutrophication whose interactions with climate warming are still poorly understood, rising temperatures will influence many aspects of nutrient processing within the systems, as well as change loading rates through its influences on hydrology.

Changes in fish communities could also complicate the direct effects of temperature increase. Fish act through predation on grazer communities of zooplankton and periphyton feeders (Moss *et al.* 1996) and may be particularly vulnerable to warming both through their limited thermal tolerance and through indirect effects of oxygen concentrations (Alabaster & Lloyd 1982). Warming may result in fish deaths or declines and changes in predation levels. Eutrophication

exacerbates such consequences by providing greater biomasses of many organisms that increase respiration rates and oxygen consumption in the vulnerable hours of the night (Bronmark & Weisner 1992). A loss of fish might result in increased grazer invertebrate communities, and hence, lower phytoplankton crops and greater macrophyte biomass (Gyllström *et al.* 2005; Meerhoff *et al.* 2007).

The literature on temperature and nutrient effects on shallow lake communities thus suggests several future scenarios. Here we performed an experiment designed to simulate new predictions of likely future temperature in conditions that closely reflect current states of eutrophication, in contrast to our first experiment with lower nutrient concentrations and lower temperature predictions. We used 48 temperature-controlled 3-m³ mesocosms factorially divided into two thermal regimes (ambient and + 4 °C), presence or absence of fish, and three nutrient-loading regimes, in hypertrophic systems. Our hypotheses were that (i) warming would increase phytoplankton or floating plant growth, particularly with increased nitrogen loading, with an alternative that warming would disfavour phytoplankton growth through increased grazing following detrimental effects on fish populations, and (ii) warming would increase or maintain submerged plant growth where loadings were not increased.

## Methods

### MESOCOSM ESTABLISHMENT

Our tank system was at Ness Gardens in north-western England (53°16′ N, 3°03′ W) and included 48 tanks, each 1 m deep and 2 m in diameter, sunk in the ground to about 20 cm of the rim (see McKee *et al.* 2000, 2003). Heating and control treatments were applied in a randomized block design with four blocks. Plastic-pipe heating elements were placed on the sediment either as a 'dummy' in 24 unheated tanks or connected to the heating system in 24 heated tanks. In the latter, water at 45 °C was pumped through submerged pipes to increase the tank temperature, which was measured every 15 min throughout the experiment by sensors at depths of 45 cm, whilst a control system ensured a 4 °C higher water temperature in heated tanks compared with the nearest unheated tank (McKee *et al.* 2000). Heated tanks thus followed the same daily and seasonal temperature cycles of the ambient temperature tanks, plus 4 °C.

In October 2005 each tank received a 20-cm deep sediment mixture containing (by volume) 50% garden loam and 50% organic material (47·5% chopped organic oat straw and 2·5% rotted organic cow manure) to simulate the sediment of a eutrophic pond. Three cubic metres of local borehole water were then added. Heating began in October 2005 and three nutrient levels were imposed in a randomized block design from January 2006, with a standard load every 2 weeks. Sixteen tanks received 2500 µg L⁻¹ N and 50 µg L⁻¹ P (high addition, N2), 16 tanks received 250 µg L⁻¹ N and 50 µg L⁻¹ P (low addition, N1) using NaNO₃ and KH₂PO₄, and 16 tanks did not receive nutrient additions (N0).

Algal and animal communities were established from well-mixed inocula from five local lakes and ponds, added to the mesocosms before January 2006. Two and three weeks after inoculation, organisms were cross-mixed between mesocosms via standardized sweep-net samples to ensure similar starting conditions. As part of the randomized

block design, four sticklebacks *Gasterosteus aculeatus* L. of both genders were added to half of the mesocosms in May 2006. These populations were supplemented by additional fish to an average biomass of 13·45 g per mesocosm in July 2006.

Aquatic plants established naturally during early summer of 2006 from seeds and fragments introduced with the animal and algal inocula. Communities were kept from full growth by cutting until July 2006 when checks were made that all species were present in each tank. Omissions were rectified by deliberate additions and the communities then allowed to establish unhindered. *Elodea nuttallii, Lemna trisulca, Ceratophyllum demersum, Lemna minor, Lemna minuta, Potamogeton berchtoldii, P. crispus* and *P. natans* were present. *Spirodela polyrhiza* appeared during 2007. Nomenclature follows Stace (1997).

All mesocosms were allowed to settle until January 2007 when sampling and monitoring of the macrophyte community began. During this period, evaporation losses from the mesocosms were replaced with de-ionized water when not compensated for by rainfall.

## MONITORING

Water samples were taken for analysis prior to nutrient dosing every 2 weeks, using a tube that integrated the water column. We used Johnes & Heathwaite (1992) for total nitrogen (TN) and total phosphorus (TP) and Mackereth, Heron & Talling (1989) for soluble reactive phosphorus (SRP), nitrate ($NO_3$-N), and ammonium ($NH_4$-N). Conductivity and pH were measured electronically. Chlorophyll-a, an estimate of phytoplankton biomass, was measured by a submersible fluorometer (Cyclops-7, Turner Designs). Oxygen was measured at mid-depth (35 cm) with a YSI Model 85 probe between 10·30 h and 12·30 h. The phytoplankton community was qualitatively examined to genus level.

Fish were monitored monthly by standardized trapping, using non-return plastic bottle traps over 24 hr. Fish were weighed fresh and returned to the mesocosms. If fish had died, new additions were made to maintain the fish treatments until summer 2006. No further additions were made during the period described in this study but numbers and biomass were monitored. Submerged and floating plants and filamentous algae were recorded every 2–3 weeks during 2007 as proportion of the water volume infested (PVI). This monitoring was performed by the same person to ensure comparability and a submersible glass-bottomed box was used to help visibility.

During the final harvest in September 2007, plants and filamentous algae were manually removed, hand-sorted, placed in mesh bags, and spun-dry at the highest setting in a domestic spin drier for a standard time of 5 min. Fresh weight was then determined. Sub-samples were retained for dry weight determination following drying in a laboratory oven at 80 °C. Dry weight contents of different species were sufficiently close (±5%), however, for fresh weights to be used in subsequent calculations. After water had been pumped out of the mesocosms, three random samples of surface sediment were taken for drying at 80 °C; loss on ignition was determined by burning at 350 °C and TN and TP were determined by acid digestion and methods as above.

## STATISTICS

Results were tested for normality (Kolmogorov–Smirnov test) and transformed to log($n$ + 1) where necessary. They were analysed by repeated measures analysis of variance (ANOVA), done in two ways
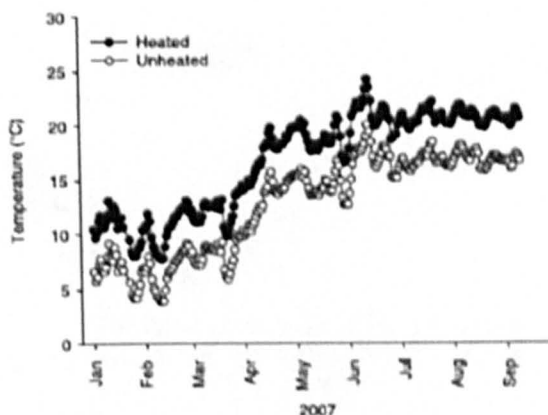


Fig. 1. Changes in noon mean water temperature between January and September 2007 in 24 mesocosms warmed by 4 °C above ambient and 24 unheated mesocosms.

for each variable: first with block as a covariate and Bonferroni tests applied, and secondly, with block removed and Tukey tests used for between-subjects factors. Where these differed, the most conservative result has been taken. Proportional values were arcsine-transformed. Repeated measures ANOVA requires that data have homogeneity of covariance, which was determined by Mauchly's test of sphericity, followed by Greenhouse–Geisser tests if the Mauchly test was not significant. Time was universally significant but block never so. Where the test showed no overall significance of treatment but there was a distinctive seasonal pattern, data were re-analysed on a date-by-date basis using one-way ANOVA. Data obtained from the final harvest were subjected to three-way ANOVA. Linear and non-linear regressions were used to investigate the shape of seasonal trends in species richness and Shannon–Weaver diversity. SPSS 15 was the main statistical package used.

## Results

The differential temperature in the experimental mesocosms was maintained as intended (Fig. 1) within ±0·2 °C. Noon temperatures reached 21 °C in unheated mesocosms and 25 °C in heated ones and did not drop below about 3 °C in any mesocosm.

Oxygen concentrations measured at a standard time in the morning were 39% lower on average in heated mesocosms (Table 1), which was mainly due to biological effects, (accounting for at least 34%), rather than to physical effects on solubility. Nutrients and fish had no significant effects on oxygen levels, but towards the end of the experiment, oxygen levels in heated and high nutrient mesocosms were very low and fish struggled to survive (Table 1, and R. J. Moran, B. Moss, H. Feuchtmayr, K. Hatton, T. Heyes and D. Atkinson, unpublished data).

SRP increased significantly in heated mesocosms (Fig. 2; Table 1). Overall SRP was not affected by fish or nutrient treatments but began to show significant decreases with fish and increases with nutrient level as the summer progressed (Fig. 2; ANOVA for individual dates) although external

Fig. 2. Changes in mean SRP concentrations between January and September 2007 in 48 mesocosms subjected to different temperature (ambient + 4 °C, ambient), nutrient addition (N0, N1, N2, see Methods) and fish (without, with) treatments. Asterisks (*) indicate significantly different results ($P < 0.05$ from ANOVAs for individual dates) for warming and predation treatments. For nutrient additions, significant differences are denoted by 02 between N0 and N2; 01 between N0 and N1; and 12 between N1 and N2.

Fig. 3. Changes in mean TP concentrations between January and September 2007 in 48 mesocosms subjected to different temperature (ambient + 4 °C, ambient), nutrient addition (N0, N1, N2, see Methods) and fish (without, with) treatments. Abbreviations are as in Fig. 2.

phosphorus loading had been kept constant. Concentrations of SRP were substantial in summer (overall means from 166 to 204 µg L$^{-1}$), suggesting major release from the sediments. TP (Fig. 3, Table 1) showed no significant difference on

warming, but increased significantly with fish, especially in the heated mesocosms (H × F interaction, Table 1). TP concentrations in the N1 treatment did not differ from those where no nutrients were added but the higher addition (N2)

Fig. 5. Changes in mean TN and NH₄-N concentrations between January and September 2007 in 48 mesocosms subjected to different nutrient addition (N0, N1, N2, see Methods) treatments. Abbreviations are as in Fig. 2.



Fig. 4. Changes in mean NO₃-N concentrations between January and September 2007 in 48 mesocosms subjected to different temperature (ambient + 4 °C, ambient), nutrient addition (N0, N1, N2, see Methods) and fish (without, with) treatments. Abbreviations as in Fig. 2.

showed significant increases (ANOVA for individual dates) from around 100 μg L⁻¹ in January to 500–600 μg L⁻¹ in late summer.

Nitrate additions (Fig. 4; Table 1) were rapidly metabolized with only trace concentrations detectable 2 weeks after

addition (Fig. 4; Table 1). Fish decreased nitrate concentrations in winter and in spring. Ammonium concentrations also increased in the N2 treatment as did TN concentrations (Fig. 5, Table 1), which were seasonally much less variable than those of ammonium and nitrate. Fish alone had no effect on ammonium and TN concentrations, and effects of heating depended mainly on nutrient and fish treatments (H × N, H × F, and H × N × F interactions, Table 1). Effects of treatments on mean pH were slight (Table 1), but conductivity decreased with time, aided by rainwater dilution, except in N2 mesocosms where it was greater than in N1 and N0, because of the increased salt addition. The significantly higher mean conductivity in heated mesocosms (Table 1) was not due to evaporative concentration because water levels were maintained by adding de-ionized water. Conductivity (Table 1) was typical of waters drawn from sedimentary rocks, as expected from the borehole source used.

Treatments caused no significant differences in sediment depth, sedimentary loss on ignition, or TN and TP concentrations at final harvest (means ± SD and ANOVA tests; for sediment depth 17·63 ± 2·15 cm, P > 0·29; for loss on ignition

Table 1. Mean effects of heating (H), nutrient (N) and fish treatments (F) on water chemistry and fish biomass in 48 mesocosms between January and September 2007 ($N = 16$ to 18 sampling dates for water chemistry, $N = 9$ for fish biomass). Abbreviations used: no addition, N0; low addition N1; high addition, N2; no fish, −F; fish present, +F; not significant, ns. Wherever results were significant, $P$ values are indicated as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $P$ values ≤ 0.08 are also given; n.a., not applicable

| | Mean value | | | | | | | Probability | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unheated | H | −F | +F | N0 | N1 | N2 | H | F | N | HF | HN | NF | HNF |
| Oxygen concentration (mg L⁻¹) | 6·6 | 4·0 | 5·7 | 4·9 | 5·9 | 5·1 | 4·9 | *** | ns | ns | ns | ns | ns | ns |
| Oxygen saturation (%) | 59·9 | 39·4 | 53·8 | 45·4 | 56·1 | 47·4 | 45·4 | *** | 0·07 | ns | ns | ns | ns | ns |
| SRP (µg L⁻¹) | 92 | 158 | 148 | 102 | 94 | 113 | 168 | * | ns | ns | ns | ** | ns | ns |
| TP (µg L⁻¹) | 352 | 381 | 298 | 435 | 353 | 329 | 418 | ns | * | ns | * | ns | ns | ns |
| Nitrate-N (mg L⁻¹) | 0·31 | 0·22 | 0·39 | 0·14 | 0·01 | 0·02 | 0·77 | ns | *** | ** | 0·06 | ns | ** | * |
| Ammonium-N (mg L⁻¹) | 0·13 | 0·09 | 0·09 | 0·13 | 0·04 | 0·05 | 0·23 | ns | ns | *** | * | ** | ns | * |
| TN (mg L⁻¹) | 2·6 | 2·2 | 2·3 | 2·5 | 1·7 | 1·8 | 3·7 | * | ns | *** | * | ** | ns | ns |
| pH | 7·38 | 7·46 | 7·44 | 7·40 | 7·44 | 7·35 | 7·47 | ** | ns | ns | ns | ns | ns | ns |
| Conductivity (µS cm⁻²) | 471 | 572 | 519 | 523 | 432 | 459 | 672 | *** | ns | *** | 0·06 | ns | ns | ns |
| Chlorophyll a (µg L⁻¹) | 66·3 | 20·8 | 18·7 | 68·5 | 50·9 | 32·1 | 47·8 | *** | *** | ns | *** | ns | 0·07 | ns |
| Fish biomass (g per tank) | 5·3 | 2·4 | n.a. | n.a. | 6·1 | 2·7 | 2·8 | *** | n.a. | *** | n.a. | ns | n.a. | n.a. |

Table 2. Effects of heating, fish and nutrient treatments on the regularly measured PVI of aquatic macrophytes in 48 mesocosms between January and September 2007. Mean values of PVI are given as fractions. There were no significant three-way (H × N × F) effects or block effects. For abbreviations used, see Table 1

| | Mean PVI (as proportion) | | | | | | | Probability | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unheated | H | −F | +F | N0 | N1 | N2 | H | F | N | HF | HN | NF |
| Total PVI | 0·35 | 0·45 | 0·39 | 0·40 | 0·50 | 0·43 | 0·26 | 0·06 | ns | ** | ns | ns | ns |
| Filamentous algae | 0·02 | 0·05 | 0·01 | 0·06 | 0·07 | 0·03 | 0·01 | * | ** | * | ns | ns | ** |
| *Ceratophyllum demersum* | 0·05 | 0·17 | 0·08 | 0·13 | 0·1 | 0·13 | 0·08 | *** | * | ns | ns | ns | ns |
| *Elodea nuttalli* | 0·18 | 0·14 | 0·18 | 0·13 | 0·23 | 0·15 | 0·09 | ns | ns | ns | ns | ns | ns |
| *Potamogeton crispus* | 0·05 | 0·06 | 0·08 | 0·03 | 0·08 | 0·07 | 0·01 | ns | * | * | ns | ns | ns |
| *P. natans* | 0·002 | <0·0001 | 0·002 | <0·001 | <0·001 | <0·001 | 0·003 | ns | ns | ns | ns | ns | ns |
| *P. berchtoldii* | 0·002 | 0·001 | 0·003 | <0·0001 | 0·004 | 0·001 | <0·0001 | ns | ns | ns | ns | ns | ns |
| *Lemna trisulca* | 0·04 | 0·01 | 0·03 | 0·02 | 0·02 | 0·03 | 0·02 | *** | * | ns | ns | 0·07 | ns |
| *L. minor/minuta* | 0·01 | 0·03 | 0·02 | 0·02 | 0·01 | 0·02 | 0·03 | *** | ns | * | ns | ns | ns |
| *Spirodela polyrhiza* | <0·0001 | 0·001 | 0·001 | <0·00001 | <0·0001 | <0·0001 | 0·001 | ns | ns | ns | ns | ns | ns |
| *Lemna* spp. cover (%) | 14 | 47 | 27 | 34 | 18 | 28 | 45 | *** | ns | ** | ns | ns | ns |

8·0 ± 1·75%, $P > 0.14$; for TN 0·83 ± 0·12 mg g⁻¹, $P > 0.08$; for TP 0·21 ± 0·04 mg g⁻¹, $P > 0.19$). Depth had decreased by 2–3 cm from the depth initially established. Loss on ignition was small (7–8%). TN and TP concentrations were well within natural ranges.

Phytoplankton biomass (Table 1; Fig. 6) was low in heated mesocosms but increased significantly in unheated ones. It was also much higher in mesocosms with fish, especially in those that were heated, but showed no systematic pattern in relation to nutrient additions. The phytoplankton communities were largely of cryptomonads and small Chlorococcales. Cyanobacteria were scarce.

Heating was associated with a trend towards increased total PVI (significant at the 6% level) (Table 2; Fig. 7), particularly through an increase in surface lemnids (predominantly *Lemna minor*), *Ceratophyllum demersum*, and filamentous algae (*Spirogyra, Ulothrix, Cladophora*). Some *Lemna minuta* was also present but could not readily be distinguished from *L. minor*. Heating increased the PVI of *Spirodela polyrhiza*,

but although the effects were very clear in mesocosms where this species occurred, the overall effect was not statistically significant since the plant came in as a late casual and did not appear in all replicates. Heating decreased the PVI of *Lemna trisulca*. Fish had relatively small effects, increasing filamentous algae and *C. demersum*, but decreasing *L. trisulca* and *Potamogeton crispus*. Nutrients had greater effects, decreasing total PVI from N0 to N2 through an effect on filamentous algae and *P. crispus*, while increasing the PVI of surface lemnids (*L. minor/minuta*).

Results from PVI were confirmed by those obtained from final biomass harvesting (Fig. 8; Table 3). Plant crops were substantial with individual mesocosms producing up to 4 kg fresh weight m⁻². Dry weight averaged 11·1 ± 1·7% of fresh weight. Heating increased total biomass slightly through its greater than eightfold increase in floating lemnids (Fig. 8), and more than doubling of *Ceratophyllum demersum*. However, among the submerged vascular plants, this increase in *Ceratophyllum demersum* contrasted with a nearly sixfold decrease in *L. trisulca*.
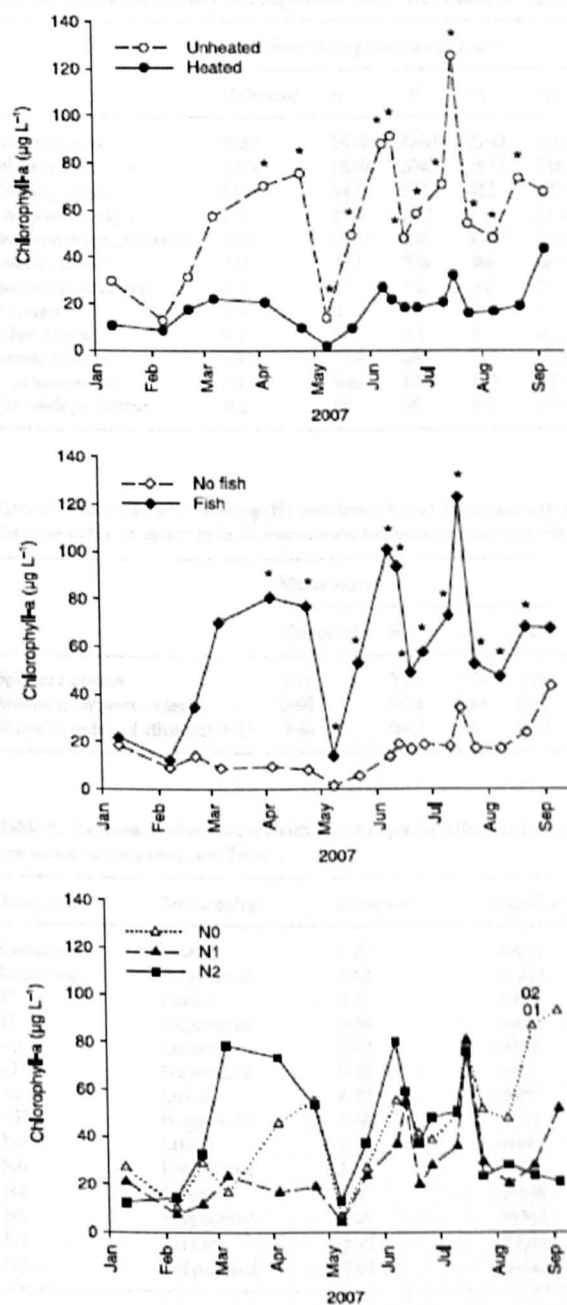
Fig. 6. Changes in phytoplankton biomass (mean chlorophyll a) between January and September 2007 in 48 mesocosms subjected to different temperature (ambient + 4 °C, ambient), nutrient addition (N0, N1, N2, see Methods) and fish (without, with) treatments. Abbreviations are as in Fig. 2.
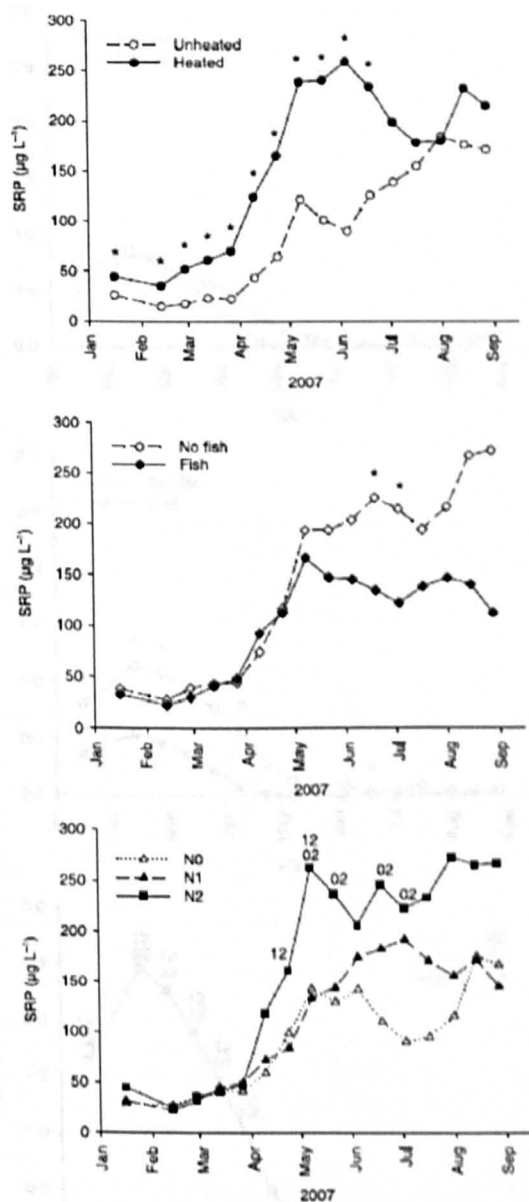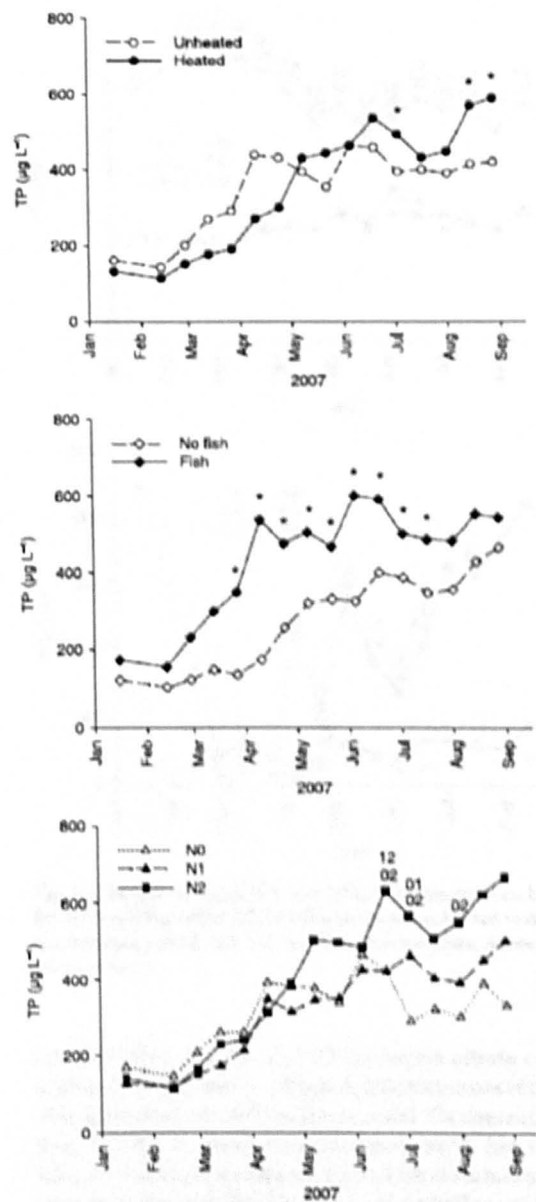
Fig. 7. Changes in mean total macrophyte PVI between January and September 2007 in 48 mesocosms subjected to different temperature (ambient + 4 °C, ambient), nutrient addition (N0, N1, N2, see Methods) and fish (without, with) treatments. Abbreviations are as in Fig. 2.

Fish increased the abundance of floating lemnids approximately threefold but were otherwise of small influence, whilst nutrients decreased total biomass slightly despite a more-than-threefold increase in floating biomass.

Heating significantly increased Shannon–Weaver diversity and the Simpson index of diversity of the macrophyte

community, but decreased species richness (Table 4). Fish affected none of these. Nutrients increased species richness and Shannon–Weaver diversity between N0 and N1, then substantially decreased them between N1 and N2 (Table 4). There were also seasonal patterns in the change in species richness (Table 5) and Shannon–Weaver diversity (not shown).

**Table 3.** Effects of heating, fish and nutrient treatments on the final fresh-weight harvest of aquatic macrophytes (g fresh weight m$^{-2}$) in 48 mesocosms between January and September 2007. There were no significant block effects. For abbreviations used, see Table 1

| | Mean biomass (g fresh weight m$^{-2}$) | | | | | | | Probability | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Unheated | H | −F | +F | N0 | N1 | N2 | H | F | N | HF | HN | NF | HNF |
| Total biomass | 2083 | 2430 | 2260 | 2260 | 2480 | 2450 | 1835 | *** | ns | ** | ns | ns | ns | ns |
| Submerged plants | 2028 | 1880 | 2040 | 1875 | 2351 | 2163 | 1355 | ns | ns | 0·08 | ns | ns | ns | ns |
| Floating plants | 56 | 547 | 220 | 382 | 132 | 291 | 480 | *** | ns | * | ns | ns | * | * |
| Filamentous algae | 3·3 | 25·8 | 25·7 | 3·5 | 21·1 | 8·7 | 13·9 | ns | ns | ns | * | ns | ns | ns |
| *Ceratophyllum demersum* | 630 | 1390 | 849 | 1170 | 1120 | 1257 | 652 | * | ns | ns | ns | ns | ns | ns |
| *Elodea nuttalli* | 735 | 357 | 706 | 386 | 881 | 435 | 321 | ns | ns | ns | ns | ns | ns | ns |
| *Potamogeton crispus* | 7·1 | 0·1 | 7·0 | 0·2 | 5·7 | 4·9 | 0·2 | ns | ns | ns | ns | ns | ns | ns |
| *P. natans* | 1·3 | 0 | 1·3 | 0 | 0 | 0 | 0 | ns | ns | ns | ns | ns | ns | ns |
| *P. berchtoldii* | 0·1 | 0 | 0·1 | 0 | 0·2 | 0 | 0 | ns | ns | 0·06 | ns | 0·06 | 0·06 | 0·06 |
| *Lemna trisulca* | 650 | 114 | 450 | 314 | 323 | 457 | 367 | *** | ns | ns | ns | ** | ns | ns |
| *L. minor/minuta* | 55 | 449 | 122 | 382 | 131 | 291 | 332 | *** | * | ns | * | ns | ns | ns |
| *Spirodela polyrhiza* | 0·1 | 99 | 99 | 0·2 | 0·2 | 0·1 | 148 | ns | ns | 0·07 | ns | 0·07 | 0·07 | 0·07 |

**Table 4.** Mean effects of heating (H), nutrient (N) and fish treatments (F) on aquatic macrophyte species richness, Shannon-Weaver index and Simpson index of diversity in 48 mesocosms between January and September 2007 (N = 13). For abbreviations used, see Table 1

| | Mean value | | | | | | | Probability | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Unheated | H | −F | +F | N0 | N1 | N2 | H | F | N | HF | HN | NF | HNF |
| Species richness | 3·78 | 3·57 | 3·78 | 3·59 | 3·87 | 3·99 | 3·19 | * | ns | *** | ns | ns | ns | ** |
| Shannon–Weaver index | 0·60 | 0·70 | 0·64 | 0·65 | 0·63 | 0·73 | 0·58 | *** | ns | *** | ns | ** | ns | ** |
| Simpson index of diversity 1-D | 0·34 | 0·42 | 0·4 | 0·37 | 0·36 | 0·43 | 0·35 | *** | ns | ns | ns | ns | ns | * |

**Table 5.** Regression relationships with time for species richness of aquatic macrophytes in 48 mesocosms between January and September 2007. For abbreviations used, see Table 1

| Treatment | Relationship | Constant | Function (x) | Function (x$^2$) | R$^2$ | P | Trend |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Unheated | Linear | 3·70 | 0·011 | | 0·01 | ns | |
| Unheated | Polynomial | 2·92 | 0·324 | −0·022 | 0·57 | ** | Mid-peak |
| H | Linear | 4·17 | −0·086 | | 0·80 | *** | |
| H | Polynomial | 3·98 | 0·012 | −0·005 | 0·84 | *** | Decline |
| −F | Linear | 3·82 | −0·006 | | 0·01 | ns | |
| −F | Polynomial | 3·29 | 0·21 | −0·015 | 0·62 | *** | Mid-peak |
| +F | Linear | 4·07 | −0·07 | | 0·49 | *** | Decline |
| +F | Polynomial | 3·65 | 0·10 | −0·012 | 0·65 | *** | Decline |
| N0 | Linear | 3·97 | −0·013 | | 0·02 | ns | |
| N0 | Polynomial | 3·29 | 0·26 | −0·019 | 0·49 | *** | Mid-peak |
| N1 | Linear | 4·4 | −0·058 | | 0·26 | ns | |
| N1 | Polynomial | 3·48 | 0·307 | −0·026 | 0·83 | *** | Mid-peak |
| N2 | Linear | 3·49 | −0·043 | | 0·43 | ** | Decline |
| N2 | Polynomial | 3·61 | −0·092 | 0·0035 | 0·46 | *** | Decline |

Heating, increase in nutrients and presence of fish all converted seasonal patterns in species richness from curves with mid-season peaks to linear declines with time (Table 5). Trends for Shannon–Weaver diversity were for heating to change a pattern of slight increase to one of decrease with time, and for nutrients to change an increase at N0 to a mid-peak relationship at N1 and then to a flat relationship at N2. Fish changed a seasonal increase in their absence to a flat or mid-peak relationship.

## Discussion

Various and complex significant effects resulted from warming these hypertrophic systems by 4 °C above ambient temperatures. A synthesis is that warming and nutrients increased the growth of plants, but favoured floating species, best able to exploit the combined high availability of light at the water surface and nutrients. Growth of these floating plants may have been enhanced by warming directly, and possibly by
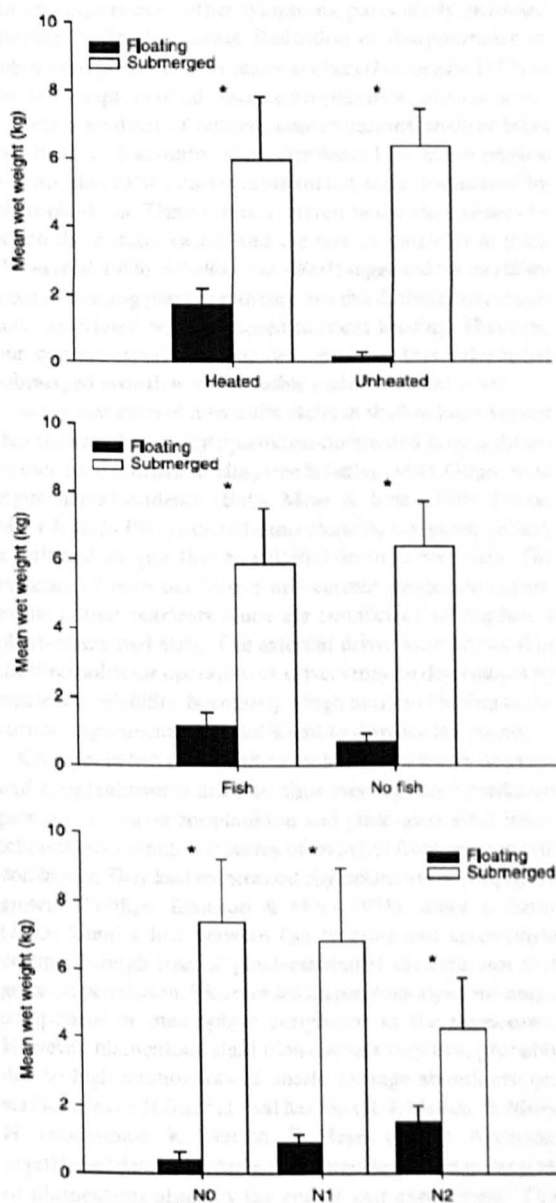
Fig. 8. Effects of warming, fish and nutrient addition on the final fresh macrophyte biomass (mean ±95% confidence limit) harvested from a set of mesocosms in September 2007. Asterisks indicate significant results ($P < 0.05$).

warming-induced SRP release from the sediment. These plants then appeared to have suppressed species just below the surface (*L. trisulca*) and probably phytoplankton. Shade-tolerant species such as *C. demersum*, which can persist under *Lemna* covers (Preston & Croft 1997), fared better under warming. Warming also reduced fish biomass (Table 1, and R. J. Moran, B. Moss, H. Feuchtmayr, K. Hatton, T. Heyes and D. Atkinson, unpublished data), probably through oxygen stress. As zooplankton biomass and the zooplankton : chlorophyll-a ratio were not significantly

higher with warming (H. Feuchtmayr, B. Moss, I. Harvey, R. J. Moran, K. Hatton, L. Connor, D. Spiller and D. Atkinson, unpublished data), it seems unlikely that zooplankton were responsible for the lower phytoplankton biomass. However, the anticipated warming-induced increase in zooplankton per capita grazing rates cannot yet be excluded as an influence on phytoplankton abundance.

Several aspects of the results deserve emphasis. These are the contrasts with a previous experiment in these mesocosms; their links with current understanding of alternative states in shallow lakes; the key role of nitrogen; and their implications for predicting ecosystem state in a warming world.

Our former experiment used deliberately designed plant communities, including *Lagarosiphon major*, *Elodea nuttallii*, and *Potamogeton natans*, a 3 °C rise in temperature above ambient, rather infertile inorganic sediments, and a fertilization regime in which maximum concentrations given were $0.5$ mg N $L^{-1}$ and $0.05$ mg P $L^{-1}$. The N1 treatment in the present experiment used a lesser and the N2 a greater addition, more in line with current loadings in countries with intensive agriculture (James *et al.* 2005). The present sediments were also richer and the plant community was self-determined. In the current experiment, a 4 °C rise over an ambient temperature that was about the same as that in 2000 (McKee *et al.* 2003) (maximum ambient temperature in 1999, 23.7 °C; in 2000, 24.8 °C; in 2007, 24.9 °C; summer mean temperature in 1999, 16.4 °C; in 2000, 15.5 °C; in 2007, 15.5 °C), thus gave slightly warmer conditions.

In the previous experiment, warming did not change phytoplankton biomass, even with increased nutrients and fish. Macrophyte communities remained dominant (McKee *et al.* 2003), as they did in the present experiment, but warming in 2007 led to lower phytoplankton crops, almost certainly because of shading by floating plants. Warming previously increased phosphorus concentrations and conductivity, decreased pH and oxygen saturation and increased the frequency of severe de-oxygenation. Currently, it had similar but more extreme effects; warming increased daytime pH, most probably due to increased photosynthetic withdrawal of $CO_2$. The rise in conductivity with warming probably reflects increased mineral weathering in the sediments. In the previous experiment, total plant abundance remained high and was unaffected by warming but the proportion of a warm-water exotic, *Lagarosiphon major*, increased. Warming had no influence on *E. nuttallii*, as in the present experiment. Effects of nutrient addition and the presence of fish were formerly independent of warming and tended to increase and decrease macrophyte abundance, respectively. This was not the case in the current experiment where warming and nutrients together increased floating lemnid growth, which had been negligible in the previous experiment although *Lemna minor* was present. Comparison of the two experiments thus suggests that symptoms of eutrophication are exacerbated by warming. Collectively, these include de-oxygenation, increased phosphorus owing to release from the sediments, reduced fish biomass, reduced plant species richness and increase in warm-water exotics. One common symptom of eutrophication,

increased phytoplankton biomass, was not shown, owing to the consequences of other symptoms, particularly increased shading by floating plants. Reduction or disappearance of submerged plants, seen by many workers (Hartmann 1977) as *per se* a symptom of advanced eutrophication, did not occur.

Over a gradient of nutrient concentrations, shallow lakes can have a clear-water state dominated by macrophytes, and an alternative undesirable turbid state dominated by phytoplankton. There is much interest in the mechanisms by which these states switch and the role of nutrients in them (Moss *et al.* 1996). Scheffer *et al.* (2003) suggested the establishment of floating plant dominance as a third, alternative stable state associated with increased nutrient loading. However, our current experiment has demonstrated that substantial submerged growth is still possible under a lemnid cover.

Many advocates of alternative states in shallow lakes assume that the switch to a phytoplankton-dominated state is driven by increased nutrient loading (see Scheffer 1998). Others note experimental evidence (Balls, Moss & Irvine 1989; Irvine, Moss & Balls 1989) that nutrients alone do not promote such a shift and suggest that an external driver is necessary. The evidence of both our former and current mesocosm experiments is that nutrients alone are insufficient to displace a plant-dominated state. The external driver view allows that the thresholds for operation of drivers may be determined by nutrient availability, but clearly a high nutrient loading in the current experiment was insufficient to displace the plants.

Changes in fish communities such that piscivores decrease and zooplanktivores increase, thus exerting more predation pressure on grazer zooplankton and plant-associated invertebrates, are common features of switches from macrophyte dominance. They lead to increased phytoplankton or periphyton growth (Phillips, Eminson & Moss 1978). Jones & Sayer (2003) found a link between fish biomass and macrophyte decline through loss of plant-associated invertebrates that graze on periphyton. We recorded filamentous algae, the major component of macrophyte periphyton in the mesocosms. However, filamentous algal biomass was very low, probably due to high abundances of snails: average abundance per surface area of 500 m$^{-2}$ at final harvest (R. J. Moran, B. Moss, H. Feuchtmayr, K. Hatton, T. Heyes and D. Atkinson, unpublished data). Fish had no significant impact on gastropods or filamentous algae by the end of our experiment. This was probably due to an increase in lemnid cover causing a decrease in oxygen levels which had a negative effect on fish biomass (R. J. Moran, B. Moss, H. Feuchtmayr, K. Hatton, T. Heyes and D. Atkinson, unpublished data). Diurnal studies in June/July 2007 showed near anaerobiosis especially in heated tanks by dawn.

Temperature increase has been suggested as a driver for loss of plants and increase in turbid conditions in a review of how global warming will affect shallow lakes (Mooij *et al.* 2005). We concur that there will be severe changes with temperature increase and we are aware that single experiments may give only one of several possible outcomes, dependent on the many combinations of starting conditions. However, for the moment, the experimental data do not suggest a loss of macrophyte dominance through temperature increase alone, even at high nutrient loadings, although a move towards floating plant abundance seems likely in small temperate hypertrophic water bodies, which parallels the preponderance of floating plants in warm temperate and tropical lakes (Meerhoff *et al.* 2007).

Temperature increase and nitrate loading reduced species richness. Currently, there is a reluctance by Government Agencies to apply standards for nitrogen in schemes for assessing ecological quality under the Water Framework Directive, on the grounds of too limited evidence (UKTAG 2007). James *et al.* (2005) produced correlative evidence that nitrate concentrations above about 1 mg NO$_3$-N L$^{-1}$ led to reduced species richness of aquatic plants and Barker *et al.* (2008) confirmed this experimentally. Our current experiment adds more evidence. Official reluctance to declare nitrate standards is misplaced.

## Acknowledgements

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**Appendix 2:** The main effects and interactions of heating (H), fish addition (F), nutrient addition (N) and season (S) on the biomass of gastropods found in the sampling of 48 mesocosms over a period of 12 months (September 2006 – August 2007).

| Treatment | Sweep | | | |
|---|---|---|---|---|
| | L. peregra | L. stagnalis | Physidae | Planorbidae |
| H | <0.001- | 0.053- | <0.001- | 0.042- |
| F | ns | ns | ns | ns |
| N | ns | ns | 0.004 | ns |
| S | <0.001 | ns | <0.001 | 0.001 |
| H * F | ns | ns | ns | ns |
| H * N | ns | ns | 0.024 | ns |
| H * S | ns | ns | ns | ns |
| F * N | ns | ns | ns | ns |
| F * S | ns | ns | ns | ns |
| N * S | ns | ns | ns | ns |
| H * F * N | ns | ns | ns | ns |
| H * F * S | ns | ns | ns | 0.050 |
| H * N * S | ns | ns | ns | ns |
| F * N * S | ns | ns | ns | ns |
| H * F * N * S | ns | ns | ns | ns |

| Treatment | Benthic | | | |
|---|---|---|---|---|
| | L. peregra | L. stagnalis | Physidae | Planorbidae |
| H | <0.001- | ns | ns | ns |
| F | ns | ns | ns | ns |
| N | ns | ns | ns | ns |
| S | 0.001 | ns | 0.002 | ns |
| H * F | ns | ns | ns | ns |
| H * N | ns | ns | ns | ns |
| H * S | <0.001 | ns | 0.044 | ns |
| F * N | ns | ns | 0.038 | 0.002 |
| F * S | ns | ns | ns | ns |
| N * S | ns | ns | ns | 0.047 |
| H * F * N | ns | ns | ns | ns |
| H * F * S | ns | ns | ns | 0.042 |
| H * N * S | ns | ns | ns | ns |
| F * N * S | ns | ns | ns | 0.009 |
| H * F * N * S | ns | ns | ns | ns |

**Appendix 3:** Primary functional feeding groups of the invertebrate groups found in the mesocosms with references. Taxa without references are invertebrates whose main mode of feeding doesn't fit into one of the two main categories or varies greatly with species and were therefore excluded from subsequent analysis.

| Group | Functional Group | Reference |
|---|---|---|
| *Chironomidae* sp. | Detritivore | Anderson & Sedell, 1979 |
| *Culicidae* | Dependent on sp. | |
| Corixid | Dependent on sp. | |
| Isopoda | Detritivore | Bjelke and Herrmann, 2005 |
| Amphipoda | Detritivore | Graça, 2001 |
| Chaoborus | Predator | Pastorok, 1980 |
| Ephemeroptera | Detritivore | Anderson & Sedell, 1979 |
| Coleoptera | Predator | Jäch & Balke, 2008 |
| Notonectidae | Predator | Papaceck, 2001 |
| Platyhelminth | Predator | Tranchida *et. al.*, 2009 |
| Bivalvia | Detritivore | Anderson & Sedell, 1979 |
| Oligochaeta | Detritivore | Anderson & Sedell, 1979 |
| Hirudinea | Predator | Young & Ironmonger 1980 |
| Odonata | Predator | Johnson, 1973 |
| Mites | Dependent on sp. | |
| Gastropoda | Dependent on sp. | |

**Appendix 4:** Effects of temperature, fish and nutrients on the biomass of gastropods in a mesocosm experiment at final harvest. ANOVA was performed on log (n + 1) transformed biomasses. Numbers are P-values. There were no 3-way interactions. Symbol (+/–) denotes the direction of the effect as increase or decrease.

| Gastropoda | H | F | N | H x N | H x F | N x F |
|---|---|---|---|---|---|---|
| *Lymnaea peregra* | <0.001– | ns | ns | ns | ns | ns |
| *Lymnaea stagnalis* | ns | ns | ns | ns | ns | ns |
| Physidae | 0.013– | ns | ns | ns | ns | 0.040 |
| Planorbidae | 0.020– | ns | ns | ns | ns | ns |

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