Acid episodes and the behavioural ecology of Atlantic salmon *Salmo salar* L.

Claire Bale

Thesis submitted for the degree of Doctor of Philosophy

Cardiff School of Biosciences

Cardiff University

September 2007

ID NUMBER	
LAST NAME	Bale
FIRST NAME(S)	Claire Louise

DECLARATION

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

Signed... (candidate) Date ...26 09·07...............

STATEMENT 1

This thesis is being submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Signed... (candidate) Date ..26·09 07...............

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references.

Signed... (candidate) Date ...26·09·07............

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed... (candidate) Date .....26·09·07............

STATEMENT 4 - BAR ON ACCESS APPROVED

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans **after expiry of a bar on access** approved by the Graduate Development Committee.

Signed... (candidate) Date .....26·09·07...........

Acknowledgements

There are many people without whom this would not have been possible – I'm sure you know who you are but I'm going to thank you anyway!

My supervisors, Siân Griffiths and Steve Ormerod for putting me forward for this project, and for your guidance and advice, particularly in times of panic!; Jo Cable, Tracey King, David Pascoe and other users of the aquarium rooms; John, Selwyn and Rob at Cynrig hatchery for your help with all things fishy (most importantly providing the fish!); Sarah Counter for getting up at silly o'clock on many a cold December morning to study salmon by the light of a red lamp (good times!); Jeff Davey for battling against dodgy equipment and even less reliable weather reports to plunge into freezing Welsh hillstreams; James Orpwood, my conference companion and all-round statistics genius; Alex, Loys, Viola and Wendy, the best in-house support system and gossip network anyone could ask for; the rest of the Behaviour Group past and present (Camilla, Ian, Keith, Michelle, Nicola, Rob, Sarah, Shai) for providing cups of tea (hippy or otherwise) and always-available shoulders to cry on; the PhD-muggles (Ben, Chris, Jodie, Rich, Rupert K, Rupert T, Sue, Tim and Yvette) for fun, games and debauchery, and nodding patiently while I rambled on about salmon and those pesky episodes…

…and finally, the people more deserving of thanks than anyone: Mum, Dad, Sarah and Philip. You have been a constant source of love, support and encouragement, and I feel lucky to call you my family. I could not have done it without you.

The best thing about fieldwork – a cup of tea when you finish!

Summary

Although the impacts of surface water acidification on Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) are well described, factors affecting recovery following pollution abatement are poorly understood. Paradoxically, despite increasing average pH over the last two decades, stocking efforts to rehabilitate damaged populations have not been successful. One possible explanation is that *average* pH measurements mask the effects of episodic fluctuations during high flow when pH is transiently reduced, metal concentrations are elevated and some organisms are affected adversely. However, because salmonid fish (i) avoid acid water and (ii) shelter within stream bed interstices, assessing the effects of acid episodes on their populations requires some understanding of how refuge use, refuge availability and refuge conditions affect recolonisation opportunities. This project therefore examined the effects of acid episodes on the behaviour of Atlantic salmon at different life stages as a possible explanation for the limited recovery of re-stocked populations. Sampling in acid sensitive-streams at low and high (episodic) flow revealed that the stream hyporheos was less acid than surface water in the same streams, with temperature and conductivity also more favourable in interstices. Laboratory experiments were then used to examine (i) the behaviour of Atlantic salmon alevins during a simulated episode in a vertical substrate-filled tank (Chapter 3); (ii) the behaviour of Atlantic salmon parr during a simulated episode in a shelter choice tank (Chapter 4); and (iii) if differences resulting from rearing environment affected the behaviour of Atlantic salmon parr during a simulated acid episode (Chapter 5). Hatchery-reared alevins avoided episodically acid conditions by moving into an area of more neutral pH; opercular rate and mortality were both greater in acid water. Hatchery-reared parr preferentially used a neutral shelter; acid exposure also resulted in increased activity and opercular rate. Fish reared in a simulated wild environment were better able to avoid acid pulses (i.e. they showed greater use of neutral refuges) than hatchery-reared fish, but other behavioural responses (activity and opercular rate) were similar. These results illustrate the potential importance of the streambed during acid episodes as chemical refuges to salmonids, which both alevins and parr are able to exploit. However, at least for hatchery-reared fish, the success of re-stocking programmes could be improved by subjecting fish to acid stress-conditioning regimes prior to release.

CONTENTS

CHAPTER 1: Introduction 12

1.1 Acidic Deposition 12

1.1.1 Characteristics of acidic deposition 12

1.1.2 Sources of acidity 13

1.1.3 Atmospheric chemistry, dispersion and deposition 14

1.1.4 Soil and water chemistry 16

1.1.5 Susceptibility to acidification 18

1.1.6 Long- and short-term changes in acidity 20

1.2 Acid Episodes 23

1.3 Effects of acidification on freshwater systems 26

1.3.1 Primary producers 26

1.3.2 Decomposers 29

1.3.3 Micro- and macroinvertebrates 30

1.3.4 Vertebrates 34

1.4 Effects of acidification on fish 35

1.4.1 Physiological changes 36

1.4.2 Population- and community-wide changes 37

1.4.3 Factors affecting acid sensitivity of fish 39

1.5 Behaviour 44

1.6 Atlantic salmon 52

1.6.1 Sheltering behaviour 52

1.6.2 Hatchery-reared vs. wild fish 54

1.7 Project context and aims 55

1.8 Preliminary experiments and pilot work 56

CHAPTER 2: **pH variations between surface and interstitial waters of Welsh hill streams during acid episodes and likely consequences for salmonids** 58

2.1 Summary 58

2.2 Introduction 60

2.3 Methods 62

2.3.1 Preliminary experiments 62

2.3.2 Study site 63

2.3.3 Sampling procedure 66

2.3.4 Statistical analysis 67

2.4 Results **67**
2.4.1 pH 67
2.4.2 Temperature 70
2.4.3 Conductivity 72
2.5 Discussion **74**

CHAPTER 3: **Exposure to acid episodes and avoidance behaviour in Atlantic salmon alevins** 77

3.1 Summary **77**
3.2 Introduction **78**
3.3 Methods **79**
3.3.1 Experimental animals 79
3.3.2 Test arena 80
3.3.3 Pilot work 81
3.3.4 Experimental protocol 83
3.3.5 Statistical analysis 84
3.4 Results **85**
3.4.1 Movement 85
3.4.2 Opercular rate 88
3.4.3 Mortality 90
3.5 Discussion **90**

CHAPTER 4: **The influence of acid episodes on the sheltering behaviour of hatchery-reared juvenile Atlantic salmon** 94

4.1 Summary **94**
4.2 Introduction **96**
4.3 Methods **97**
4.3.1 Preliminary experiments 97
4.3.2 Experimental animals 98
4.3.3 Test arena 98
4.3.4 Pilot work 99
4.3.5 Experimental protocol 101
4.3.6 Statistical analysis 103
4.4 Results **103**
4.4.1 Shelter use 103
4.4.2 Activity 103
4.4.3 Opercular rate 105
4.5 Discussion **109**

CHAPTER 5: Effects of rearing environment on acid avoidance in juvenile Atlantic salmon 113

5.1 Summary 113

5.2 Introduction 115

5.3 Methods 116

5.3.1 Experimental animals 116

5.3.2 Rearing conditions 117

5.3.3 Test arena 118

5.3.4 Experimental protocol 118

5.3.5 Statistical analysis 120

5.4 Results 121

5.4.1 Shelter use 121

5.4.2 Activity 122

5.4.3 Opercular rate 123

5.5 Discussion 125

CHAPTER 6: Discussion 130

References 140

Appendices 167

1: Chapter 2 – Preliminary Field-Work (Summer 2005) 168

2: Chapter 2 – Statistics 175

3: Chapter 3 – Statistics 176

4: Chapter 4 – Preliminary Experiment (Winter 2004) 177

5: Chapter 4 – Pilot Work 183

6: Chapter 4 – Statistics 202

7: Chapter 5 – Statistics 203

Tables and Figures

Chapter 1

Figure 1.1 The acidification process in lakes (Mason, 2002, after Henriksen, 1989). 17

Figure 1.2 Historical reconstruction of pH using sediment diatom assemblages of (a) Lake Gårdsön, Sweden, and (b) Round Loch of Glenhead, south-west Scotland (Mason, 2002, after Renberg and Hedberg, 1982; Flower and Battarbee, 1983). 22

Figure 1.3 Variations in pH and water level in the Videkal River, Norway, February – July 1983 (Mason, 2002, after Henriksen *et al.*, 1984). 26

Figure 1.4 Sequential disappearance of fish in a Scandinavian lake (Almer *et al.*, 1974). 40

Figure 1.5 Atmospheric emissions of sulphur dioxide (SO_2) by sector in the UK, 1970 – 2002. 55

Figure 1.6 Atmospheric emissions of nitrous oxides (NO_x) by sector in the UK, 1970 – 2002. 56

Figure 1.7 UK Salmon rod and net catch statistics between 1960 and 2000. 56

Chapter 2

Figure 2.1 Map showing location of sample streams in the River Wye catchment, mid-Wales: chronically acidified (A), limed (L), circumneutral (C) and main river sites (M) (total of $n = 8$; per site type $n = 2$). 64

Table 2.1 Summary of sampling site features, including L/W/D (m) at low and high flow, stream bed characteristics, surrounding vegetation and dominant land use. 65

Figure 2.2 Mean pH ± SE ($n = 6$) in surface water (stippled columns) and the hyporheic zone (shaded columns) across four stream types at high and low flow. 69

Figure 2.3 Mean temperature (°C) ± SE ($n = 6$) between surface water (stippled columns) and the hyporheic zone (shaded columns) across four stream types at high and low flow. 71

Figure 2.4 Mean conductivity (μS) ± SE ($n = 6$) between surface water (stippled columns) and the hyporheic zone (shaded columns) across four stream types at high and low flow. 73

Chapter 3

Figure 3.1 Representation of the vertical substrate-filled tank used to study alevins movement during a simulated acid episode, with astro-turf-covered Perspex shelves and inlet/outlet pipes at each side (after Gunn *et al.*, 1987). 81

Table 3.2 Number of alevins (mean ± SE) on the top two, middle six and bottom two shelves in the pre-treatment, treatment and post-treatment periods of the control group (stippled columns) and acid group (shaded columns) (total of $n = 140$ fish). 87

Figure 3.3 Opercular rate (mean ± SE) of sentinel fish (n = 7 in each case) in the pre-treatment, treatment (1 – 4) and post-treatment periods. Data are presented separately for the control (○ = upper sentinel; □ = lower sentinel) and acid (● = upper sentinel, ■ = lower sentinel) treatment groups. 88

Chapter 4

Figure 4.1 Representation of the test-tank, with downstream inlet, centrally positioned shelter, upstream outlet and self-contained cooling pipe around the tank perimeter. 99

Table 4.1 Number and quantity of acid applications during 40 minute treatment period in Acid tank/Neutral shelter and Acid tank/Acid neutral groups. 101

Table 4.2 pH conditions in the test-tank water and shelter water during the 40 minute treatment period; neutral pH = 7, acid pH = 4 – 5. 102

Figure 4.2 Mean shelter use as a proportion of corresponding test period ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter). 104

Table 4.3 Proportional increase in time spent sheltering (mean ± SE) between the pre-treatment/treatment and pre-treatment/post-treatment periods of the Neutral/Neutral and Acid/Neutral groups. 105

Figure 4.3 Mean activity minute^{-1} ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter). 106

Figure 4.4 Interaction between treatment group (Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter) and test period (pre-treatment, treatment and post-treatment) (mean activity minute^{-1} ± SE). 106

Figure 4.5 Mean opercular rate ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter). 108

Figure 4.6 Interaction between treatment group (Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter) and test period (pre-treatment, treatment and post-treatment) (mean opercular rate ± SE). 108

Chapter 5

Table 5.1 pH conditions in the test tank water and shelter water during the 40 minute treatment period; neutral pH = 7, acid pH = 4 – 5. 119

Table 5.2 Number and quantity of acid applications during 40 minute treatment period in the Hatchery Acid (H2) and Wild Acid (W2) groups. 120

Figure 5.1 Mean shelter use as a proportion of corresponding test period ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (H1: Hatchery Control; H2 = Hatchery Acid; W1 = Wild Control; W2 = Wild Acid). 122

Figure 5.2 Mean activity minute^{-1} ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (H1: Hatchery Control; H2 = Hatchery Acid; W1 = Wild Control; W2 = Wild Acid). 123

Figure 5.3 Mean opercular rate ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (H1: Hatchery Control; H2 = Hatchery Acid; W1 = Wild Control; W2 = Wild Acid). 124

Figure 5.4 Interaction between treatment group (H1: Hatchery Control; H2 = Hatchery Acid; W1 = Wild Control; W2 = Wild Acid) and test period (mean opercular rate ± SE). 125

CHAPTER 1

Introduction

Acidic deposition (commonly termed 'acid rain') is one of the major threats to lakes and streams in many industrialised parts of the world. In the UK, acidification of soils and freshwater systems has occurred over large upland areas during the past 150 years, and has received considerable attention over the last 30 years. The main pollutants responsible for acid rain are sulphur dioxide and nitrogen oxides, which are derived primarily from the combustion of fossil fuels (coal and oil) and agricultural sources of ammonia. These acidifying pollutants can be carried over long distances prior to deposition, either directly, or in rain (Elder, 1992). The effects of acid water on aquatic life have been well documented in many studies on a wide range of species, for example microscopic algae (Muniz, 1991), zooplankton (Tessier and Horowitz, 1988), larger aquatic plants (Jackson and Charles, 1988), invertebrates (Raddum and Fjellheim, 1984), riverine birds (Ormerod *et al.*, 1986) and mammals (Mason and Macdonald, 1987, 1989).

1.1 ACIDIC DEPOSITION

1.1.1 Characteristics of acidic deposition

The term acid rain is often used synonymously with acidic deposition. However, acidic deposition is associated with many forms of air-borne acidic pollutants (Elder, 1992). In addition to rain, acidifying pollutants are transported and deposited via the atmosphere as snow, cloud, fog as well as gases and dust during dry periods. Rain and snow are termed 'wet deposition', cloud and fog are 'occult deposition', and gases and dust are 'dry deposition'. The proportion of the acidic materials that are deposited by each mechanism varies by location and altitude. For example, at higher elevations in southern Quebec, occult deposition of sulphate and nitrate (i.e. due to cloud and fog) is equivalent to that deposited by wet deposition at lower elevations (Schemenauer *et al.*, 1988).

Rain and snow are most commonly monitored for quantity and chemical composition. Uncontaminated rainwater in equilibrium with atmospheric carbon dioxide has a pH of 5.6 (Likens and Bormann, 1974), although the pH of rain and snow is lower than this throughout most of the world (Mason, 2002). Strong acids may occur naturally in the atmosphere; for example, sulphur dioxide is produced during periods of volcanic activity and oxides of nitrogen are formed during electrical storms, culminating in an increase in sulphuric and nitric acid. However, these natural conditions have been present for millions of years in the environment, so they alone cannot be responsible for the increase in acidic deposition that has been observed more recently.

1.1.2 Sources of acidity

The main pollutants responsible for acidic deposition are sulphur dioxide (SO_2) and oxides of nitrogen (NO_x). Although natural sources of sulphur and nitrogen exist, the majority of acidifying emissions are produced by anthropogenic activities, for example, the use of fossil fuels for heating, locomotion, power generation and chemical feedstocks. These activities tend to be centralised rather than evenly distributed (i.e. they are grouped around centres of population); most of the acid gases are therefore generated and released in the most densely populated areas.

To date, much of the atmospheric acidity has been attributed to SO_2 (NO_x being responsible for a smaller proportion of the total acidity), but as measures are taken to reduce the amount of SO_2 released, NO_x is assuming more importance (Sullivan *et al.*, 1997). In the UK, approximately one-third of NO_x emissions come from vehicle exhausts, and half from power stations. Power stations are responsible for two-thirds of SO_2 released, the bulk of the remainder in the UK coming from heavy industry (Mason, 2002). In some regions where intensive livestock rearing is common, the ammonia released may enhance deposition of NO_x. As a source of nitrogen, the ammonia may also lead to changes in the natural vegetation of sensitive areas (Fangmeier *et al.*, 1995), though it is alkaline rather than acidic in reaction. At the same time, its uptake may result in the acidification of soils (Miller, 1983). All forms of deposited nitrogen are plant nutrients, and may cause enhanced growth of trees (in the initial stages of acidification) and eutrophication.

1.1.3 Atmospheric chemistry, dispersion and deposition

When the sulphur and nitrogen oxides reach the atmosphere they react with water, oxygen, carbon dioxide and sunlight, forming sulphuric acid (H_2SO_4) and nitric acid (HNO_3). Sulphur emissions may be hydrated to sulphurous acid (H_2SO_3) in clouds before being oxidised to H_2SO_4, or they may be oxidised in the dry state before being hydrated. If the gas is emitted near the ground it may be deposited dry on to surfaces and plants, or be adsorbed on to dust particles and later precipitate as dry deposition; in both cases it is ultimately oxidised to H_2SO_4. Similar processes occur for NO_x, culminating in the production of HNO_3. The chemical reactions that occur are summarised below.

Atmospheric reactions: $CO_2 + H_2O \rightarrow H_2CO_3$

Sulphur and nitrogen oxides: $SO_2 + H_2O_2$ (clouds) $\rightarrow H_2SO_4$

$SO_2 + O_3$ (clouds) $\rightarrow H_2SO_4$

$SO_2 + OH + O_2$ (air) $\rightarrow H_2SO_4$

SO_2 + oxidants (wet surfaces) $\rightarrow H_2SO_4$

NO_x + sunlight + $OH \rightarrow HNO_3$

NO_x + VOC (volatile organic compounds) + sunlight $\rightarrow O_3$

VOC + sunlight + $HO_2 \rightarrow H_2O_2$

Chemical processes in the atmosphere require the presence of other chemicals acting as photo-oxidants. NO_x and hydrocarbons perform this role, forming highly reactive radicals (free radicals) in the presence of sunlight. The hydrocarbons are found in vehicle exhaust fumes or fuel leakages, and their concentration is highest in the air over densely populated and industrialised areas (Irwin, 1985; Lee and Longhurst, 1992).

The majority of acidic deposition exists mainly in clouds, and falls to earth in rain or snow (wet deposition). The conversion from SO_2 and NO_x to acids in a wet atmosphere is very rapid, being approximately 100% per hour in summer and 20% per hour in winter. In a dry atmosphere, complex chemical reactions involving highly reactive oxidizing agents lead to acid production, with conversion rates approximately 16% per day in summer and 3% per day in winter – much slower than the reactions in a moist atmosphere. The conversion of the precursor gases to acids may be limited by the availability of oxidising agents, hydrocarbons and ultraviolet radiation (Mason, 2002).

Sulphuric acid contributes approximately 70% to the mean total annual acidity of precipitation in north-west Europe, and about 60% in eastern North America (Mason, 2002); the remainder is due primarily to nitric acid. Most acidic pollution will fall in close proximity to the area of production; however, air masses can transport pollutants thousands of kilometres from their original source prior to deposition. Because air mass and storm movements tend to follow regular patterns, there is a strong correlation between the sources of pollutants and the areas that receive acidic deposition (Elder, 1992). In eastern North America, the air mass movements and storm tracks are generally from the south-west towards the north-east – emitted pollutants are carried from the rapidly growing 'industrial heartland' of the south (Arizona, Texas, New Mexico and Oklahoma) over the more rural and comparatively pristine area of north-east America (Vermont and Alaska) and south-east Canada (Environment Canada, 1990). In Europe, it is estimated that approximately 17% of acid precipitation falling on Norway is derived form Britain, and 20% of that falling on Sweden is from eastern Europe (Mason, 2002).

1.1.4 Soil and water chemistry

The acids normally present in soil (organic and carbonic acids) are weak acids, and contribute significantly to the overall acidity of the soil (Mason, 2002). However, they do not dissociate into their respective anions and cations to the same extent as strong acids (H^+, SO_4^{2-} and NO_3^-) which are derived from acid precipitation. The acidification of soils, and consequently lakes and streams, is caused by the input of acidity (H^+ or compounds that release H^+, such as NH_4^+) to the catchment (Hendershot *et al.*, 1991). The transfer of acidity from soil to surface waters requires mobile negative ions to bind to the acid hydrogen ions. This process is termed 'The Mobile Anion Concept'. Studies on the chemical processes involved actually pre-date concern about acid precipitation and the detrimental effects of acidified fresh waters on aquatic organisms (Hendershot *et al.*, 1991). For example, McColl and Cole (1968) proposed that cation movement in soil solutions depended on the production of a mobile anion; for instance, the production of H_2CO_3, its dissociation into H^+ and HCO_3, the exchange of H^+ for a base cation[1] and the leaching of the cation and HCO_3^-.

In soil water, as the rate and supply of dissociated hydrogen ions increases (i.e. from increased precipitation), the rate of exchange with base cations (such as Na^+, K^+ and Mg^{2+}) is increased. Cations are displaced from the soil cation exchange sites, and move through the soil, provided that negative mobile ions are available to transport them. These are present in acid precipitation as SO_4^{2-}, and in combination with the cations, form neutral salts which are washed into water courses, thus consuming in-soil produced alkalinity. If weathering of the underlying bedrock is too slow to replace the displaced cations, then the dissociated acids will not be able to form neutral compounds, and acidification will occur.

---

[1] Base cations are those which do not form complexes with hydroxide ions (OH-) in acidic soils, although they are not bases in the chemical sense (Binkley and Sollins, 1990), and are critical in neutralising acids.

In addition to the problems caused by increasing acidity, as soil pH declines, toxicity of metals may increase, in particular aluminium (Andelman and Miller, 1986). Aluminium is a ubiquitous metal, found either in its inorganic form in clays, or tightly bound in complexes with organic matter. In these inert forms it is relatively harmless, but in solution can be toxic to aquatic organisms (Voigt, 1980), and has been blamed for fish kills during acid events (Grahn, 1980; Dietrach and Schlatter, 1989). Heavy metals may also be mobilized (Vesely, 1994).

The extent to which a body of water becomes acidified is determined by the buffering capacity, i.e. the concentration of bicarbonate ions. Henriksen (1989) states that the acidification process occurs in three stages, which are outlined below (Figure 1.1).

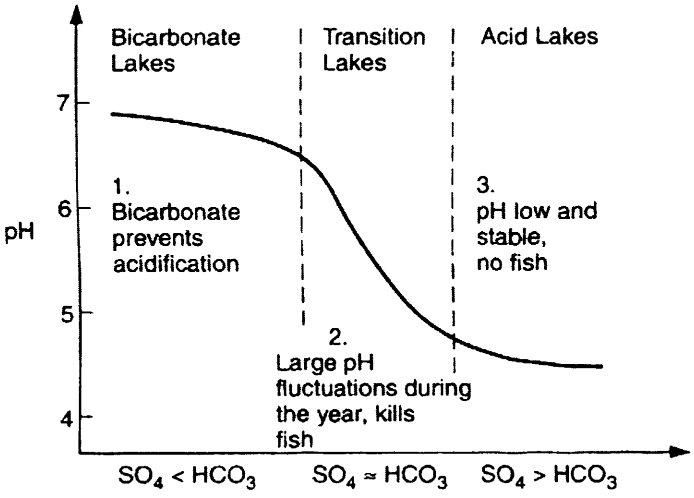


Figure 1.1: The acidification process in lakes (Mason, 2002, after Henriksen, 1989).

Initially, the dissolved bicarbonate buffers the inputs of strong acids. During long periods of acid inputs, the bicarbonate buffer may be lost, resulting in large fluctuations in pH and periodic fish kills. Finally, the loss of alkalinity is complete, and the lake retains a low, stable pH.

1.1.5 Susceptibility to acidification

The impact of acid precipitation on fresh waters is dependent on the acid neutralising capacity (ANC), which is defined as the ability of a solution to neutralise inputs of strong acid to a pre-selected equivalence (Henriksen *et al.*, 1992). Elements such as calcium, potassium, and magnesium are base nutrients that help buffer acid inputs. Higher amounts of these nutrients increase the ANC of an ecosystem. The extent to which water within a catchment is able to buffer itself against acidification is used to monitor the effect of acid rain on watersheds (Stoddard *et al.*, 2003). Acid rain primarily affects sensitive bodies of water, i.e. those that cannot neutralise unnatural levels of acidity due to a reduced ANC. A variety of ecological, chemical, physical and anthropogenic factors determine the vulnerability of an ecosystem to acid deposition.

The impact of acid rain on the environment depends greatly on the geological nature of the catchment area. Calcareous rocks and soils are able to neutralize acid inputs and prevent deleterious effects on the ecosystem; even very small amounts of limestone in a drainage basin can prevent the damage caused by acidification by reaily releasing neutralising bicarbonate ions. In contrast, granite and gneiss weather slowly and do not produce neutralizing chemicals. In Scandinavia, North America and the UK, the areas most affected by acid rain typically have granite bedrock and agriculturally poor soils (Krug *et al.*, 1985; Davis *et al.*, 1978; Edmunds and Coe, 1986).

Areas with thin soils are unable to neutralize acid depositions due to a shortage of base cations. Acidity leaches minerals and nutrients from the soil and alters its chemistry (Ulrich, 1983; Bache, 1984). Soil chemistry is extremely complex. However, in general, the acidity of biologically formed soils is greatest near the surface, where plant growth and decay is taking place, and declines in the more mineral horizons of parent material. The rapid runoff typical of thin soils will therefore tend to be more acidic, as it has been in contact with only the most superficial soil layers. Rainfall which can percolate into deeper soil horizons will be neutralized to a greater extent, or will be less acidified, depending on the original pH and alkalinity.

Land use also influences the acidification of fresh waters; several studies suggest that changes in land use can be directly linked to increasing acidity of surface waters (Krug *et al.*, 1985; Rosenqvist *et al.*, 1980). It has been found that, in areas impacted by acid deposition, lakes and streams in forested catchments are more acidic than those in unforested areas (Harriman and Morrison, 1982; Burns *et al.*, 1984). Vegetation scavenges dry deposition and pollutants held in mists and fogs very efficiently; the pollutants are subsequently washed from leaves and branches and leached out of the plants. Rainfall reaching the soil via vegetation is therefore much more acid than freely falling rain. For example, Mason and Macdonald (1987) recorded a minimum pH of 4.4 in the forested stretch of the River Severn, compared with pH 5.5 several kilometres downstream. In the River Dulas (a tributary of the Severn), which drains through unforested open moorland, a minimum pH of 5.0 was recorded near the source, and most of the river had a minimum pH of 5.5 (Mason and Macdonald, 1987).

These results are supported by a study carried out by Helliwell *et al.* (2001) in the Galloway area of south-west Scotland. The combination of large-scale afforestation efforts and acid-sensitive soils and geology have contributed to a highly acid-sensitive environment (Wright *et al.*, 1994; Rees and Ribbens, 1995; Lees, 1995; Puhr *et al.*, 2000). In a regional study of the long-term response of surface waters to changes in sulphur deposition and afforestation, it was found that surface waters draining two afforested catchments were more acid than those draining an area of open moorland. Both these studies highlight the differences in evapotranspiration rates between trees and grassland – runoff is reduced in forested areas and contains a higher concentration of pollutants (Helliwell *et al.*, 2001).

Scavenging efficiency (i.e. uptake of particulates from the atmosphere) varies with species – conifers in particular are highly efficient, and much work has been carried out over the last two decades to identify the effect of conifer plantations on surface water acidification. In the Llyn Brianne catchment in Wales, a comparison of sulphur deposition and pH beneath different types of vegetation showed that both increased beneath Sitka spruce (*Picea sitchensis*) (Gee and Stoner, 1988).

In forested areas, trees absorb CO_2 (an acid gas) from the atmosphere, and build it into cellulose, stored as wood, which is neutral. Basic materials from the soil are used up in neutralizing the acidity, and the soil itself becomes more acidic (Hornung, 1985; Nilsson *et al.*, 1982). The accumulation of litter as forests develop also leads to natural acidification of soils due to the acidity of the scavenged materials they contain (e.g. Hughes-Clarke and Mason, 1992), although felling and burning will often improve the alkalinity of upper soil layers (Krug and Frink, 1983), by releasing bases.

The presence of trees may alter the drainage pattern of the soil. Drainage channels are intended to minimise contact time between runoff and soil, which results in more rapid runoff, with greater acidity. Freely draining sandy soils are more rapidly affected than more slowly draining soils. In addition, sulphur and nitrogen may become exposed to the atmosphere and form acids by oxidation. In a comparison between an unforested catchment and one with 30% forest cover (mainly Sitka spruce), Harriman and Wells (1985) showed that pH declined and both sulphate and aluminium increased in the forested catchment. Similar results were obtained by Ormerod *et al.* (1989) following an extensive survey in Wales.

1.1.6 Long- and short-term changes in acidity

Acidification processes can be broadly divided in two groups. Chronic acidification generally refers to streams, lakes, and soil ecosystems that have lost their ability to counteract acidifying events. Base nutrients such as calcium, potassium, and magnesium (in addition to other neutralizing chemicals) buffer changes in ecosystem acidity. However, when ecosystems are exposed to excessive, long-term acid deposition these chemicals become depleted. This can make the system more vulnerable to episodic acidification events and may lead to chronic surface water acidity. Episodic acidification is characterized by short, intense acidic events (for example winter snowmelt and heavy rains), in which large acid loads are delivered to ecosystems in a short period of time.

Analysis of any long-term trend in acidification is extremely difficult, for several reasons. Water records are typically incomplete and in the past have been taken for purposes other than to monitor pH; also, historical assessments of water quality have focused more on lowland, buffered areas of rivers (Mason, 2002).

Despite these difficulties, several methods to track long-term changes in acidity do exist. Analysis of sediment cores has been traditionally used to track progressive eutrophication, although this technique has more recently been used to monitor the course of acidification in lakes (Mason, 2002). Cores typically contain pollen grains (which indicate the predominant vegetation type and hence land use at the time), the Pb^{210} isotope which can be used to estimate the core age, and diatoms. Diatoms have siliceous frustules which fall to the lake floor following cell death; the nature of the cell wall means that decomposition does not take place as with other organisms. Examination of a time series through a sediment core allows the reconstruction of changes that have occurred in the waters of a lake according to the depth distribution of diatom species within the core; species dominance is strongly correlated with pH.

There have been several investigations into the timescale of acidification using diatom assemblages. In an early study by Renberg and Hedberg (1982), sediment cores of several Swedish lakes were used to reconstruct inferred pH values since the last ice age, although the most noticeable changes have occurred within the last 100 years. In Lake Gårdsön, pH declined from 6.0 in the 1950s to pH 4.5 by 1980 (Figure 1.2a).

Analysis of sediment cores in the UK has provided similar results. Areas of Scotland are estimated to have suffered from acidic deposition since the 1800s (Flower and Battarbee, 1983). The Galloway region in south-west Scotland is one of the most seriously impacted areas of the UK; several lochs are now devoid of fish and many others have markedly reduced fish stocks. Galloway is a granitic area, which has been extensively planted with coniferous forest since the Second World War. Analysis of sediment cores from several lochs demonstrates that in the mid-1800s, diatom species typically found in acid waters (for example, *Tabellaria binalis* and *T. quadriseptata*) began to increase, while those less tolerant of acid conditions (*Anomoeonis vitrea* and *Fragilaria virescens*) declined in abundance. Species indicative of very low pH dominated in various sites between 1930 and 1950 (Flower *et al.*, 1987). In the Round Loch of Glenhead, pH reconstructions indicate that until the 1820s, pH was fairly consistent at 5.5, but over the next 130 years, gradually declined to 4.5 (Figure 1.2b). More severe changes have been observed in recent decades (Flower and Battarbee, 1983). These results suggest that although fish stocks within the UK have apparently been declining since the middle of the 20th century, lakes, rivers and soils have been exposed to acidic deposition for much longer.

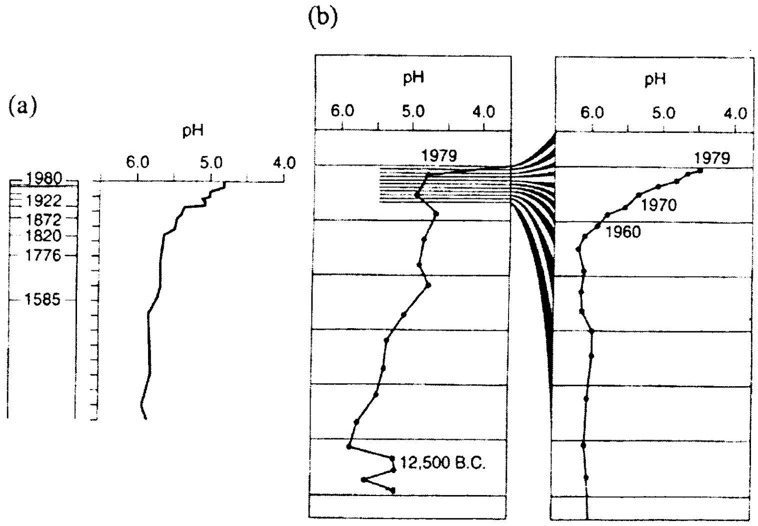


Figure 1.2: Historical reconstruction of pH using sediment diatom assemblages of (a) Lake Gårdsön, Sweden, and (b) Round Loch of Glenhead, south-west Scotland (Mason, 2002, after Renberg and Hedberg, 1982; Flower and Battarbee, 1983).

The use of organisms as ecological indicators has increased and been further refined over the last 20 years. Statistical procedures such as Canonical Correspondence Analysis can be used to make predictions about environmental conditions from the biological structure of communities, and these have been used with invertebrates (Walker *et al.*, 1991; Hann and Turner, 2000), diatoms (Cumming and Smol, 1993), chrysophytes (Cumming *et al.*, 1992), which like diatoms have decay-resistant siliceous wall parts, and benthic filamentous algae (Kinross *et al.*, 1993), to indicate not only pH, but heavy metals, salinity and phosphorus (Dixit *et al.*, 1992, 1998). When coupled with analysis of sediment cores, these techniques allow the estimation of conditions in a lake in an undisturbed state. This is particularly important if the ultimate aim of improving water quality is to restore the aquatic ecosystem to 'good ecological status', ideally their original pristine state.

1.2 ACID EPISODES

Acid episodes are characterised by changes in stream-water chemistry associated with increased stream flow (Wigington *et al.*, 1990), and are considered a major management issue throughout upland areas of Europe (Lepori *et al.*, 2003). During acid episodes, pH decreases and the concentration of potentially toxic metals such as aluminium increases. Acid episodes most commonly occur following spring snowmelt or orographic rainfall, in which an airstream is forced to rise over a mountain range, though episode duration varies depending on the driving weather conditions – snow melt episodes typically last days to weeks, whereas rainfall episodes take place over the course of hours to days (Davies *et al.*, 1992; Bradley and Ormerod, 2002).

Previous assessments of acid rain effects on the chemistry of lakes and streams have focused largely on base flow conditions, i.e. the periods between storms when stream flows tend to be low, and chemical conditions are stable (Lawrence, 2002). Surface waters that are acidic during base flow are also likely to be acidic during high flow, and are therefore considered to be chronically acidified, whereas waters that are only acidified during high flow are considered to be episodically acidified. Episodic acidification is most common during seasons of high precipitation (and during spring snowmelt in regions where snow accumulates), and is least common in summer, when evapotranspiration reduces soil moisture.

Episodic acidification is controlled by a combination of natural acidification processes and anthropogenic acid deposition, and is a process that occurs in environments that have both low and high levels of acidic deposition (Wigington *et al.*, 1990, 1992). In areas that receive low levels of acidic deposition, episodes are controlled primarily by natural factors, but in areas where acidic deposition is high, episodes are influenced by a combination of natural processes and deposition. The processes and factors that control episodic acidification vary between catchments and regions.

One natural factor influencing the nature of acid episodes is dilution, in which low ionic strength rainwater is mixed with higher ionic pre-event water in a catchment. For example, a study of episodic acidification in Alpine streams showed that autumn episodes were caused by rainwater dilution of stream water alkalinity (Lepori *et al.*, 2003). However, dilution alone rarely leads to episodes of reduced pH, but is often combined with other natural processes such as nitrification within organic-rich upper soils, and the production of organic acids through natural carbon and nitrogen cycling (Galloway *et al.*, 1987; McAvoy, 1989). These acids accumulate in soils during dry periods, and are washed into streams and lakes during heavy rainstorms and snowmelt events. In the Alpine study, spring episodes involved a combination of dilution and titration of nitrate ions. Nitrates are an important contributor to acid episodes in other European locations, such as the Storgama catchment in Norway (Seip *et al.*, 1979; Sullivan *et al.*, 1987), and are also strongly associated with episodes in north-east America (Wigington *et al.*, 1990).

Acidic deposition can affect episodes in a variety of ways. Acidic precipitation may enter a body of water without prolonged contact with watershed soil, which can occur when (i) precipitation falls directly into lakes and streams; (ii) snow and ice on lakes melt directly into lake water; (iii) snow melts and travels over frozen catchment surfaces and (iv) precipitation travels through soil macropores directly into water bodies (LaZerte and Dillon, 1984; Roberge and Plamondon, 1987). However, direct inputs are not usually major contributors to acid episodes. A more common occurrence is the delivery through wet and dry deposition of acidifying compounds to a catchment, which accumulate during dry periods. Following large hydrological events such as snowmelt and rainstorms, these compounds are flushed into the water system (Rosenqvist, 1978; Lynch and Corbett, 1989). An additional mechanism of acidification can occur in watersheds with low pre-episode ANC levels, where increased sulphate concentrations exacerbate small depressions in acid neutralising capacity caused by other processes, leading to low pH values during episodes (Galloway *et al.*, 1987). This type of acidic event is most common in mid-Atlantic USA, Canada and Europe. A survey of episodic acid events in Canada caused by snowmelt recorded pH depressions of between 0.4 and 2.6 units; minimum pH in one lake was recorded as 3.2 (Tranter *et al.*, 1994). Similar changes have been observed in most regions affected by acidification. In the Videkal River in south-west Norway, a continuous monitor installed in the river showed that large fluctuations in pH were related to changes in water flow (Figure 1.3); during periods of heavy rainfall, pH fell by more than half a unit within three hours (Henriksen *et al.*, 1984). In both these cases, the major cause of the acidic event was an increased concentration of sulphate ions, derived from anthropogenic sources.

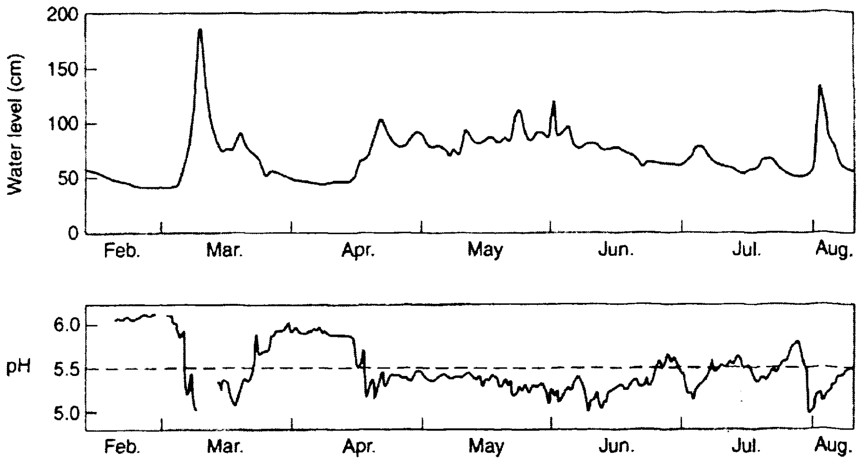


Figure 1.3: Variations in pH and water level in the Videkal River, Norway, February – July 1983 (Mason, 2002, after Henriksen *et al.*, 1984).

1.3 EFFECTS OF ACIDIFICATION ON FRESHWATER ECOSYSTEMS

When considering the effects of acidification on freshwater organisms, two factors need to be taken into account. Firstly, the differences between lentic and lotic habitats and their biota, and secondly, whether the acidification event is chronic or episodic. Organisms that are exposed to a slow acidification may be able to tolerate changes, within limits. More sensitive species are gradually replaced by more acid-tolerant ones, while ecosystem functions such as biomass (standing crop) and productivity may not be affected. In contrast, acid episodes (which can occur after snowmelt, or with the first rain after a dry spell in which dry deposition has accumulated) may have a considerable impact on all aspects of freshwater biology. Similarly, in experimental studies of responses to artificial acidification, the rate of pH change must be taken into account.

1.3.1 Primary producers

Photosynthetic microscopic freshwater algae are extremely important in aquatic systems, both as a food source for many herbivorous animals, and as nutrient recyclers. Species composition and production/biomass estimates have been determined in many lakes with a range of pHs (Almer *et al.*, 1978; Siegfrid *et al.*, 1989a, b); it is clear that increasing acidity alters the nature of the phytoplankton community. Species richness declines from 30 – 80 in cirum-neutral (oligotrophic) lakes, to 10 – 20 species in acid lakes (Muniz, 1991), and in some cases fewer than 10 species have been reported (Almer *et al.*, 1974, 1978). During acidification, the percentage of green algae decreases, while the species-poor assemblages of acid-tolerant phytoplankton include dinoflagellates (which often account for 30 – 50% of total biomass), and yellow green algae (Stokes, 1986; Brettum, 1996). Despite the decline in species richness, biomass and photosynthesis are often unaffected if nutrient supplies are maintained (Olaveson and Nalewajko, 1994).

In the littoral zone, periphyton is of greater significance than phytoplankton, and in shallow lakes may account for most of the productivity (Wetzel, 1964a, b). Periphyton are photosynthetic micro algae that grow on a variety of submerged substances. The changes that occur in periphyton communities are markedly different to those observed in phytoplankton. Several field studies have been carried out in which standing crops of periphytic algae have been examined in relation to pH, but results do not show any consistent pattern. An early study within the UK suggested that epilithic flora in Lake District becks were greatly reduced at pH < 5.7 (Sutcliffe and Carrick, 1973); these results are supported by a later study in New Zealand, where epilithic biomass in circum-neutral streams was greater than that in acid streams (Collier and Winterbourne, 1990). However, several other studies indicate that primary productivity and biomass increase in acid conditions (Hendrey, 1976; Findlay and Saesura, 1980; Hall *et al.*, 1980; Allard and Moreau, 1985; Mulholland *et al.*, 1986; Parent *et al.*, 1986).

Periphyton growth begins to increase as pH falls to below 6 and is extensive below pH 5.5 (France and Wellbourn, 1992). Acidifying lakes are characterised by a proliferation of filamentous green algae as periphyton, or as metaphyton (floating mats of filamentous algae), dominated by Zygnematacean algae (acid tolerant species such as *Mougeotia*, *Zygogonium* and *Spirogyra*) (Grahn *et al.*, 1974; Stevenson *et al.*, 1985; Muniz, 1991). These types of growth can have a detrimental effect on public/recreational use of a water system and have attracted considerable public interest; they are widely regarded as an early sign of acidification (Turner *et al.*, 1987). In Welsh streams, the green algae *Mougeotia*, *Ulothrix* and *Stigeoclonium* were most frequent at low pH (Ormerod and Wade, 1990), and in the Experimental Lakes area of Canada, 30 – 50% of the epiphyte habitat in one lake was occupied by *Mougeotia* (Turner *et al.*, 1987). The prolific growth of this alga may be the primary cause of increased biomass per unit area with increasing acidity (Mulholland *et al.*, 1986), although other factors may be partly responsible. Acidification usually leads to a reduction in grazing pressures as invertebrate communities decline (Howell, 1988, in Muniz, 1991), and acid waters typically have increased clarity due to the precipitation of humic substances.

Some reports, however, indicate little change in biomass (Arnold *et al.*, 1981; Winterbourn *et al.*, 1992) or decreased biomass in acidified streams (Maurice *et al.*, 1987), and discount the decreased grazing hypothesis (Junger and Planas, 1993). Although the decrease in number of algal species and proliferation of filamentous green algae have been widely observed, it is possible that early reports of increased standing crop were mistaken, due simply to more noticeable green algae (Marker and Willoughby, 1988).

In some oligotrophic acidified waters blue-green algae become dominant, or have an increased biomass with Dinophyceae and Chrysophyceae dominating (Conway and Hendrey, 1982; Findlay and Kaisan, 1990). Diatoms also show changes in species composition. Such changes are of significance because the persistence of their siliceous frustules within lake sediment and known correlations with changing acidity can be used to infer the pH history of a lake over many years (Battarbee, 1984).

Macrophytes (which include large algae, mosses and liverworts, and higher vascular plants) provide food and habitat for a variety of aquatic organisms. As with periphyton, the effects of acidification on macrophyte communities are inconsistent. Some studies indicate that species richness declines with increasing acidity (Roberts, 1984; Jackson and Charles, 1988), but this association is often absent in others (Yan *et al.*, 1985). In general, species richness is reduced in lakes of lower pH. Dominant macrophytic species such as *Lobelia* and *Isoetes* are reduced, and are largely replaced by the moss *Sphagnum* (Hendrey and Vertucci, 1980; Grahn, 1986). Increases in *Sphagnum* may accelerate the acidification process; *Sphagnum* spp. exchange metabolically produced hydrogen ions with nutrient and metal ions, thus accumulating potentially toxic metals and acidifying their own micro-habitat (Clymo, 1984; Peverly, 1984; Muniz, 1991).

1.3.2 Decomposers

In contrast with other groups of organisms, little is known about the relationship between stream water and acidity in organisms associated with decomposition (e.g. bacteria and detritivores). Productivity of epilithic and epiphytic bacteria has been shown to be positively correlated with stream water pH (Palumbo *et al.*, 1987a, b; Osgood and Boylen, 1990); some studies have indicated that decomposition rates are reduced in acid waters. For example, Ormerod and Wade (1990) showed that there was a marked reduction in the decay rate of rice grains added to acid waters compared with circum-neutral waters.

Early experimental work suggests that in addition to reduced decomposition rates, there is usually a shift in dominance from bacteria to fungi (Bick and Drews, 1973; Traaen, 1980), but not all studies support this observation; in some cases there is a decline in the diversity and abundance of hyphomycetes (Hall *et al.*, 1980). Experimental work in the USA suggested that decomposition of leaf litter in streams was reduced because the number of detritivores was reduced, but other work suggests that microbial activity is also reduced (Chamier, 1987). Mats of fungal hyphae were reported covering the bottom of experimentally acidified streams, presumably because of the accumulation of organic debris and the reduction in normal decomposer organisms, but later reports indicate that these 'hyphae' are actually mats of filamentous algae (Hendrey and Vertucci, 1980).

In circum-neutral waters, allochthonous matter (leaves and twigs from terrestrial vegetation) is broken down by leaching, invasion by microbes, and the activity of detritivores (Andersson, 1985; Stenson and Eriksson, 1989). Because acidification results in slower decomposition rates, accumulations of organic matter such as leaves and twigs are often observed in acid lakes, and are used as indicators of acidification (Grahn *et al.*, 1974). Slow decomposition rates also lead to lowered rates of mineralization (Burton *et al.*, 1985), which may limit important plant nutrients such as phosphous – primary production could also therefore be reduced.

1.3.3 Micro- and macroinvertebrates

Zooplankton communities can be directly affected by toxic conditions, and indirectly by changes in the activity of primary producers at a lower trophic level, and of predators at higher levels (including larger invertebrates, fish and higher vertebrates). The primary roles of zooplankton in freshwater systems are (i) transfer of energy through food chains and (ii) recycling of elements. As with lower trophic levels, species diversity decreases with increasing acidity. This has been demonstrated at a range of spatial scales, including laboratory experiments, field surveys and whole stream/lake manipulations (Almer *et al.*, 1974; Carter *et al.*, 1986; Tessier and Horowitz, 1988). Harvey *et al.* (1981) showed that pH below 5.5 – 5.0 is particularly detrimental. While most analyses of invertebrate communities in acidic streams show a correlation between pH and species density and diversity, there is much variation among taxa in response to acidic conditions (Burton *et al.*, 1982, 1985; Simpson *et al.*, 1985; Hall and Ide, 1987; Weatherly and Ormerod, 1987; Feldman and Connor, 1992; Rosemond *et al.*, 1992). This may be due to differences in sensitivity between species (Bell, 1971; Hall *et al.*, 1980; Hall and Ide, 1987; Allard and Moreau, 1987; Hopkins *et al.*, 1989), or varying sensitivity among life stages within the same species (Bell, 1971; Camargo, 1995).

In general terms, acidification results in a reduction of sensitive organisms, while acid tolerant species increase following an acid event, although the dominance of a particular species varies with location. In North America, predatory crustaceans are more sensitive, whereas in Scandinavia, Daphnids are the first to disappear, while predators are more acid resistant. Common dominant species in acidic lakes in North America include *Diaptomus minutus*, *Bosmina longirostris* and *Keratella taurocephela*; these species often represent the entire zooplankton community (Mierle *et al.*, 1986; Brett, 1989). In Scandinavia, typical species include *Keratella serrulata*, *Eudiaptomus gracilis* and *Eobosmina longispina*. Such variations in the responses of functional groups make comparisons between lakes difficult, even though they may be chemically similar.

There are several factors that may explain the changes in zooplankton community structure following acidification. The disappearance of sensitive zooplankton such as Daphnids seems to be directly related to ambient water quality. Death in Daphnids by acid stress is linked with a number of physiological changes, including reduced oxygen uptake (Ailbone and Fair, 1981), and failure of osmoregulation (Potts and Fryer, 1979), as shown by a net loss of body ions such as sodium (Na) and chloride (Cl). Hàvas and Likens (1985a, b) studied the combined effects of acidity and aluminium on mortality and sodium balance in *Daphnia magna*, and concluded that increased concentrations of H^+, in combination with aluminium, interfered with sodium balance. At pH 6.5, increased Al lead to a net loss of Na; at pH 4.5, net loss of Na was much reduced, whether Al was present or not. However, in the presence of Al ions, net Na loss was reduced, thus prolonging survival of *Daphnia*. A net loss of sodium ions at low pH has also been demonstrated in stoneflies and mayflies (Frick and Hermann, 1990; Twitchen, 1990), while increases in aluminium have been shown to decrease sodium ions in body fluids. These results highlight the complex relationship between acid water and metal ions. In some cases, metals (and not reduced pH) have been responsible for the loss of zooplankton biomass (e.g. Yan and Strus, 1980); in studies where metal toxicity is the primary factor responsible for a loss of species, toxic effects are usually exacerbated by reduced pH. Metals are generally more soluble at low pH, and several laboratory experiments have demonstrated increased metal uptake by invertebrates under acid conditions, though few field studies support this observation (Wren and Stephenson, 1991; Gerhardt, 1993).

There are other factors that may influence the response of invertebrates to acid conditions. Invertebrate populations demonstrate reduced growth rates when exposed to acid water – this may be the result of wider food web changes leading to availability of an inferior food source. Shifts in species composition (such as a reduction in edible species or changes in the availability of bacterioplankton and detritus) could reduce the density and diversity of prey items (Muniz, 1991). In an artificially acidified lake, the population of the dominant crustacean *Holopedium* collapsed due to the disappearance of phytoplankton, in particular *Oocystis* (Hessen and Lydersen, 1996). Changes in the predator community may also affect zooplankton communities, for example the elimination of larger invertebrates and fish (Brett, 1989). In acidified enclosures, the abundance and mean body size of *Bosmina longirostris* increased, which was attributed to a decline in its predator, *Mesocyclops edax* (Havens and DeCosta, 1985).

Benthic macroinvertebrates also exhibit marked changes during acidification. Molluscs, crustaceans and many groups of aquatic insects break down organic material, regenerate nutrients and are a food source for many fish and bird species. Many species are acid-sensitive and they have proved useful as indicators of acidification (Økland and Økland, 1986). Changes in their populations are relatively easy to assess due to short generation times. Studies have shown that populations of mayflies consistently decreased with increases in acidity, and when a critical level of acidity is reached they become extinct (Harriman and Morrison, 1982). Raddum and Fjellheim (1984) showed that in Norwegian lakes, 60% of mayfly species were lost when pH reached 5.5. Declines in species richness and invertebrate diversity are closely related to decreases in pH observed in acidified lakes; however, the loss of sensitive species is sometimes partially offset at moderate acidity by the positive response of the more acid-tolerant species (Muniz, 1991).

Amphipod responses are similar to those observed in mayflies. In North America, the most abundant lake amphipod, *Hyalella azteca* is acid sensitive, and was recorded in 69 out of 70 lakes in Ontario with positive alkalinity, but was absent in nine acidified lakes (Stephenson and Mackie, 1986). As with zooplankton communities, sensitivity and species dominance vary with location; in Scandinavia, the common freshwater shrimp *Gammarus lacustris* is absent, or found very rarely at pH 6.0, while *Asellus* spp. are more tolerant, and found at greater acidities (Økland and Økland, 1986). Crayfish are also known to be vulnerable to acidification, suffering from delayed hardening of the exoskeleton, parasitism, egg mortality and recruitment failures at low pH (McMahon and Stuart, 1989; Siewert and Buck, 1991). However, changes in the crustacean and mollusc populations must be interpreted with care, because crayfish and molluscs require calcium to build their calcareous scales (Hunter, 1964), shells and carapaces (Malley, 1980). A shortage of calcium may be more important than reduced pH, though the calcium content and pH of surface waters are often strongly correlated (Økland, 1983). Declining numbers of mollusc species with decreasing pH has been observed even when the effects of Ca are taken into account (Økland, 1990).

The decline in macroinvertebrate species diversity could be attributable to a combination of physiological stress, a change in food supply or a reduction in predation. The active uptake of sodium, chloride, potassium and calcium ions is paramount to survival, and uptake is dependent on external conditions. Sensitivity to changing ion concentrations varies with species; crustaceans and gastropods are particularly susceptible to low ion concentrations (probably because they are more permeable), and arthropods are vulnerable whilst moulting, when permeability is increased (Mason, 2002). Currently, food supply and predation are not considered important factors in mediating change in invertebrate populations. While decreases in food supply have coincided with declining invertebrate numbers in some studies, invertebrate losses have been attributed to direct mortality rather than availability of food (Burton *et al.*, 1985).

1.3.4 Vertebrates

Although amphibians are generally more tolerant than fish to increasing acidity (Mason, 2002), severe conditions can impact amphibian populations. Many species of amphibian are susceptible because the areas used for reproduction (temporary pools and shallow waters) are vulnerable to acidification (Pough, 1976; Pough and Wilson, 1977; Clark and Euler, 1982); in addition, amphibian spawning usually occurs in early spring, when the risk of episodic events is particularly high. Lethal pH varies typically between 3.4 and 4.5 (Freda, 1986), although other effects are observed above this level, most commonly reproductive failure. Andrén *et al.* (1988) reported increased egg mortality and embryonic development time in three species of frog (*Rana arvalis*, *R. temporaria* and *R. dalmatina*) with increasing acidity. Increased aluminium concentration had no effect on the eggs, but pH and Al both influenced larval mortality. The interaction between pH and aluminium has been demonstrated in other studies, for example Tyler-Jones *et al.* (1989) showed that in the absence of aluminium, pH had no effect on the survival of embryos and free-swimming larvae of *R. temporaria*, but embryo survival decreased with increasing Al concentration.

There are few records of the direct toxic effects of acidification on birds, although they may be adversely and indirectly affected by changes in food supply. Several studies have reported reduced population densities in areas where fish and invertebrate populations have been impacted by acidification. For example, the dipper (*Cinclus cinclus*) feeds on aquatic invertebrates such as mayfly and caddis larvae; dipper densities are much reduced along acid streams that would otherwise provide a suitable habitat (Ormerod *et al.*, 1986; Vickery, 1991; Logie, 1995; Buckton *et al.*, 1998). Breeding density (number of pairs per 10 km) is also highly correlated with mayfly and caddis abundance (Ormerod and Tyler, 1993). A long-term (1960 – 1984) study of the River Irfon, Wales, showed that dippers declined as acidity increased (by 1.7 units) (Ormerod and Tyler, 1987). Piscivorous birds are also affected by acidification; for example, the high level of brood mortality in common loons (*Gavia immer*) in Canada has been attributed to a shortage of suitable foods (Alvo *et al.*, 1988). However, in other areas, the decline in fish populations may result in an increase in avian invertebrate-feeders, due to reduced competition (Hunter *et al.*, 1986). The increased clarity of acidic waters may also favour predatory species which hunt using visual clues (Eriksson, 1984).

Mammals are not usually directly affected by acidic water, but indirect changes such as a reduction in food supply can impact populations. In the UK, the otter (*Lutra lutra*) is an ecologically important species and has been the subject of some acidification studies. In the UK, areas where otters are known to thrive are also those which may be susceptible to acid deposition; distribution of otters within these areas therefore reflects the water quality and food supply. For example, otters are common on a tributary of the River Severn, the Dulas, which rises in open moorland; in contrast, they are rare along the top 15 km of the main river, which flows through a forestry plantation. In a study by Mason and Macdonald (1987), fish were shown to be absent from the top of the Severn, whereas higher populations were recorded in the Dulas; the authors argue that the abundance and distribution of otters in these areas was therefore influenced by food supply. These observations are supported by a later study carried out in Scotland, in which otters were rarely found at sites where the minimum pH was low enough to adversely affect fish populations (Mason and Macdonald, 1989).

1.4 EFFECTS OF ACIDIFICATION ON FISH

Acidification was first reported in Norway in the early 1900s following Atlantic salmon (*Salmo salar*) kills. In the 1970s, a decline in salmon numbers was observed in seven southern Norwegian rivers, all known to receive acid precipitation. In contrast, in rivers not receiving acid precipitation, there was no overall change in numbers (Henriksen, 1989). Other fish species have also been affected by acid precipitation. In the 1920s and 1930s reductions in brown trout (*Salmo trutta*) numbers were observed, and by the 1970s a complete lack of fish in lakes in much of southern Scandinavia was reported. The elimination of fish from thousands of lakes in acid-sensitive areas of Scandinavia (Muniz, 1984) and more widely in Europe and North America (Dillon *et al.*, 1984; Baker *et al.*, 1990) is a powerful indicator of the impact of acid deposition on freshwater systems. Acidification is associated with two types of fishery problem: (i) adult fish may disappear from acid waters due to periodic mortality during short term increases in acidity, and (ii) poor recruitment due to mortality in the susceptible early stages of development and growth, so that the population cannot maintain itself (Rosseland, 1986).

1.4.1 Physiological changes

Fish exposed to acid water experience respiratory distress, disruption of ionoregulation, and acid-base imbalance (Laurent and Perry, 1991).. Also affected are osmoregulation and nitrogen excretion (Brakke *et al.*, 1994). The severity of these disturbances is related to the extent of the acidification and concentration of calcium within the water (Wood and McDonald, 1982, McDonald, 1983). Following acidification, mucus secretion on gill epithelium increases (Daye and Garside, 1977; Laurent and Perry, 1991), which inhibits oxygen diffusion across the gill surface. This process may be further complicated by aluminium, which precipiates in acid solution, and accummulates on the gill surface. In the presence of calcium, ion loss is reduced but respiratory stress is still severe. When sodium and chloride ions decrease by approximately one-third, body cells swell and extracellular fluids become more concentrated. Potassium is transported from within cells to compensate, but must be removed from the body quickly, or will lead to fatal depolarisation of nerve and muscle cells (Mason, 2002). Degradation of the gill surface has been shown to occur with increasing acidity in brook trout (*Salvelinus fontinalis*) (Daye and Garside, 1977), thus increasing permeability to ions and water. Sodium and chloride are found in high concentrations in the blood plasma of fish, and ions lost in excretion must be replaced by active transport across the gills against a concentration gradient. If the gill surface is damaged, then sodium will be lost in higher concentrations; the excessive loss of sodium (which cannot be replaced quickly enough via active transport) is a common cause of mortality in acid waters (Mason, 2002). Calcium ions reduce sodium and chloride ion egress; however, calcium regulation of gill permeability is inhibited by aluminium.

1.4.2 Population- and community-wide changes

There are many recorded examples of the loss of individual species and changes in species composition of fish communities in acid-sensitive waters (e.g. Watt *et al.*, 1983; Beggs and Gunn, 1986; Carline *et al.*, 1992; Rask, 1992; Simonin *et al.*, 1993; Pinder and Morgan, 1995; Baker *et al.*, 1996; Barlaup and Åtland, 1996; Stallsmith *et al.*, 1996; Baldigo and Murdoch, 1997; Heard *et al.*, 1997; Baldigo and Lawrence, 2000, 2001).

In North America, a study of the chemical and biological changes that occurred in five streams in areas not typically at risk from long-term acidification (i.e. undisturbed, sandstone bedrock catchments) showed that following storm events, pH declined by as much as 1.2 units, and total aluminium concentrations increased. Following storm events, brook trout were eliminated from the most acidic stream, and sculpins (*Cottus bairdi* and *C. cognatus*) were collected only in those streams where episodes were mild (Carline *et al.*, 1992). Radio-tagged fish moved downstream when aluminium concentrations reached toxic levels; in addition, brook trout were recorded near areas of upwelling groundwater, where pH was higher, and aluminium lower[2]. *In situ* bioassays showed that mortality ranged from 0 to 80% in streams during acid episodes and was positively related to aluminium concentrations (Carline *et al.*, 1992).

---

[2] Movement of fish away from acid water is a widely recognized behavioural response, and will be discussed in greater detail in a later section of this Chapter.

In a later study, bioassays using brook trout and blacknose dace (*Rhinichthys atratulus*) showed that survival decreased during acid episodes, and blacknose dace were more sensitive than brook trout. Aluminium concentration was also a primary factor influencing mortality in both species (Simonin *et al.*, 1993). These results are supported by observations made in a more extensive survey carried out as part of the Episodic Response Project. Baker *et al.* (1996) studied three different regions in north-eastern America (Adirondack Mountains, Catskill Mountains and the northern Appalaichan Plateau). In streams with suitable low-flow conditions but moderate/severe acidification during high flow, fish mortality was higher, downstream movement was recorded, and acid-sensitive species (blacknose dace and sculpins) were absent. Movement of fish into refugia (areas with higher pH and lower aluminium) was also observed, and is thought to have at least partially mitigated the adverse effects of the episodes (Baker *et al.*, 1996). These results show how habitat quality may influence survival during acid episodes.

Spatial and temporal variability of habitats, in combination with water quality, complicate the effects of acidification (Baldigo and Lawrence, 2000, 2001). The characteristics of any population are a result of the responses of individual fish to the interaction of several factors, including (1) stream chemistry; (2) chemical inputs from the terrestrial environment; (3) physical and hydrological parameters such as water velocity, stream depth and width, pool/riffle ratio, amount of cover and shade; (4) basin characteristics such as drainage area, stream gradient, site elevation; (5) water temperature; (6) recruitment success (e.g. rates of reproduction and emigration); (7) anthropogenic factors such as rates of fishing and stocking; and (8) natural ecological factors, for example predation and competition (Baker *et al.*, 1990, Beauchamp *et al.*, 1992; Baldigo and Lawrence, 2000). Many habitat and water-chemistry factors are difficult to quantify due to the spatial and temporal fluctuations that frequently occur in aquatic ecosystems. However, it is important to understand how habitat quality might influence the behaviour of recovering fish populations with the additional limits imposed by water quality.

The loss of fish species from a community is reflected in decreasing densities and biomasses (Beamish, 1970, in Muniz, 1991; Hultberg and Stenson, 1970), and productivities and yields (Beggs *et al.*, 1985; Harriman *et al.*, 1987). Before extinction occurs, some fish species may exhibit increased growth and larger weight/length ratios, as a result of decreased predation and competition for food (Almer, 1972; Harvey, 1982). The point at which a particular species is eliminated from a community is influenced by a variety of factors, including the level of acidity, the life history and longevity of different species, and their tolerance to acid conditions. The disappearance of acid-sensitive species with short life-cycles such as minnows will occur more quickly than those with longer life cycles, for example lake trout *Salvelinus namaycush* (Muniz, 1991).

1.4.3 Factors affecting acid-sensitivity of fish

Species

The progressive elimination of different fish species following acidification can be used as an general indicator of species sensitivity. In the LaCloche Mountain region in Canada, as acidification progressed during the 1950s and 60s fish populations gradually declined (Beamish and Harvey, 1972; Beamish *et al.*, 1975; Harvey and Lee, 1980). Prior to 1950, eight species of fish were recorded in one lake – by 1970, all species had been eliminated, in the following sequence: yellow perch (*Perca flavescens*), burbot (*Lota lota*), lake trout, slimy sculpin, white sucker (*Catostomus commersoni*), lake herring (*Coregonus arteedi*), trout-perch (*Percopsis omiscomaycus*) and lake chub (*Couesius plumbeus*). An additional survey in the same region confirmed the link between declining fish numbers and pH (Harvey and Lee, 1982; Matuszek and Beggs, 1988); disappearance thresholds for lake trout and brook trout have also been established in the Adirondack Mountains (pH 5.4 and 5.1, respectively) (Schofield and Driscoll, 1987). The sequential disappearance of fish in Scandinavia follows a similar trend, as shown in Figure 1.4 (Almer *et al.*, 1974).

Most sensitive Roach (*Rutilus rutilus*)
Minnow (*Phoxinus phoxinus*)
Arctic charr (*Salvelinus alpinus*)
Brown trout
European Cisco (*Coregonus albula*)
Perch (*Perca fluviatilis*)
Pike (*Esox lucius*)
Least sensitive Eel (*Anguilla anguilla*)

Figure 1.4 Sequential disappearance of fish species in a Scandinavian lake (Almer *et al.*, 1974).

In the UK, a study on the occurrence of fish species in streams with various pH ranges demonstrated the sensitivity of certain species to increasing acidity. Brown trout were found in only 28% of streams with pH less than 5, but in 95% of streams with pH greater than 6.5. Atlantic salmon were also absent from streams with pH less than 5.5. Population densities and biomass were also reduced in streams with pH less than 5 (compared with streams of pH 6.5). Eel distribution was largely unaffected by water quality (although biomass decreased in acid streams), while minnow and bullhead (*Cottus gobio*) distribution decreased with increasing acidity (Turnpenny, 1989). Fish also differ in their sensitivity to aluminium; laboratory experiments with newly hatched fry showed sensitivity to pH and aluminium in the order roach (most sensitive) → zander (*Stizostedion lucioperca*) → whitefish (*Coregonus peled*) and perch → pike (Rask, 1992; Vuorinen and Vuorinen, 1992).

Age

The primary reason for population decline is usually recruitment failure (Mills *et al.*, 1987; Brezonik *et al.*, 1983). For example, an experimental lake in Canada (Lake 223 in the Experimental Lakes Area) was artificially acidified from pH 6.7 in 1976 to pH 5.1 in 1983, and then allowed to recover to pH 6.7 between 1984 and 1996. During acidification, recruitment for all species declined to zero, and two species were eliminated. As pH increased again, recruitment gradually improved for all remaining stocks, and one species re-colonised the lake (Schindler *et al.*, 1985; Mills *et al.*, 2000).

Acidification threatens all life stages in freshwater salmonids (Skogheim *et al.*, 1984), although in general, effects on the early life stages are often the main cause of fish disappearance (Jensen and Snekvik, 1972; Rosseland *et al.*, 1980). Spawning failure and impaired egg development, rather than mortality, are contributory factors; there are several reports of reduced reproductive potential of fish in acidified lakes (Almer, 1972; Beamish *et al.*, 1975; Frenette and Dodson, 1984). This may be attributed to an inability to produce/release eggs or sperm, or unsuccessful fertilisation (Sayer *et al.*, 1993). However, evidence for impaired reproductive function following acidification is inconclusive, as there are several conflicting studies. For example, Valtonen and Laitinen (1988) observed reduced gonadal maturation in female perch taken from an acidified lake, compared with those from a circum-neutral lake. In contrast, Vuorinen *et al.* (1992) reported that gonad development in males was more advanced in acidified lakes. Other sudies do not use gonad size as an indication of reproductive potential due to potentially misleading results (e.g. Beamish, 1976; McCormick *et al.*, 1989); gamete development and subsequent release are more commonly used but are also subject to misinterpretation. In a study of brook trout, females subject to deleterious conditions (acid, soft-water with aluminium) exhibited higher gonad maturation after spawning than those maintained in more favourable conditions (Mount *et al.*, 1988). However, mature gametes were still contained within the ovary after manual stripping, which may indicate delayed maturation in the acid/aluminium stressed fish. While gamete production may be minimally affected by acid conditions, delayed spawning is a widely reported occurrence (Tam and Payson, 1986; Hutchinson and Sprague, 1986; Vourinen *et al.*, 1990, 1992). It has been suggested that delayed gamete release could be an adaptation to unfavourable water conditions (Sayer *et al.*, 1993), although this may be irrelevant if fertilisation is not able to take place. There are no direct studies concerning the quality of the spawning environment and its influences on egg/sperm quality and their ability to fuse. Brown (1982) found no effect of fertilising eggs of brown trout in low pH solutions; however, Carrick (1979) and Parker and McKeown (1987) both reported reduced embryo-larval survival of eggs fertilised in acid waters. In all of these studies, eggs were obtained from stock not originating from acidic areas. Johnson and Webster (1977) demonstrated that indigenous adults avoid low pH water, suggesting that higher pH levels may be beneficial to successful fertilisation.

Eggs are especially sensitive to acidity. Several freshwater fish species bury their eggs in the gravel of the river or lake bed in late autumn, where they are left to incubate over the winter. Burying fertilised eggs within gravel may protect eggs by providing insulation from acidic surface water, and increasing exposure to upwelling groundwater of higher pH (Lacroix, 1985; Gunn, 1986). Exposure to acid water has been shown to disrupt key processes (e.g. oxygen consumption and ionoregulation) in egg development in several species of fish, including Atlantic salmon (Peterson and Martin-Robichaud, 1982; Eddy and Talbot, 1985; McWilliams and Shephard, 1991), perch (Runn *et al.*, 1977; Rask, 1983; Shephard, 1987) and brown trout (Runn and Sohtell, 1982). In a study of survival of brown trout eggs, Brown and Sadler (1989) showed that percentage survival of freshly fertilised eggs after eight days was 100% at pH 5.1, and was not affected by calcium concentration; high calcium concentrations, irrespective of pH, also had no effect. At low pH (4.2) and low calcium concentrations, all eggs died. In brook trout, Hunn *et al.* (1987) demonstrated increased egg mortality at low pH; at pH 4.5, 80% of eggs died. When pH was increased to 7.5, mortality declined to less than 2%. Following hatching, all larvae died within 30 days at pH 4.5. Embryos exposed to acid water are known to experience hatching difficulties. Successful hatching relies on rapidly rupturing and discarding the egg capsule (chorion); low pH has been shown to reduce the activity of the necessary hatching enzyme chorionase. This may result in the head and tail remaining within the chorion, a condition which may be temporary or terminal, due to yolk sac damage (Nelson, 1982; Kwain and Rose, 1985; Sayer *et al.*, 1991a) or a reduced oxygen supply at a critical period (Thomsen *et al.*, 1988). Encapsulation has been observed in many salmonid species (Sayer *et al.*, 1993).

Newly hatched larvae and fry just starting to feed are also particularly sensitive to acidification. Larval size is known to be dependent on water quality (Sayer *et al.*, 1993). In the period between hatching and exogenous feeding, the yolk sac is converted into cell tissue, a process which may be inhibited by low pH (Howells, 1990). Some studies have shown differences in whole-body mass of fish kept in acid conditions compared with control animals (Kwain and Rose, 1985; Cleveland *et al.*, 1986; Peterson and Martin-Robichaud, 1986; Hunn *et al.*, 1987; Thomsen *et al.*, 1988; Ingersoll *et al.*, 1990), although several studies found no change (Brown, 1982; Parker and McKeown, 1987; Reader *et al.*, 1988, 1989; Wood *et al.*, 1990a, b; Dalziel and Lynam, 1991; Sayer *et al.*, 1991a; Steingraeber and Gingerich, 1991). Whole-body mass is also unaffected by calcium concentration and trace metal content of the test media. Although acid waters are known to retard development and increase mortality, the conflicting evidence regarding body mass suggests that it is not a reliable indicator of acid stress. Other physiological factors are often used to monitor effects of acidification in larvae and young fish, including whole-body mineral content and skeletal calcification, which are both thought to be disrupted by acid water. In soft, acid water, reduced uptake of minerals such as calcium, magnesium, sodium and potassium has been observed in brown trout (Reader *et al.*, 1988, 1989; Sadler and Lynam, 1989; Dalziel and Lynam, 1991) and brook trout (Wood *et al.*, 1990a, b), although reduced uptake was not correlated with increased mortality in any of these studies. Uptake of calcium is particularly important as it is required for skeleton formation, and some studies have shown reduced calcium uptake at low pH (Sadler and Lynam, 1989; Sayer *et al.*, 1991a; Steingraeber and Gingerich, 1991); however, reduced uptake may not lead to reduced calcification, and disruptions in skeletal development are often small (Reader *et al.*, 1988, 1989; Dalziel and Lynam, 1991). Reduced calcium uptake is most apparent when acid water treatments are supplemented with trace metal elements, such as aluminium or copper; whole-body ion content is reduced and skeletal deformities occur (e.g. Reader *et al.*, 1988, 1989; Sayer *et al.*, 1991b). Where different responses between species have been observed, differences in vulnerability to metal ions has been cited as the main cause – it is possible that higher ion concentrations are needed to have a deleterious effect on mineral balance in some species (Sayer *et al.*, 1993).

The physical appearance of larvae subject to acid stress and increased metal ion concentrations may also vary. Increased mucus production from the gills has been observed in several species, including Atlantic salmon (Daye and Garside, 1977), and brown trout (Reader *et al.*, 1988). Impaired gill development has also been observed in these species (Daye and Garside, 1980; Kwain and Rose, 1985; Jagoe *et al.*, 1987), and may result in a reduction of the surface area available for gaseous exchange or ion regulation. Behavioural abnormalities are also common, for example impaired swimming activity and range and frequency of movement. These traits have been observed in several species, including brook trout (Cleveland *et al.*, 1986, 1989; Hunn *et al.*, 1987; Jordahl and Benson, 1987), brown trout (Reader *et al.*, 1988; Sayer *et al.*, 1991a) and Atlantic salmon (Daye and Garside, 1979). If swimming ability is impaired, fish are less likely to survive in the field because individuals may not be able to emerge from the substrate in which they hatched. Feeding may also be inhibited, and fish will be more vulnerable to predation. However, several studies have shown that larvae nearing the end of the yolk resorption period exhibit an avoidance response when exposed to short term pulses of low pH, behaviour which may enhance survival in areas subject to acid episodes (for example, brook trout alevins: Gunn and Noakes, 1986). Similar behavioural changes have been observed in many other species, both in newly-hatched and older fish.

1.5 BEHAVIOUR

The responses of animals to changes in their environment are many and varied. Exposure to acutely toxic substances can result in immediate mortality, whereas sub-lethal exposures can elicit a different, and more subtle, response. Traditionally, studies on the stress response[3] have focused on physiological responses, for example the work pioneered by Seyle (1936, 1946, 1950, 1956). However, there is increasing evidence that behavioural responses are equally important in re-establishing homeostasis, thus ensuring survival (Johnson *et al.* 1992). One of the most obvious behavioural compensation mechanisms is to avoid the stressor. In polluted waters, avoidance responses can prevent fish from experiencing potentially toxic conditions (Gray, 1990). Several studies have demonstrated that fish are able to detect and avoid detrimental physical and chemical changes to their environment (Atland, 1998). Gas supersaturated water and effluents of cooling water (thermal effluents) have been shown to induce avoidance reactions in fish (Gray, 1983, 1990), and laboratory studies have shown that salmonids in particular avoid several toxicants, including metals (Atchison *et al.*, 1987) and organic pollutants (Carr *et al.*, 1990).

Among the important chemical stressors for fish in Welsh streams (and indeed, throughout most of the United Kingdom) are low pH and elevated aluminium concentrations, caused by anthropogenic acidification. Avoidance behaviour in acidic waters has been demonstrated in laboratory tests of brook trout (Gunn and Noakes, 1986; Pedder and Maly, 1986), lake trout (Gunn *et al.*, 1987), Arctic charr and rainbow trout (*Oncorhynchus mykiss*) (Peterson *et al.*, 1989), although little is known about avoidance behaviour in Atlantic salmon. Previous studies have demonstrated contradictory results. For example, Hoglund (1961) used a gradual gradient choice tank (i.e. a trough in which distinct compartments create a range of water

---

[3] The concept of biological stress, in which a stimulus acts upon a biological system causing that system to respond, has been studied for many years, although there are varying definitions of what constitutes stress. Early studies considered stress in terms of the stimulus (Canon, 1935), whereas subsequent reports define the applied stimulus as the stressor, and stress as the response of the system (Seyle, 1956; Esch *et al.*, 1975). However, most studies continue to refer to stress in terms of the stimulus, and the change in the animal as the stress response (Pickering, 1981). The occurrence of compensation mechanism (i.e. a stimulus and subsequent reaction) is a basic feature of most biological processes, but it is sometimes difficult to distinguish between a stress response and the normal adaptive responses of an animal. The process of quantifying a stress response can use two approaches, firstly, studying the immediate physiological and morphological responses of an animal to stress, and secondly, long term changes in the performance capacity of an animal, in which performance capacity is measured as survival (Brett, 1958). These two approaches are not mutually exclusive, and it may be easier to classify stress responses as primary (neural and neuro-endocrine), secondary (the physiological consequences of primary responses) and tertiary (changes in behaviour and decreased growth rate) (Mazeaud *et al.*, 1977; Wedemeyer and McLeay, 1981). However, this is a system of convenience and it should be noted that stress responses can involve changes at all levels.

conditions) to test the response of salmon parr to acidifed water; fish were seen to move away from compartments with pH less than 5.3. Conversely, Peterson *et al.* (1989) tested the response of several fish to a pH gradient (typically pH 4 – 10) using the same apparatus, and found that Atlantic salmon fry did not significantly avoid any of the low pH areas. However, the difference in ages of the fish used in these two studies may account for the contradictory results. The lack of avoidance in juvenile Atlantic salmon may have been due to the tendency of these fish to remain stationary for long periods of time, and possible immobilisation of salmon at very low pH.

In some cases, increased Al concentration, and not low pH, has resulted in avoidance behaviour (Van Coilie *et al.*, 1983). Current studies suggest that the most widely exhibited behavioural response to increased acidity is avoidance behaviour of some type. Several laboratory studies have shown that when given an opportunity, fish move away from water of low pH and high Al concentration (Johnson and Webster, 1977; Jones *et al.*, 1985a; Gunn and Noakes, 1986; Nakamura, 1986; Atland and Barlaup, 1996; Atland, 1998; Ikuta *et al.*, 2001), and it is thought that behavioural avoidance of acid waters could increase the probability of survival during acid episodes (Breck *et al.*, 1988).

Atland and Barlaup (1996) tested the avoidance behaviour of Atlantic salmon in waters with different pH levels and aluminium concentrations, using a steep gradient choice tank comprising two distinct chambers in which water of two different qualities could be separated, whilst allowing the fish free movement between the two areas (Hoglund, 1961). When the pH in one side of the tank was reduced to 4.0 and 4.5, avoidance behaviour occurred at significantly higher levels than at pH 6.9 (control), but not at pH 5.0. Avoidance behaviour was not significantly affected with the addition of labile Al. The absence of avoidance behaviour at pH 5.0 suggests that there is a threshold concentration at which point avoidance is triggered, but above which avoidance will not enhance survival. The avoidance threshold has been reported in several salmonid species, e.g. Arctic charr (pH 5.5, Jones *et al.*, 1985a; pH 5.2, Peterson *et al.*, 1989) and rainbow trout (pH 5.9, Peterson *et al.*, 1989). However, behavioural studies carried out on fish under laboratory conditions are not directly applicable to fish living in their natural habitat – it is likely that laboratory studies underestimate the threshold concentrations for avoidance due to the stress of handling and confinement (Pickering *et al.*, 1982; Waring *et al.*, 1992; Atland and Barlaup, 1996; Atland 1998).

Atland (1998) tested the behavioural responses of brown trout juveniles in waters of different pH, with and without Al, using the steep-gradient choice tank described by Atland and Barlaup (1996). Fish were studied for one hour, which included a 5 minute acclimation period, followed by the addition of acid/acid and Al dosing over a 15 minute period, and a 40 minute test period. In addition to avoidance/attraction behaviour (expressed as time spent in the acid side, analysed in 5 and 10 minute intervals), other behavioural measures included proportion of time spent actively swimming in the acid and control side, and number of times the fish crossed the centre-line of the tank. Although the test apparatus and methodology follow that of the previous study (Atland and Barlaup, 1996), the results were contradictory. No avoidance response was observed at pH 4.0, pH 4.5 and pH 5.0; rather, the fish showed significant attraction to low pH water, spending 67%, 74% and 68% in the acid side, respectively. No attraction or avoidance was demonstrated at pH 5.5. With the addition of Al at pH 5.0, the fish showed significant avoidance at the highest Al concentration, and spent on average only 32% of the time in the acid, Al-rich side of the tank. The lack of avoidance in the low pH treatments could be partly explained by the activity patterns of the fish – a lower proportion of time was spent actively swimming in the acid than in the control side, i.e. fish remained more passive in the acid side. Previous exposure experiments have shown that brown trout are less sensitive to acid water than other species of salmonids (Grande *et al.*, 1987; Rosseland and Skogheim, 1984); in addition, the concentration of free CO_2 can be important for the attraction to low pH water. Laboratory studies on Arctic charr have shown that fish were attracted to increased concentrations of free CO_2 (Jones *et al.*, 1985b);

therefore, the slight elevation of free CO_2 within the acid side of the test apparatus may also be relevant.

Ikuta *et al.* (2001) studied the effects of low pH on upstream migratory behaviour in hime (land-locked sockeye) salmon (*Oncorhynchus nerka*) using 2-way flow-through channels in which 2 small upper ponds were connected (using 2 narrower pipes) with a large lower pond, semi-divided to prevent water from mixing. Acidic conditions were simulated with the addition of sulphuric acid to the connecting pipe in one half of the tank. When neutral (pH 6.7) water was allowed to flow through both channels, fish were observed in both sides, and there was no significant difference in swim-up frequency between the two groups. When the pH of one half of the channel was reduced to 6.0, the number of fish exhibiting upstream swimming behaviour decreased from 20% to 10% of fish in the acid side. With further pH reductions, fish were completely absent from the acid side of the tank. In addition to examination of the upstream migratory behaviour, the distribution of fish within the test tank was also recorded. When neutral water was flowing in both channels, there was no significant difference in the distribution of fish (approximately 50% in either side). When pH 6.0 water flowed through one side of the channel, the average pH of the acidic side of the lower pond was recorded as 6.2, and fewer fish were recorded in the acidic side than in the neutral side (approximately 67% neutral *vs.* 33% acid). Further reductions in pH of water flowing into the acidic side resulted in a decrease in pH in both the acidic and neutral sides of the tank, which corresponded with a decline in the average percentages of fish distributed in the lower pond in both halves of the tank. This study clearly demonstrates the sensitivity of land-locked sockeye salmon to acidity, and their tendency to avoid even slight changes in acidity during upstream migrations.

There are few detailed *in situ* studies of avoidance reactions to acidic water in salmonids (Gunn and Noakes, 1986; Gagen *et al.*, 1994), although there are some reports suggesting that salmon avoid acidic water. Skogheim *et al.* (1984) observed downstream movement of

Atlantic salmon spawners during an acid episode in a river, but did not distinguish between active swimming or passive drift. A similar type of directional avoidance has been observed in other salmonids, where acidic episodes have resulted in a downstream movement (Leivestad and Muniz, 1976; Hall *et al.*, 1980; Gagen *et al.*, 1989), and congregations of fish near points of convergence with more alkaline tributaries (Muniz and Leivestad, 1981; Watt *et al.*, 1983). Atland and Barlaup (1995) demonstrated that Atlantic salmon and brown trout may also avoid potentially toxic mixing zones where an acidic tributary enters a limed river (characterised by a rapid increase in pH and polymerisation of Al from the acid tributary), causing Al precipitation onto fish gills.

Gagen *et al.* (1994) tested the response of brook trout to episodes of low pH (in combination with high Al concentration) in two groups of streams, one of which experienced severe acid episodes, thus allowing a comparison between acidic and cirum-neutral (reference) streams. Following insertion of miniature radio transmitters, fish were confined in holding cages and subsequently released to the study streams. Location of the fish was then determined either daily, or every other day, for 30 days, to within a circular area of 1 m radius, from which water samples were taken. However, water samples were not collected if a fish was within 3 m of its previously sampled location, and in cases where two fish were within 3 m of each other, a single water sample collected at a point midway between them was used for both fish. In the reference streams, some downstream movement was observed; however, some individuals also moved upstream, thus the median net movement (relative to where each fish was initially released), was zero. In the acidified streams, median net movement was downstream. However, about one-third of the fish died as they moved downstream during the spring episodes, so downstream movement may not always ensure survival of fish during acidic episodes. Furthermore, the potential effect of acid conditions on small scale movement patterns (< 1 m) may not be revealed by this study, since water quality measurements were not taken in close proximity to the location of the fish. It is still possible therefore that movement into interstitial spaces within the stream bed (rather than mass downstream movement), could provide an alternative means by which fish can shelter from changes in surface water conditions. Interstitial spaces may offer areas of improved water quality and allow fish to minimise energy losses and mortality rates.

In addition to avoidance, acid water can induce other behavioural responses. Disruption of normal feeding behaviour and metabolism (i.e. reduced feeding and increased metabolic rate) have been reported in guppy fish (*Lebistes reticulatus*) (Urban-Jezierska, 2002) and in juvenile rainbow trout (Dockray *et al.*, 1998), although in the latter of these two studies, low pH conditions were combined with elevated temperatures (2 °C above the control summer temperature range of 16.5 – 21 °C) so the direct effect of reduced pH is unknown. Exposure to short pulses of acidified water has recently been shown to reduce feeding activity in Atlantic salmon smolts (Magee *et al.*, 2003).

Acid episodes can result in disruption or cessation of spawning behaviour. A number of reports have shown that exposure of maturing adults to sub-lethal low pH water affects gametogenesis and/or ovulation of the oogonia in salmonids, which may lead to reduced survival and growth and teratogenesis in the offspring (Menendez, 1976; Kennedy, 1980; Ikuta and Kitamura, 1995). Fish populations can therefore potentially be damaged by the negative effects of acidification on the reproductive process in adult fish; in addition to this, recent laboratory studies have demonstrated that the behavioural responses of fishes (i.e. reproductive behaviour) are also subject to change in acidic water, in particular those of salmonid species.

Acidification has been shown to disrupt and in some cases lead to the complete cessation of reproductive behaviour in some salmonid fishes, for example, Johnson and Webster (1977) showed that brook trout avoided spawning in low pH (below 4.5) upwelling water. Many species of fish are most vulnerable to low pH during early ontogenesis (Almer *et al.*, 1974; Peterson *et al.*, 1982); Peterson *et al.* (1982) observed that the period between hatching and the onset of exogenous feeding was particularly sensitive to low pH in most species. Following a comparison between the acid tolerance of larvae of six salmonid species, Ikuta *et al.* (1992) suggested that larvae of migratory species such as hime salmon are more vulnerable to acidity than non-migratory species such as charrs (*Salvelinus* spp.) and brown trout.

In a laboratory study on the effects of an acidic environment on the female sexual behaviour of hime salmon, fish were found to be extremely sensitive to the acidity of ambient water (Kitamura and Ikuta, 2000). Nest-digging behaviour was severely inhibited by only slight acidification (pH below 6.4) of the water, and below pH 6.0, almost no digging was observed. When ambient water was returned to nearly neutral (pH 6.6), only 4 out of 6 fish resumed digging behaviour. In their study comparing the female sexual behaviour of brown trout and hime salmon, Kitamura and Ikuta (2001) observed that spawning brown trout were also sensitive to the acidity of ambient water, and showed almost no digging at pH below 5.0. However, when water was returned to nearly neutral conditions, digging activity reappeared in all 6 fish tested. In these studies, the temporal acidification of the aquatic environment and subsequent return to a nearly neutral state is characteristic of acid episodes. The results of the first experiment suggest that in their natural environment, hime salmon may not select even slightly acidic areas for spawning. In combination with the second experiment, the lack of normal behaviour in some hime salmon indicates that following temporal acidification, the return of water pH to its original level may not result in spawning behaviour in all fish species. This idea supports the theory that migratory species are more vulnerable than non-migratory species – it has been suggested that the observed difference in behavioural responses reflect the species difference in terms of vulnerability to acidification (Ikuta *et al.*, 1992). The failure to resume normal spawning behaviour in hime salmon may apply to other behaviour types (for example, feeding and avoidance) following acid episodes, in other salmonid species. However, little is known about the behaviour of fish following acid episodes – previous studies have focused on changes during acidification, not after the event.

The ability of fish to resume normal behaviour may be as important to their survival as is the disruption of normal behaviour patterns during a pollution incident.

1.6 ATLANTIC SALMON

Fish play pivotal roles in almost all freshwater ecosystems, as well as being economically important. Stocks of Atlantic salmon and brown trout are worth over £15 million to the Welsh economy alone each year, yet wild Atlantic salmon are considered an endangered species worldwide. The anadromous lifecycle of wild salmon means that whilst oceanic conditions are an important factor influencing the survival of salmon populations (Friedland *et al.*, 1998), the quality of freshwater streams used for spawning, migration and growth in the juvenile stage are paramount for long term population survival (Bardonnet and Baglinière, 2000). The reported decrease in Atlantic salmon stocks can be largely attributed to anthropogenic factors, and their influence on freshwater habitat quality (Mills, 1989). Such factors may act as a barrier to migration (e.g. dams), or cause detrimental changes in water quality which preclude salmon survival (several types of pollution). Acidification has been responsible for the loss of many fish populations, however, salmonid fish are particularly sensitive to low pH for several reasons: (i) salmonids are more physiologically susceptible to acid toxicity than other fish; (ii) juveniles occur largely in upland areas where soils and rocks are more sensitive to acid deposition, and (iii) adults use headwaters disproportionately during spawning. The combination of high susceptibility and economic value make salmonids, particularly brown trout and Atlantic salmon, a focus for conservation and management efforts, with both species emphasised in the EU Habitats and Species Directive. This work will focus on Atlantic salmon as the most threatened and economically important of these two species.

1.6.1 Sheltering behaviour

The use of shelters is a behavioural trait commonly observed in many animals; in particular, several species of fish are known to shelter. These include European minnows (Greenwood and Metcalfe, 1998), coho salmon *Oncorhynchus kisutch*, steelhead trout *Oncorhynchus mykiss* (Walbaum) (Bustard & Narver, 1975), Atlantic salmon (Cunjak, 1988) and coelacanths *Latimeria chalumnae* (Fricke *et al.*, 1991). Sheltering behaviour has been perhaps best described in terms of stream-dwelling salmonids; Atlantic salmon provide a particularly good example of refuge use among fishes (Orpwood, 2005). As water temperatures decline during winter, there is a switch in behaviour from the 24-hour foraging typically observed in summer to daytime sheltering and nocturnal foraging (Rimmer *et al.*, 1983; Heggenes *et al.*, 1993; Fraser *et al.*, 1993, 1995; Valdimarsson *et al.*, 1997, 2000; Valdimarsson and Metcalfe, 1998). Work by Valdimarsson *et al.* has shown that this shift in behaviour is triggered as water temperatures decline to approximately 6 – 8°C (Valdimarsson *et al.*, 2000), although several species of salmonid are known to shelter at 10°C (Greenwood and Metcalfe, 1998).

Three primary functions of shelter use have been suggested (Orpwood *et al.*, 2003); possibly the most important of these is predator avoidance. Alexander (1979) suggests that mortality of stream-dwelling juvenile salmonids is strongly influenced by predation (Heggenes and Borgstrøm, 1988; Valdimarsson and Metcalfe, 1998). Fish are ectothermic and become more vulnerable to predation from endothermic organisms (such as piscivorous birds and mammals) at low temperatures due to slower swimming speed, reduced reaction times (Fraser *et al.*, 1995) and reduced mobility (Heggenes and Borgstrøm, 1988). Sheltering during periods of low temperature may also avoid the dangers of ice formation (Cunjak, 1988; Heggenes *et al.*, 1993; Whalen *et al.*, 1999). However, sheltering occurs at temperatures above zero (Fraser *et al.*, 1993) and in areas where ice formation is rare (Metcalfe *et al.*, 1999). It is likely that the impact of ice formation will be more pronounced at higher latitudes and altitudes. A third advantage of sheltering behaviour may be respite from stream currents, and the opportunity to conserve energy at low temperatures (Rimmer *et al.*, 1984; Heggenes *et al.*, 1993). However, juvenile Atlantic salmon have been shown to prefer dark refuges that allow them to hide, and the location of these refuges is not related to current (Valdimarsson and Metcalfe, 1998).

Functional explanations for sheltering become more complicated when spatial and temporal variation in water quality as a result of acid episodes are considered. To date, sheltering has only been considered in terms of the reasons give above, of which predator avoidance is the most widely accepted. However, in wild fish, sheltering might also reflect either a direct response to pulses of acid water during episodes and a movement to more favourable areas of water quality, or an advantage gained as a result of sheltering for other reasons. Thus, the interstitial spaces previously considered in terms of physical sheltering might also have more favourable water quality. This is currently unknown, due to the scarcity of studies on the interaction between surface and groundwater and the effects of acid episodes (characterised by increases in discharge following rainfall or snowmelt) on water chemistry in these zones.

1.6.2 Hatchery-reared vs. wild fish

An additional aspect of sheltering behaviour that may have implications for fisheries management is the differences that may arise as a result of rearing environment. Previous work has shown that wild salmon aggressively defend stream bed refuges, and the availability of refuges may strictly limit population size (Armstrong and Griffiths, 2001); whereas hatchery fish can be reared in large densities due to their willingness to share shelters. Other studies have demonstrated further behavioural differences between hatchery-reared and wild fish, including predator avoidance (Álvarez and Nicieza, 2003), movement (Bachman, 1984), foraging (Sosiak *et al.*, 1979), dispersal within a novel environment (Symons, 1969) and aggressive social interactions (Fenderson *et al.*, 1968), and reach the same general conclusion that as a result of such hatchery-induced changes, fish will have lower survival rates when released into natural streams than resident wild fish. However, differences between wild and hatchery-reared fish can sometimes be overcome by a period of 'training', in which hatchery fish are subjected to conditions mimicking those encountered in the natural environment in the hope that this will improve post-release survival (e.g. Berejikian *et al.*, 2000). It is possible therefore that there may also be differences in the behaviour of wild and hatchery-reared fish in response to acid episodes, and while sheltering within the stream bed may be an important behaviour type in avoiding surface water acidification, this behaviour may only develop following repeated exposure to acid episodes.

1.7 PROJECT CONTEXT AND AIMS

In the UK, the decline of commercially important species such as Atlantic salmon and brown trout has prompted the rehabilitation of damaged populations; up to ten million juvenile salmon are released annually. However, despite the decline of acidifying emissions and long-term increases in stream pH that have also occurred over the last three decades (Figures 1.5 and 1.6), these substantial stocking efforts have not been successful. Annual assessments of salmon stocks and fisheries published by the Environment Agency indicate a continuing decline in the number of salmon caught each year (Figure 1.7). One possible explanation for this is that in predicting the extent of recolonisation, routine pH monitoring does not take into account the short-term fluctuations that occur during acid episodes, which may be as damaging to fish populations as the long-term effects of acidity.

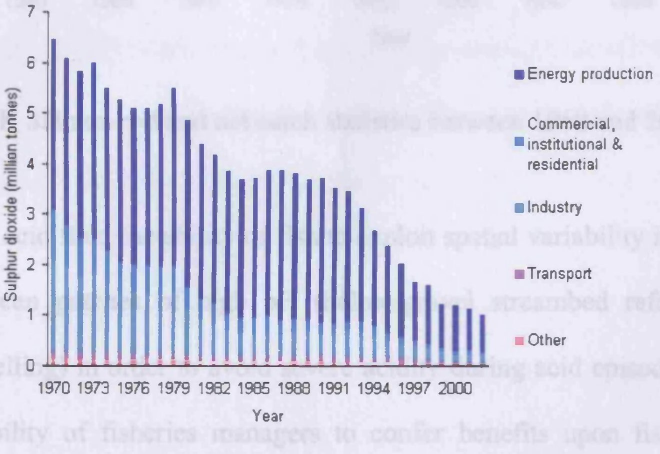
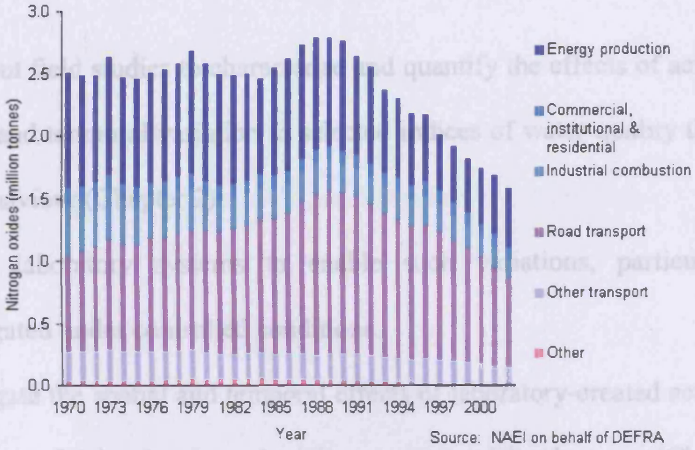


Figure 1.5: Atmospheric emissions of sulphur dioxide (SO_2) by sector in the UK, 1970 – 2002.



Source: NAEI on behalf of DEFRA

Figure 1.6: Atmospheric emissions of nitrous oxides (NO_x) by sector in the UK, 1970 – 2002.

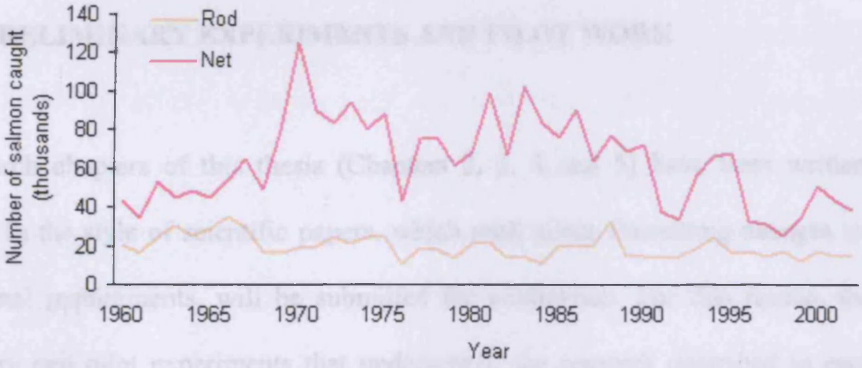


Figure 1.7: UK Salmon rod and net catch statistics between 1960 and 2000.

During periods of acid flux, the ability of fish to exploit spatial variability in habitat quality – i.e. moving between patches of high pH (below-gravel streambed refuges, or areas of groundwater upwelling) in order to avoid severe acidity during acid episodes – may become restricted. The ability of fisheries managers to confer benefits upon fish in the wild by manipulating habitat structure will be possible only when we understand the basis for differences in behaviour among environments, particularly with respect to additional constraints imposed by water quality and rearing environment.

Against this background, the main aims of this project were to:

1. Carry out field studies to characterise and quantify the effects of acid episodes on the spatial and temporal variation in selected indices of water quality that may influence fish behaviour (Chapter 2).
2. Design laboratory systems to enable such variations, particularly pH, to be investigated under controlled conditions.
3. Investigate the spatial and temporal effects of laboratory-created acid episodes on the behaviour of Atlantic salmon in different stages of development (Chapters 3 and 4).
4. Assess the effect of rearing environment on behaviour and survival of fish during acid episodes (Chapter 5).

1.8 PRELIMINARY EXPERIMENTS AND PILOT WORK

The research chapters of this thesis (Chapters 2, 3, 4 and 5) have been written and are presented in the style of scientific papers, which with minor formatting changes to conform with journal requirements, will be submitted for publication. For this reason, the various preliminary and pilot experiments that underpinned the research described in each chapter have been summarised within the relevant chapter, with examples of methods and the data acquired included as an appendix where appropriate.

CHAPTER 2

pH variations between surface and interstitial waters of Welsh hill streams during acid episodes and likely consequences for salmonids

2.1 SUMMARY

Evidence that streams have been recovering from chronic acidification over the last three decades has made it possible for fisheries managers to consider rehabilitating damaged salmonid populations by stocking streams and rivers with hatchery-reared fish. However, despite increases in average stream pH and other chemical changes consistent with recovery, substantial stocking efforts have not been successful and some salmon populations have continued to decline in acid-sensitive areas. An increasingly favoured hypothesis is that average measurements of pH mask short-term episodes of acidification that occur regularly in upland streams during rainstorms during which surface water pH may drop to $< 4.5 - 5.5$. Moreover, in western Britain the greatest frequency of episodes coincides with critical periods in the salmon life cycle (i.e. eggs and fry). This study aimed to evaluate the extent to which the hyporheic zone could provide a refuge from episodic effects. More specifically, it tested the hypothesis that variation in pH between interstitial and surface waters increases in streams during acid episodes. Surface and hyporheic water samples were taken during periods of high and low flow in four stream types (chronically acidified, treated by catchment liming, circumneutral and control [main river] streams) to test primarily for differences in pH between water depths, discharge conditions and stream type. The results supported the hypothesis: there was a significant difference in pH between high and low flow ($P < 0.001$), between stream types ($P < 0.001$); the difference between the surface and hyporheic samples was near-significant ($P = 0.144$). Importantly, pH in the surface water was lower at high flow

than low flow across all stream types (pH 5.04 ± 0.14 compared to pH 5.95 ± 0.06), i.e. the surface water was more acidic. The difference in pH between surface water and the hyporheos was significantly greater at high flow than at low flow (0.39 ± 0.14 vs. 0.27 ± 0.07; $P < 0.001$), suggesting that (i) acid episodes lead to greater variability in water chemistry between surface waters and the hyporheos of sensitive streams and (ii) there could be consequences for fish survival during episodes. These potential consequences were investigated in a series of laboratory experiments, presented in Chapters 3, 4 and 5.

2.2 INTRODUCTION

The effects of acidification on fish are potentially severe. Fish kills in Norway in the early 1900s, and declining salmon numbers in the 1970s, demonstrate how acidification can affect fish populations (Beamish and Harvey, 1972; Almer *et al.*, 1974; Beamish *et al.*, 1975; Harvey and Lee, 1980; Matuszek and Beggs, 1988; Turnpenny, 1989). The elimination of fish from thousands of lakes in acid-sensitive areas of Scandinavia (Muniz, 1984), and more widely in Europe and North America (Dillon *et al.*, 1984; Baker *et al.*, 1990) illustrates the impact of acid deposition on freshwater systems as a whole. While acidification has been responsible for the loss of many fish populations (Beamish and Harvey, 1972), salmonids such as Atlantic salmon, brown trout and Arctic charr are particularly sensitive to low pH, and are often the first to disappear. Not only are salmonids physiologically more susceptible to acid toxicity than other fish (Milner and Varallo, 1990), but also spawning adults and juveniles occur largely in upland areas where soils and rocks are most sensitive to acid deposition. This combination of high susceptibility and economic value has made salmonids, particularly brown trout and Atlantic salmon, a focus for conservation and management efforts.

In recent years, reductions in the acidifying emissions responsible for acid rain have been recorded (Jenkins *et al.*, 1998; Cooper and Jenkins, 2003), and fisheries managers have attempted to rehabilitate damaged populations through stocking. However, despite marked increases in stream pH, such stocking efforts have not been successful. One possible explanation is that the continued occurrence of acid episodes negatively affects salmonids despite increasing mean pH. Acid episodes are characterised by changes in stream-water chemistry associated with increased stream flow (Wigington *et al.*, 1990), and are considered a major management issue throughout upland areas of Europe (Lepori *et al.*, 2003). During acid episodes, pH decreases and the concentration of potentially toxic metals such as aluminium increases. Acid episodes most commonly occur following spring snowmelt or orographic rainfall, in which an airstream is forced to rise over a mountain range, though episode duration varies depending on the driving weather conditions – snow melt episodes typicall last days to weeks, whereas rainfall episodes take place over the course of hours to days. It is likely that average measurements of pH used to predict recolonisation may therefore mask these short-term fluctuations to pH < 4.5 – 5.5 that could increase acid toxicity to salmonids and other organisms.

While all the foregoing evidence suggests that episodes would have negative effects on salmonids, other reasoning suggests effects might not be so clear cut. The hydrological and hydrochemical factors that give rise to episodes are complex, reflecting the balance between (i) the addition to runoff of accumulated organic or mineral acidity from soils and (ii) the dilution of base-cations and alkalinity at high flow (e.g. Wigington *et al.*, 1990; Kowalik *et al.*, 2007). However, variations between stream habitats or reaches in flow paths can create further variability (Baldigo and Lawrence, 2000; 2001; Evans *et al.*, 2006). In particular, differences in flow velocity and residence times between the surface and hyporheos could limit dilution in the latter to create a zone of relatively stable pH. Similar variations in other aspects of water quality can have disproportionate importance for fish by affecting the survival of early life stages associated with interstices – namely fry and eggs (Malcolm *et al.*, 2003a, b, 2004a, b, 2005; Youngson *et al.*, 2004; Soulsby *et al.*, 2005). In addition, adults could take advantage of any such conditions with a behavioural trait observed in many salmonid species: sheltering in interstitial spaces or under large rocks. To date, sheltering has only been interpreted, for example, as protection from predators. However, sheltering could also reflect either a direct response to areas where stream water quality is locally more favourable (due to higher pH) or an incidental advantage gained as a consequence of sheltering for other reasons. Is it possible that the interstitial spaces previously considered in terms of physical shelters may also be those areas with favourable water quality? Information on the interaction between surface and ground-water, and the effect of increases in discharge (i.e. acid episodes) on water chemistry in these zones, is scarce.

In addition, although the large-scale movement of fish away from acid water and towards areas of higher pH is well documented, there are currently no studies that consider the smaller scale movement into streambed shelters due to the possible differences in chemistry between the hyporheic water and surface water. This variation in chemical quality may have critical importance in the rehabilitation of damaged fish stocks, but to date, information is severely lacking. The ability of fisheries managers to confer important benefits upon fish in the wild by manipulating habitat structure will be possible only when we understand the basis for differences in behaviour among environments, particularly with respect to additional limits imposed by water quality. This study therefore aimed to quantify differences in pH that may arise between the hyporheic zone and surface water as a result of increased discharge. Specifically, the work addressed whether pH varies between the surface and the hyporheos in streams differing in acid sensitivity as a result of acid episodes.

2.3 METHODS

2.3.1 Preliminary experiments

It was hypothesised that differences in stream chemistry could exist between the surface water and hyporheos in acid-sensitive streams and that such differences would create separate and distinct habitats, both accessible by Atlantic salmon. Preliminary work was therefore carried out in June – July 2005 to primarily determine if such differences existed, but also to allow suitable sites to be chosen for further work, and to enable testing of equipment and time periods over which sampling would take place. These initial studies were used (i) to design laboratory experiments to test the acid avoidance and sheltering abilities of Atlantic salmon and (ii) to modify the methods used in fieldwork the following year. Methods and results of the preliminary study are given in Appendix 1.

2.3.2 Study site

The main part of the study was carried out in the upper reaches of the River Wye catchment, near Llangurig, Wales, in September and December 2006. Four stream types were surveyed within a 4 km radius (such that the same weather conditions would similarly impact each site), either along the main river or in smaller tributaries that were chosen to represent the following stream types: chronically acidified, treated by catchment liming, circum-neutral and main river (Figure 2.1). Site selection was informed on the basis of data available from other studies (Lewis *et al.*, 2006). The aim was to compare water chemistry between streams that were likely to differ in short-term pH fluctuations due to the surrounding geology and land use, and between the surface and hyporheic water within the same stream type. On the basis of the 2005 study (Appendix 1) two replicate streams within each type were sampled, to give a total of 8 streams, the location of which is shown in Figure 2.1. A summary of site characteristics is given in Table 2.1.

Figure 2.1: Map showing location of sample streams in the River Wye catchment, mid-Wales: chronically acidified (A), limed (L), circumneutral (C) and main river sites (M) (total of $n = 8$; per site type $n = 2$).

Table 2.1: Summary of sampling site features, including Length/Width/Depth (m) at Low and High flow, stream bed characteristics, surrounding vegetation and dominant land use.

Site Code	**Stream Type**	**~Length**	**~Width (Low/High)***	**~Depth (Low/High)****	**Stream bed material**	**Vegetation*****	**Land Use**	**OS reference*******
L1	Limed	10 m	1.10 m 1.80 m	0.20 m 0.95 m	Silt, gravel, pebbles, cobbles and some boulders	Grassland, shrubs, overhanging deciduous trees	Farming (sheep)	883795
A1	Chronic	10 m	1.50 m 1.90 m	0.10 m 0.30 m	Mostly pebbles, some larger cobbles and boulders	Flat grassland, some low shrubs and deciduous trees	Farming (sheep and cows)	871808
M1	Main	10 m	6.90 m 16.00 m	0.15 m 1.25 m	Silt, gravel, and pebbles	Flat grassland, some low shrubs and overhanging shrubs and deciduous trees	Farming (sheep and cows)	872701
L2	Limed	10 m	7.35 m 9.20 m	0.30 m 0.90 m	Mostly silt, some larger pebbles	Grass, low shrubs	Forestry Commission	840826
A2	Chronic	10 m	2.10 m 3.90 m	0.10 m 0.60 m	Mostly silt, some larger pebbles	Grass, low shrubs, coniferous woodland	Forestry Commission	853814
M2	Main	10 m	8.75 m 19.50 m	0.25 m 0.95 m	Silt, gravel, pebbles, cobbles, few large boulders	Grass, low shrubs, gorse	Forestry Commission	845824
C1	Circum-neutral	10 m	1.10 m 1.40 m	0.15 m 0.35 m	Exposed bedrock, gravel, pebbles	Little riparian vegetation, flat grassland	Farming (sheep), recreational rally driving	823892
C2	Circum-neutral	10 m	2.20 m 3.70 m	0.25 m 0.50 m	Exposed bedrock, gravel, pebbles	Little riparian vegetation, flat grassland	Farming (sheep), recreational rally driving	824856

* Width measured from one bank to the other, across the centre of each site (i.e. 5 m from either end)

** Depth measured at point above central hyporheic tube

*** Indicates primary vegetation on either bank along 10 m site length

**** All taken from OS Landranger sheet 136

2.3.3 Sampling procedure

The aim of this study was to compare surface water with the hyporheic zone before and after an acid episode (i.e. following a prolonged period of rainfall and increased discharge); prior to sampling, three hyporheic extraction tubes were therefore installed at each sampling site. These consisted of a 25 mm air-stone (typically used to oxygenate fish tanks) connected to a 500 mm (6 mm internal diameter) length of polyethylene tubing, of which the lower 100 – 150 mm was inserted below streambed level. Following installation, the hyporheic tubes were left undisturbed for 4 weeks before any water quality sampling commenced. Each site was approximately 10 m long, and sampling tubes were positioned equidistantly apart (3 m from one another), with the lowermost and uppermost pipes each 2 m in from the site boundary. At each site, two samples were collected (surface and hyporheic water) from three locations according to the position of the hyporheic tubes (termed upstream, middle and downstream) using the protocol detailed below.

Sites were sampled from the downstream position first moving in an upstream direction, so as to avoid disturbing filtration tubes by dislodging sediment. When filling sample bottles, the containers were held with the neck facing upstream, keeping hands and feet downstream. Surface water was collected from the water column in the region above the groundwater tubes by immersing the sample bottle under the surface of the water until the bottle had filled. Hyporheic water was collected using a 50 ml plastic syringe attached to the extraction tube. For both the surface and hyporheic water samples, a 250 ml bottle was rinsed with river water then filled to the brim with sample water excluding air (separate bottles were used for each sample); the sample was unfiltered. Following collection, three variables were measured in the field for each sample; pH, temperature (± 0.1 °C) and conductivity (± 0.1 μS/cm) using a Hanna HI 9024 pH/temperature meter in combination with a Hanna HI 1053B low ionic strength pH electrode, and a Hanna HI 98308 conductivity meter.

Sites were sampled once in September and once in December according to low and high flow conditions, which were determined through continued monitoring of six-hour weather reports over the August – December period. Low flow sampling took place following a two-week period in which no precipitation fell on the sample sites; such that sampling occurred at very low flow, and was not affected by an acid episode. High flow sampling took place following three days in which heavy rain fell on the Llangurig area, and streams were at their highest point since the Septmember samples were collected. This 'targeted' approach has previously been shown to be effective at characterizing event chemistry in biological studies (Lepori *et al.*, 2003) and also avoids the difficulties of accurately determining mean or extreme chemistry at acid-sensitive sites from random sampling (Brewin *et al.*, 1996).

2.3.4 Statistical analysis

Where appropriate, mean pH values were calculated directly from the pH frequency distribution rather than after conversion to Hydrogen ion concentration, since pH values are normally distributed, rather than log-normally distributed. Over the range pH 5-7, typical of surface water, differences in outcome for the two procedures are only around 1-3%. Variations in pH, temperature (°C) and conductivity (µS) among streams, stream classes and depth (hyporheos versus surface water) were assessed using a General Linear Model. Any significant interactions between all combinations of these factors were also noted using multiple Tukey-Kramer *post-hoc* pairwise comparisons. A significance level of $P < 0.05$ was used throughout the analyses. Mean values are shown ± standard error throughout the results discussion. Complete statistical results are given in Appendix 2.

2.4 RESULTS

2.4.1 pH

pH differed significantly between low and high flow ($F_{1,89} = 23.65$, $P = < 0.001$) (Figure 2.2); pH was generally lowest under high flow conditions though this varied with depth – the average pH in the surface water was pH 5.04 ± 0.1 compared to pH 5.95 ± 0.1 under low flow conditions. There was a significant difference between stream type ($F_{3,89} = 16.80$, $P < 0.001$); in general, the chronic and limed streams were more acidic than the main and circumneutral streams, particularly at high flow. The difference between surface and hyporheic water was near-significant ($F_{1,89} = 2.18$, $P = 0.144$); the difference depended on flow conditions (described below). The interaction between factors (flow level*stream type*sample depth) was not significant ($F_{3,89} = 1.10$, $P = 0.356$).

At low flow, although the difference in pH between surface water and hyporheic water varied with stream type, pH remained consistently higher in surface water than in hyporheic water. At high flow, this situation was reversed; surface water pH was consistently lower across stream types than hyporheic water pH – surface water was more acidic than hyporheic water. The overall difference between surface and hyporheic water pH within stream type was significantly greater ($F_{1,41} = 17.04$, $P < 0.001$) at high flow (0.39 ± 0.1 pH units) than at low flow (0.27 ± 0.1 pH units).

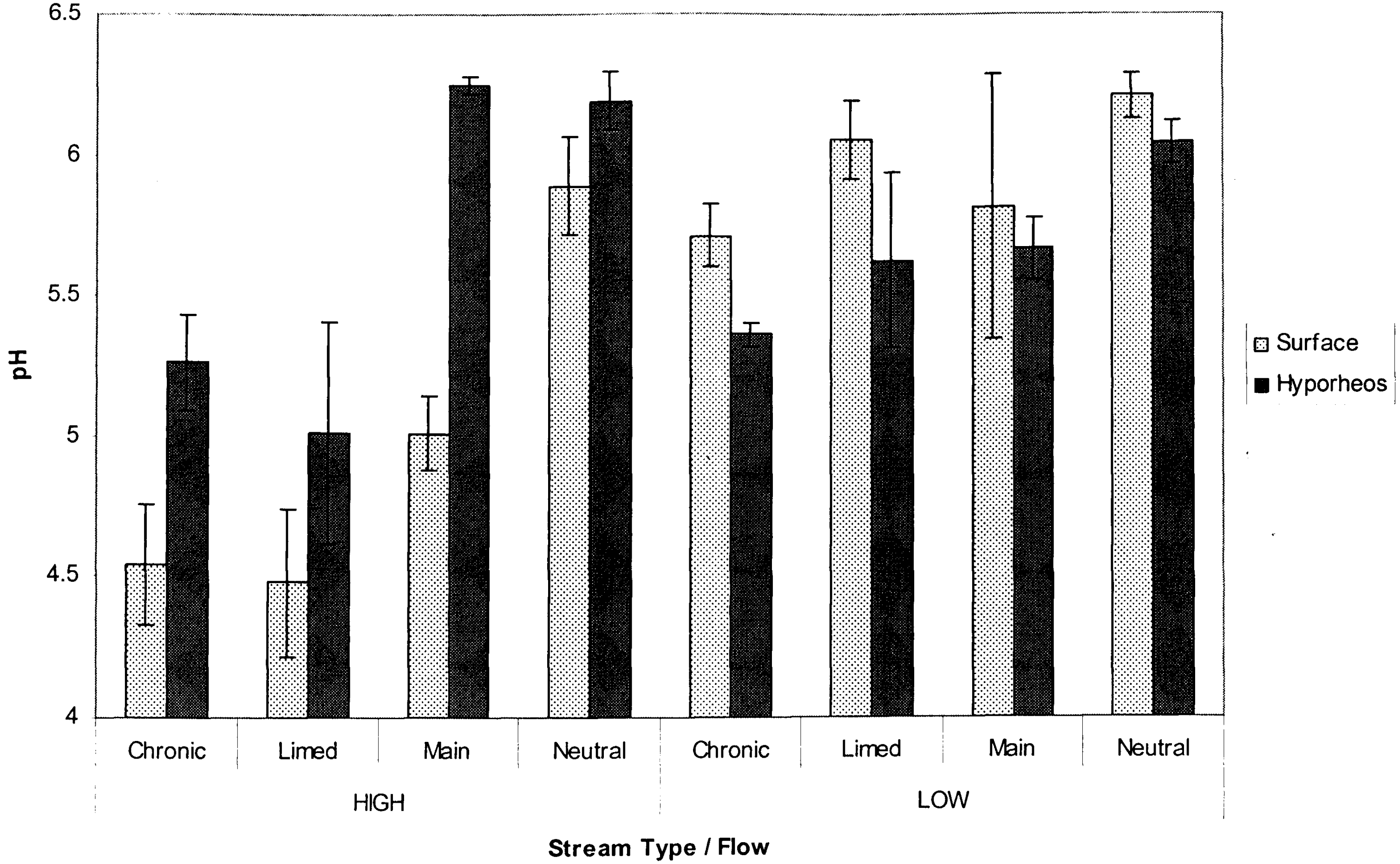


Figure 2.2: Mean pH ± SE ($n = 6$) in surface water (stippled columns) and the hyporheic zone (shaded columns) across four stream types at high and low flow.

2.4.2 Temperature

Temperature differed significantly between low and high flow ($F_{1,89} = 1717.67$, $P < 0.001$); in general, temperatures were higher during low flow (Figure 2.3). There was a significant difference between stream type ($F_{3,89} = 30.33$, $P < 0.001$), with temperatures being highest in the main river samples. The difference between surface and hyporheic water was also significant ($F_{1,89} = 52.26$, $P < 0.001$) – temperature was consistently higher in the hyporheic samples, though the degree of difference varied, as described below. There was a significant interaction effect between flow level*stream type*sample depth ($F_{3,89} = 7.97$, $P < 0.001$).

At both high and low flow, the difference in temperature between surface and hyporheic water varied with stream type, though the greatest differences were observed in the main river samples in each case (high: 3.3 ± 0.2 °C; low: 0.5 ± 0.1 °C between the main river surface water and hyporheos). Although the hyporheic zone was consistently warmer than the surface water, the magnitude of the difference varied between flow levels. At high flow, there was an average difference of 1.10 ± 0.3 °C, which was significantly higher ($F_{1,41} = 10.03$, $P < 0.005$) than the difference in the low flow samples (0.3 ± 0.1 °C). These results lend further support to the theory that acid episodes may lead to increased spatial variability between habitat patches.

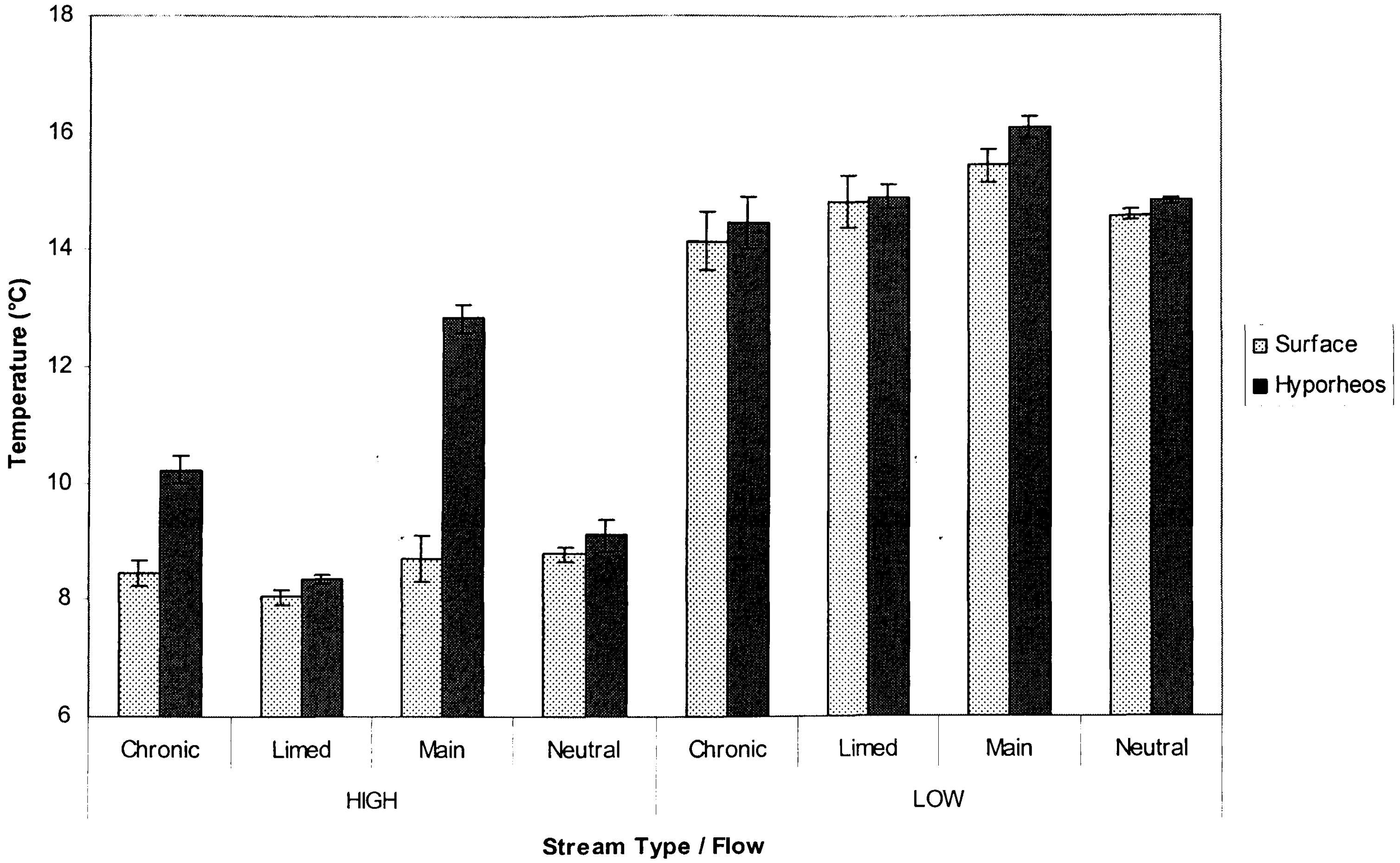


Figure 2.3: Mean temperature (°C) ± SE (n = 6) between surface water (stippled columns) and the hyporheic zone (shaded columns) across four stream types at high and low flow.

2.4.3 Conductivity

Conductivity differed significantly between low and high flow ($F_{1,89} = 84.18$, $P < 0.001$); on average, conductivity was higher under high flow conditions across all stream types and depths (high flow: 58.0 ± 1.2 µS vs. low flow: 42.2 ± 1.2 µS) (Figure 2.4). There was no significant difference between stream type ($F_{3,89} = 1.52$, $P = 0.217$) or between surface and hyporheic water ($F_{1,89} = 0.08$, $P = 0.772$), though some interesting trends were observed: at low flow, hyporheic water had consistently higher conductivity than surface water (hyporheic: 43.6 ± 2.1 µS vs. surface 41.0 ± 1.2 µS). This situation was reversed at high flow: with the exception of the neutral stream type (where hyporheic conductivity was still greater than that of the surface water), all stream types showed higher conductivity in the surface water (surface: 58.6 ± 1.8 µS vs. hyporheic: 57.4 ± 1.6 µS). The level of difference varied according to stream type at both flow levels, but was not significantly greater at high or low flow (high: 5.1 ± 1.8 µS vs. low: 3.2 ± 1.4 µS; $F_{1,41} = 0.72$, $P > 0.5$). The interaction between factors (flow level*stream type*sample depth) was not significant ($F_{3,89} = 0.35$, $P = 0.789$).

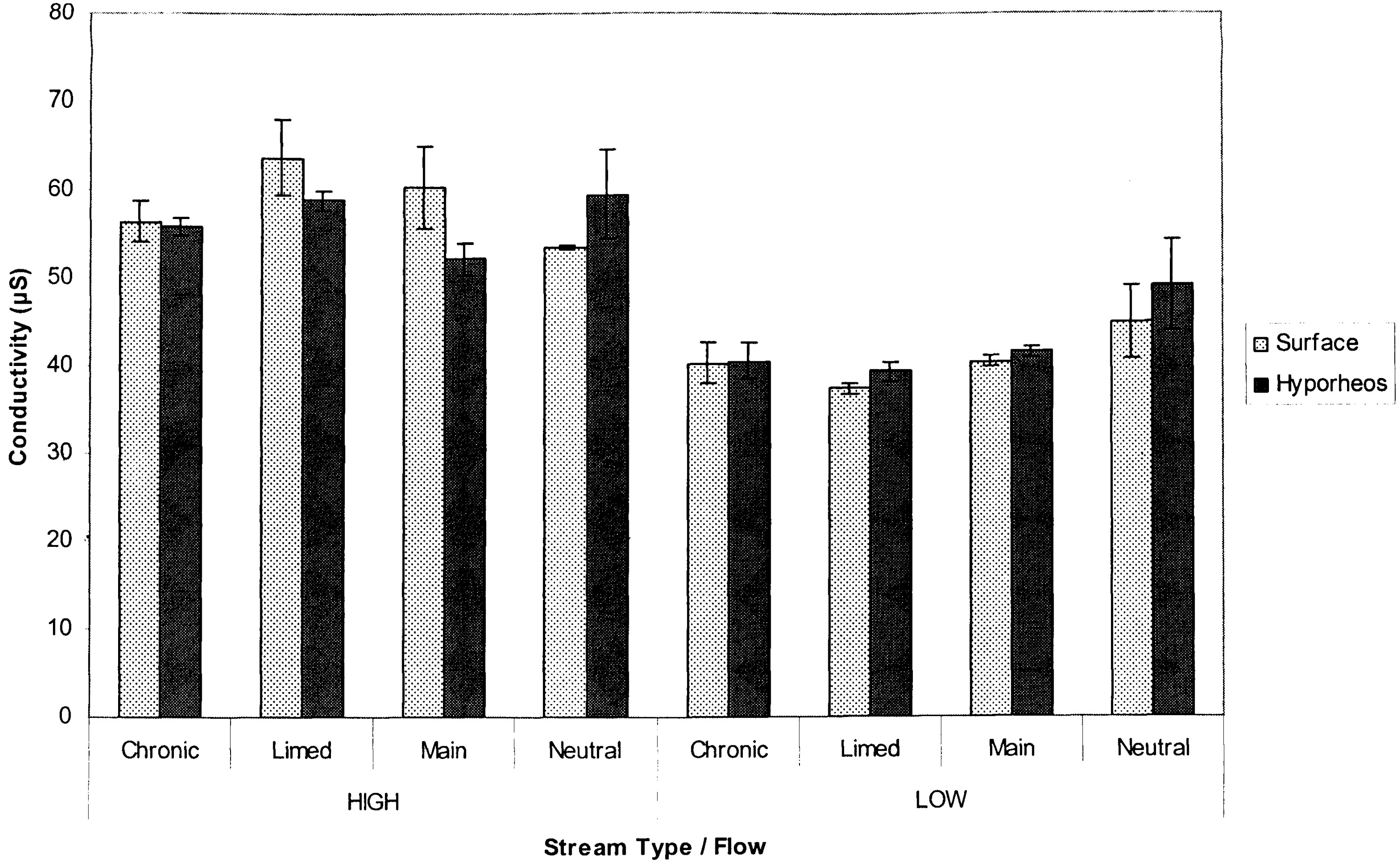


Figure 2.4: Mean conductivity (µS) ± SE (*n* = 6) between surface water (stippled columns) and the hyporheic zone (shaded columns) across four stream types at high and low flow.

2.5 DISCUSSION

The aim of this study was to examine the extent to which differences in chemistry between the surface water and hyporheic zone are affected by increased discharge and acid episodes, by taking water samples from within these two zones before and after an episode. The results indicate that there was a difference in chemistry between the surface and hyporheos. Significant differences or near-significant differences were observed for pH and temperature, but not for conductivity (though differences were still recorded in measurements of conductivity between the surface water and hyporheic zone, flow level and stream type). These results support the view that these two areas are indeed separate and distinct habitats. In addition, differences between the surface and hyporheos increased following a prolonged period of discharge; surface water across all stream types appeared to be more vulnerable to changes in chemistry during an acid episode, whereas the conditions in the hyporheic zone were more consistent, providing a stable environment for fish within this habitat patch.

There are currently no other studies on the inter-habitat variability (i.e. surface *vs.* hyporheic) in water chemistry in relation to increased discharge and acid episodes, so it is difficult to reach a conclusion as to whether these findings might be general and exactly what the implications might be for salmonids. However, some Scottish studies suggest that the interaction between surface and groundwater within the hyporheic zone and differences in water quality between the surface and groundwater (including temperature and dissolved oxygen) may be of critical importance in salmonid egg and embryo survival (Malcolm *et al.*, 2003a, b, 2004a, b, 2005; Youngson *et al.*, 2004; Soulsby *et al.*, 2005), though the additional impact of increased discharge was not considered.

The results of this study are particularly important in relation to the life history of Atlantic salmon and the timing of events such as hatching, emergence and smoltification, in addition to behavioural traits such as sheltering. Atlantic salmon are known to be found in the streams sampled in this study (personal experience of electrofishing with the Environment Agency), and will therefore experience and be extremely vulnerable to the changes in chemistry observed in this experiment. Adult salmon spawn during winter (typically November and December) and eggs then hatch in spring, after which the young fish (alevins) remain within streambed interstices in the hyporheic zone, relying on their yolk sac for nutrition. Following yolk sac absorption (a short transitional period of just a few weeks), fry become parr, and emerge from their gravel refuges to commence independent feeding for the first time. These critical periods all coincide with the times of year when episodes are most likely to take place – during autumn and winter rainfall, and following snowmelt in the spring. Mortality during the early life stages of salmonids is high, and may occur during the egg stage (MacKenzie and Moring, 1988), when the alevins are still in the gravel (Coble, 1961; Phillips et al., 1975), when salmon first emerge to feed (Elliot, 1994), and later (Elliott, 1997). High spring floods may affect mortality at all these stages (Jensen and Johnsen, 1999), often reducing entire year classes of fish (Elwood and Waters, 1969), since fish populations within waters subject to acid episodes may be close to their survival threshold, and even a slight deviation from optimum conditions may result in the decline of a whole population (Sayer *et al.*, 1993).

In a study on the functional relationship between peak spring floods and survival and growth of juvenile salmonids in Norway (Jensen and Johnsen, 1999), it was observed that mortality of Atlantic salmon and brown trout increased significantly in years with high discharge during the alevin stage. The increased mortality was attributed to the effect of increased flow, and not chemical changes within the surface water as a result of increased discharge. 'Washout' effects – where young fish with limited swimming ability are pushed downstream by increased flow – have been observed in other studies (Heggenes and Traaen, 1988). However, this study has shown that variability between surface and hyporheic chemistry increases significantly following prolonged discharge; it is therefore possible that the increased mortality observed in the Norwegian study could also be attributed to changes in pH that occurred as a result of increased discharge.

For juvenile salmon that do survive the first critical stages of life, acid episodes may still be a limiting factor in the persistence of wild populations. However, the study mentioned previously also stated that for one-year-old fish or older, the extent of the peak spring floods did not influence mortality significantly. This may in part be due to sheltering behaviour, which has been widely observed in many species, including Atlantic salmon (Rimmer *et al.*, 1983; Cunjak, 1988; Fraser *et al.*, 1993; Valdimarsson *et al.*, 1997, 2000; Valdimarsson and Metcalfe, 1998; Metcalfe *et al.*, 1999; Bremset, 2000; Orpwood *et al.*, 2003, 2004). Sheltering (seeking refuge on the streambed in gravel interstices) is typically thought to provide protection primarily from predators (Valdimarsson and Metcalfe, 1998; Metcalfe *et al.*, 1999), though it is possible that it may also take place in response to changing water conditions. Avoidance of acidified water has been widely studied in Atlantic salmon, though there are few *in situ* studies (Gunn and Noakes, 1986; Gagen *et al.*, 1994). Downstream movement of Atlantic salmon during an acid episode has been reported, but no distinction was made between active swimming or passive drift (Skogheim *et al.*, 1984). Directional avoidance has been observed in other salmonids, where acidic episodes have resulted in a downstream movement (Leivestad and Muniz, 1976; Hall *et al.*, 1980; Gagen *et al.*, 1989) though whether salmon are able to actively avoid acid water with the intention of making use of a more favourable streambed shelter is unknown. The high flow results observed in this study, particularly those within the main river, suggest that there is a real refuge within the hyporheos in exactly the location where salmonid densities will be greatest – it almost certainly means that salmonids can persist in the upper catchment of the main river and if salmon seek refuge within the hyporheos, the otherwise detrimental effects of an acid episode may be mitigated.

CHAPTER 3

Exposure to acid episodes and avoidance behaviour in Atlantic salmon alevins

3.1 SUMMARY

Acidified surface waters are characterised by the gradual decline of fish populations and by fish kills during episodes of increased acidity in rivers. During acid episodes, free-swimming salmonid fish can detect changes in water chemistry and commonly exhibit avoidance behaviour. Fish are known to be most vulnerable to acid conditions during life cycle changes (i.e. hatching, smoltification), though there are few studies on Atlantic salmon. This experiment focussed on newly emerged salmon alevins, in which marked sensitivity reflects their transition from yolk-sac to independent feeding. A vertical substrate-filled tank with a pH gradient was used with post yolk-sac alevins to test the hypotheses that (i) alevins move away from acid into circumneutral water and (ii) opercular rates during exposure to acid increased relative to alevins in neutral water. The results supported both hypotheses: alevins tended to avoid acid pulses of pH 4 – 5 by moving downwards into less acid regions of the tank ($P < 0.005$), and the opercular rate of those fish exposed to acid water (approximately 130 beats per minute) was significantly higher than fish in circumneutral conditions (approximately 80 beats per minute) ($P < 0.005$). These results confirm acid sensitivity in salmon alevins, but also reveal avoidance behaviour that could have survival value if acid-base gradients occurred in the stream hyporheos.

3.2 INTRODUCTION

Freshwater acidification threatens all life stages in salmonids (Skogheim *et al.*, 1984), although effects on the early life stages are often the main cause of population decline and loss (Jensen and Snekvik, 1972; Rosseland *et al.*, 1980). The physiological effects of chronically low pH on freshwater life stages of Atlantic salmon are well documented (Fay *et al.*, 2006) and deleteriously affect sexual maturation (Valtonen and Laitinen, 1998), spawning (Tam and Payson, 1986; Hutchinson and Sprague, 1986; Vourinen *et al.*, 1990, 1992), fertilisation (Sayer *et al.*, 1993), egg development (e.g. in Atlantic salmon: Peterson and Martin-Robichaud, 1982; Eddy and Talbot, 1985; McWilliams and Shephard, 1991), and hatching and survival of larvae (Carrick, 1979; Parker and McKeown, 1987). In waters where acidification is most likely, the early life stages of many freshwater fishes will be close to their survival threshold and only slight acidification can result in the loss of complete year classes, which would affect recruitment and eventual population status (Sayer *et al.*, 1993).

Alevins (following yolk sac absorption) are among the most susceptible life stages, during which transitional period mortality is high even where healthy populations occupy optimum habitat (Fay *et al.*, 2006), possibly due to the behavioural stresses related to the change in feeding patterns, i.e. from absorbing nutrients from a yolk sac to foraging for water-borne food items. Vulnerability increases once dependence on the yolk has come to an end (Sayer *et al.*, 1993) and mortality during alevin emergence is high even in undisturbed, normal conditions (Gustafson-Marjanen and Moring, 1982; MacKenzie and Moring, 1988; Pauwels and Haines, 1994). At the end of the yolk absorption period, the young fish move from incubation sites in the stream hyporheos into the open water. Here, water chemistry is likely to fluctuate more than in the substratum, for example due to pH fluctuation during rainstorms or snowmelt (Chapter 2). Emergence of several salmonid species coincides seasonally with the onset of such episodic pH depressions (e.g. brook charr: Gunn and Noakes, 1986; Curry *et al.*, 1991; lake charr: Gunn *et al.*, 1987; Atlantic salmon: Magee *et al.*, 2003) with potential consequences for behaviour, physiology, survival and population dynamics. Changes in swimming activity/range and frequency of movement have been observed in brook trout (Cleveland *et al.*, 1986, 1989; Hunn *et al.*, 1987; Jordahl and Benson, 1987), brown trout (Reader *et al.*, 1988; Sayer *et al.*, 1991a) and Atlantic salmon (Daye and Garside, 1979) following exposure to acidified water. If swimming ability is impaired, fish are less likely to survive in the field because individuals may either not be able to emerge from the substrate in which they hatched, or not be able to seek shelter in more favourable environments following emergence. Feeding may also be inhibited (Haya *et al.*, 1985), and fish will be more vulnerable to predation.

Some studies have shown that fish nearing the end of the yolk absorption period exhibit an avoidance response when exposed to short term pulses of pH, which may enhance survival in areas subject to acid episodes (for example, brook charr alevins: Gunn and Noakes, 1986). Similar behavioural changes have been observed in other species, for example, brown trout (Atland, 1998), though little is known about the role avoidance behaviour may play in enhancing survival of Atlantic salmon alevins in acid-sensitive waters, or those now recovering from chronic acidification (e.g. Kowalik *et al.*, in press). This study therefore tested whether salmon alevins are able to avoid pulses of acidified water by moving into an area of patches with more favourable water chemistry. Opercular rate and mortality (if any) were also examined as indicators of physiological stress brought on by exposure to acidified water.

3.3 METHODS

3.3.1 Experimental animals

Fertilised Atlantic salmon eggs from wild-caught fish were collected from the Environment Agency hatchery at Abercynrig (OS Explorer sheet 12, grid reference 064264), near Abergavenny, Wales in March of 2006. Prior to collection, the eggs were housed in shallow tanks on top of a mesh shelf, supplied with Afon Cynrig river water (circum-neutral), and nearing hatching. Following transportation to the temperature-controlled facilities at Cardiff University (11.9 ± 0.1°C, n = 47 [measurements taken during incubation period and experiment]) the eggs were housed in a tank of free running de-chlorinated mains water, and monitored for hatching progress. After hatching, alevins were allowed to absorb almost all of the yolk-sac to become free swimming fish ready to begin independent feeding (Mills, 1971), after which the experiment commenced. Once used in the experiment, alevins were kept in a separate tank to avoid re-use. Following completion of the study, fish were humanely euthanased (Home Office PIL 30/6656).

3.3.2 Test arena

The alevins were observed in a purpose-built, vertical, substrate tank similar to that used by Gunn *et al.* (1987). The 45 H x 40 W x 6 D cm tank contained 9 artificial 'shelves' consisting of a layer of Perspex covered with 'astro-turf' spaced at 1.5 cm intervals between the top and bottom of the tank along two runners at either side. Each shelf was perforated with equal numbers (eight per shelf) of evenly spaced 1 cm diameter holes to allow the fish free movement between different levels. The tank had two inlet/outlet pipes at each side, to allow aliquots of tank water to be sampled from different depths within the tank as described in 'Pilot work' below.

Throughout the experiment, de-chlorinated mains water (pH 7) flowed through the tank from an inlet pipe at the bottom of the tank on one side, and drained from an outlet pipe at the top of the opposite side of the tank, thereby creating an upwelling groundwater effect. Water in the test tank did not cool to the same temperature as water in the holding tank due to the different volumes and flow rates in each tank, so temperature was controlled using a LEEC closed-circuit cooling device (11.5 ± 0.2°C, n = 14 [measurements taken during experiment in test arena]); the inlet pipe was repeatedly coiled around the cooling pipe of an LEEC closed-circuit cooling device, though there was no direct contact between water used in the experiment and coolant fluid. It is possible that the cooling element may have resulted in a vertical temperature gradient within the tank. Though the low flow rate of water through the tank makes it unlikely that any such gradient would have developed, any influences of temperature would have been identical across treatment groups.

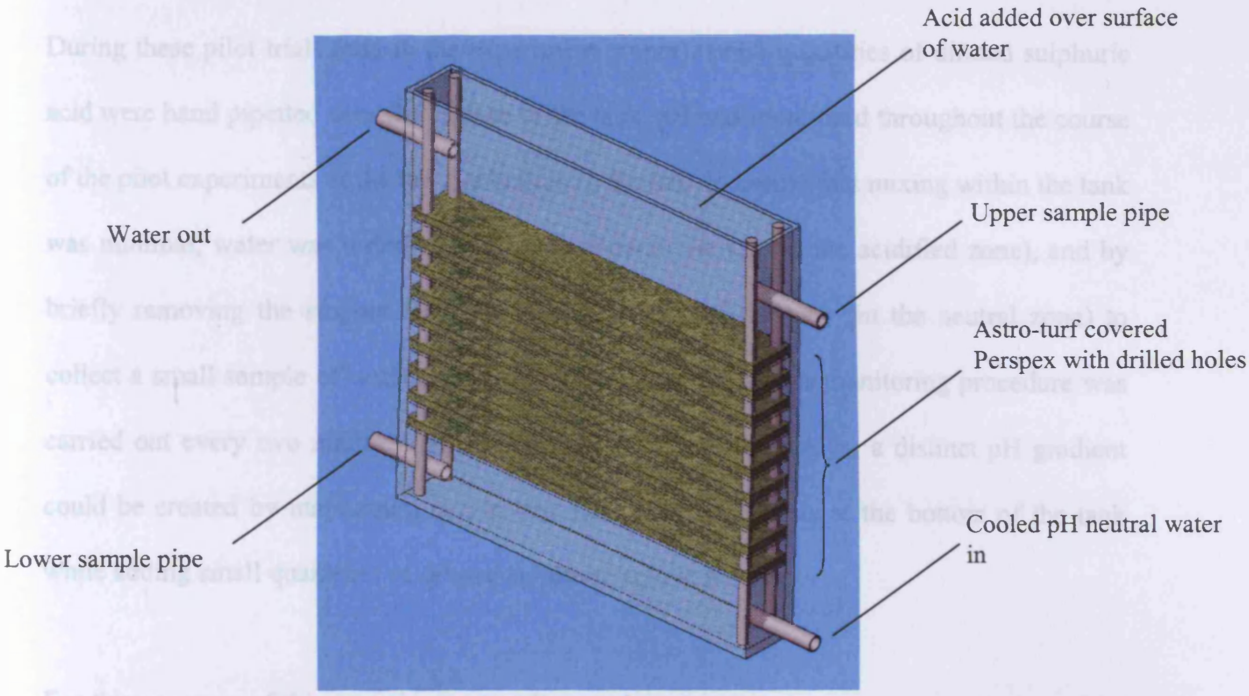


Figure 3.1: Representation of the vertical substrate-filled tank used to study alevins movement during a simulated acid episode, with astro-turf-covered Perspex shelves and inlet/outlet pipes at each side (after Gunn *et al*., 1987).

3.3.3 Pilot work

In preparation for the experiment, it was necessary to create a pH gradient within the tank, such that the surface water was 'acid' and the deeper water 'neutral', while ensuring that vertical mixing between these two zones was minimised. This mimicked the situation in real streams where episodically acid surface water flowed over a circumneutral hyporheic zone. The method by which a pH gradient could be created and maintained was first tested using cochineal red dye, a common food colouring. The dye was hand pipetted over the surface of the tank over the course of 40 minutes to determine whether a divide between surface and deeper water could be created and if so, the necessary quantities of coloured water required.

These trials showed that the combination of upwelling groundwater from the bottom of the tank with the addition of small quantities (5 ml or less) at the top of the tank over the course of the test period would create the necessary divide; this protocol was then replicated using 5M sulphuric acid (H_2SO_4) diluted to pH 3 with the same de-chlorinated mains water used in the control treatments, before any fish were tested.

During these pilot trials (and in the experiment proper), small quantities of diluted sulphuric acid were hand pipetted over the surface of the tank. pH was monitored throughout the course of the pilot experiments at the top and bottom of the tank to ensure that mixing within the tank was minimal; water was tested in the tank outflow at the top (in the acidified zone), and by briefly removing the stopper from the bottom section of the tank (in the neutral zone) to collect a small sample of water before replacing the stopper. This monitoring procedure was carried out every two minutes, and established that, as with the dye, a distinct pH gradient could be created by maintaining a constant flow of neutral water at the bottom of the tank while adding small quantities of diluted sulphuric acid at the top.

For the purposes of this experiment, 'acid' was classed as pH 4 – 5, a range which avoided lethal conditions while creating the necessary simulation and stress. Previous laboratory and *in situ* studies by other authors have shown Atlantic salmon to be acid sensitive, with a lethal pH of between 3.5 – 4.0 according to age (Daye and Garside, 1977) and an avoidance threshold of pH 5.0, above which avoidance is not triggered (Atland and Barlaup, 1996). The field investigation described in Chapter 2 suggested that this pH range is also environmentally relevant, and is likely to simulate the conditions juvenile fish are exposed to in the natural environment. The term 'neutral' refers to the de-chlorinated mains water (pH 7) supplied to the aquarium room in which the experiments took place.

3.3.4 Experimental protocol

At the start of each trial, 10 alevins were selected at random from the holding tank by scooping a group of alevins into a net and transferring the first 10 to the test tank. Alevins were not size-selected; all fish were approximately 1.5 – 2 cm at the time the experiment took place. Alevins were placed in the top of the tank, in the surface water above the uppermost shelf. For the first 10 minutes (pre-treatment acclimation period) the tank was supplied with neutral water; for the next 40 minutes (treatment period), either sulphuric acid or neutral water was hand pipetted over the surface of the tank (according to the pre-determined timescale established in pilot experiments described above). In the last 10 minutes (post-treatment), no additions took place. Though acid episodes in the natural environment can take place over the course of hours – weeks (depending on the driving climatic conditions), this time scale was chosen to allow observation of behavioural responses to, and avoidance of, episodic conditions, rather than an evaluation of the full ecotoxicological effect of prolonged exposure. Trials where neutral water was added during the treatment period acted as a control, to eliminate the possibility that alevins movement took place in response to external cues (e.g. movement outside the tank).

A total of 7 replicate trials within each treatment was carried out, using a naïve group of 10 alevins in each (total n = 140 fish). Treatments were carried out at random by choosing a folded piece of paper indicating either 'Control' or 'Acid' from a box, until 7 trials within each group had been completed. Fish were observed under artifical 'strip' lights, and though this may have casued additional disturbance to the fish, it was not possible to carry out the experiment in the low-light conditions used in other experiments. The light regime prior to and during the experiment was controlled with a timer, such that each group of fish observed experienced the same conditions. For each group of fish tested, two variables were recorded every minute; the number of alevins on each shelf and the opercular rate of two randomly chosen sentinel fish (calculated by counting the number of gill openings over a 12 second period and multiplying by 5 to obtain a 1 minute count); one in the surface water (i.e. within the top two shelves if possible, as this was the zone determined to be 'acid' in the pilot trials), and one in the deeper water (the bottom two shelves) in each treatment.

If applicable, the number of fish dead at the end of each trial was also recorded. In trials where the sentinel fish initially chosen for measurement of opercular rate rolled onto its side (an early indicator of death) the next closest fish subsequently became the sentinel, such that the gradually declining opercular rate of a dying fish would not compromise the results.

3.3.5 Statistical analysis

To make the data set more manageable, movement data (number per shelf over time) data were treated as follows:

(i) the number of alevins were not considered shelf-by-shelf, instead, groups were formed to include fish on the following shelves: 'top 2', 'middle 6' and 'bottom 2'.

(ii) the treatment phase of each trial was split into 4 separate time periods of equal length (10 minutes), rather than one much longer period. The number of alevins on each shelf differed between the start and end of the treatment phase, and subtle changes in number per shelf group might be masked were the phase to be considered as a whole. At the other extreme, to analyse data minute-by-minute may not reveal general changes in number within each shelf group.

Opercular rate data were also grouped into the 4 treatment phases described above. Movement data (number per shelf over time) and sentinel-fish opercular rates from each trial were analysed using a General Linear Model to test for significant differences between position in the tank (either t, m, b or upper/lower sentinel as applicable), treatment type (control vs. acid) and time period (pre-treatment, treatment, and post-treatment), and for any interaction effects between these factors. Results were investigated in more detail where possible, using a

Tukey-Kramer *post hoc* test to look for specific significant differences (i) within treatment type and (ii) between treatment types. Data for the number of dead fish the end of each trial were analysed using a one-sample *t*-test (alternative = greater than), to compare mortality in the acid treatment group against a standard value (zero) in the control group. A significance level of $P < 0.05$ was used throughout the analyses. Mean values are shown ± standard error throughout the results discussion. Complete statistical results are given in Appendix 3.

3.4 RESULTS

3.4.1 Movement

The number of alevins (mean ± SE; Figure 3.2) differed significantly between shelf group ($F_{2,2519} = 339.37$, $P < 0.005$), though this depended on treatment and is explained in more detail below. Treatment type ($F_{1,2519} = 0.00$, $P = 0.959$) and time period ($F_{5,2519} = 0.00$, $P = 1.000$) were not significant. However, although these factors were not significant when considered in isolation, a significant interaction effect was observed when all three factors (shelf group, treatment type and time period) were considered in combination ($F_{10,2519} = 5.01$, $P < 0.005$). A Tukey-Kramer *post hoc* pair-wise comparison of means highlighted the following points:

(i) within treatment

In the control group, there was a significantly higher number of alevins on the top two shelves than on the middle six or bottom shelves throughout the trial; however, the number of alevins on the top two shelves decreased with time as fish moved downwards through the tank. The pre-treatment count (number of alevins) was significantly higher than the post-treatment count ($T = -4.78$, $P < 0.005$). The downwards movement of fish throughout the trial resulted in a significant difference in the number of alevins on the middle six shelves between the pre- and post-treatment periods ($T = 5.910$, $P < 0.005$), though there was no difference in the number of fish on the bottom two shelves between the start and end of the experiment ($T = -1.130$, $P = 1.000$).

In the acid group, there was a significant difference in the number of alevins between the top two shelves and the bottom two shelves throughout the trial. The difference in number between the top two and middle six shelf groups was only significant in the pre-treatment period ($T = -4.52$, $P < 0.005$), after which alevins became more evenly distributed over the upper eight shelves, with no significant difference between shelf groups. The pre-treatment periods for both the top two and bottom two shelf-groups were significantly different to all other time periods – alevins seemed to move rapidly in the first treatment period, leading to a reduction/increase in numbers accordingly, but little subsequent movement took place, thus the significant difference between the pre- and post-treatment periods was maintained.

(ii) between treatments

There was a significant difference in the number of alevins on the top two shelves between the control and acid groups in all time periods. The number of alevins was significantly higher in the control group throughout the trial. Conversely, the number of alevins on the middle six and bottom two shelves was significantly higher in the acid group in the majority of time periods – alevins were more likely to move lower through the tank, and a higher proportion of fish had taken up residence on the bottom two shelves by the end of the trial. Rate of movement also varied between groups; control fish moved slowly and continued to move between shelves throughout the trial. Fish in the acidic treatment group moved more quickly at the start of the experiment and with the onset of acid exposure; by the middle of the treatment period very little movement took place, fish tended to remain stationary at a particular depth.

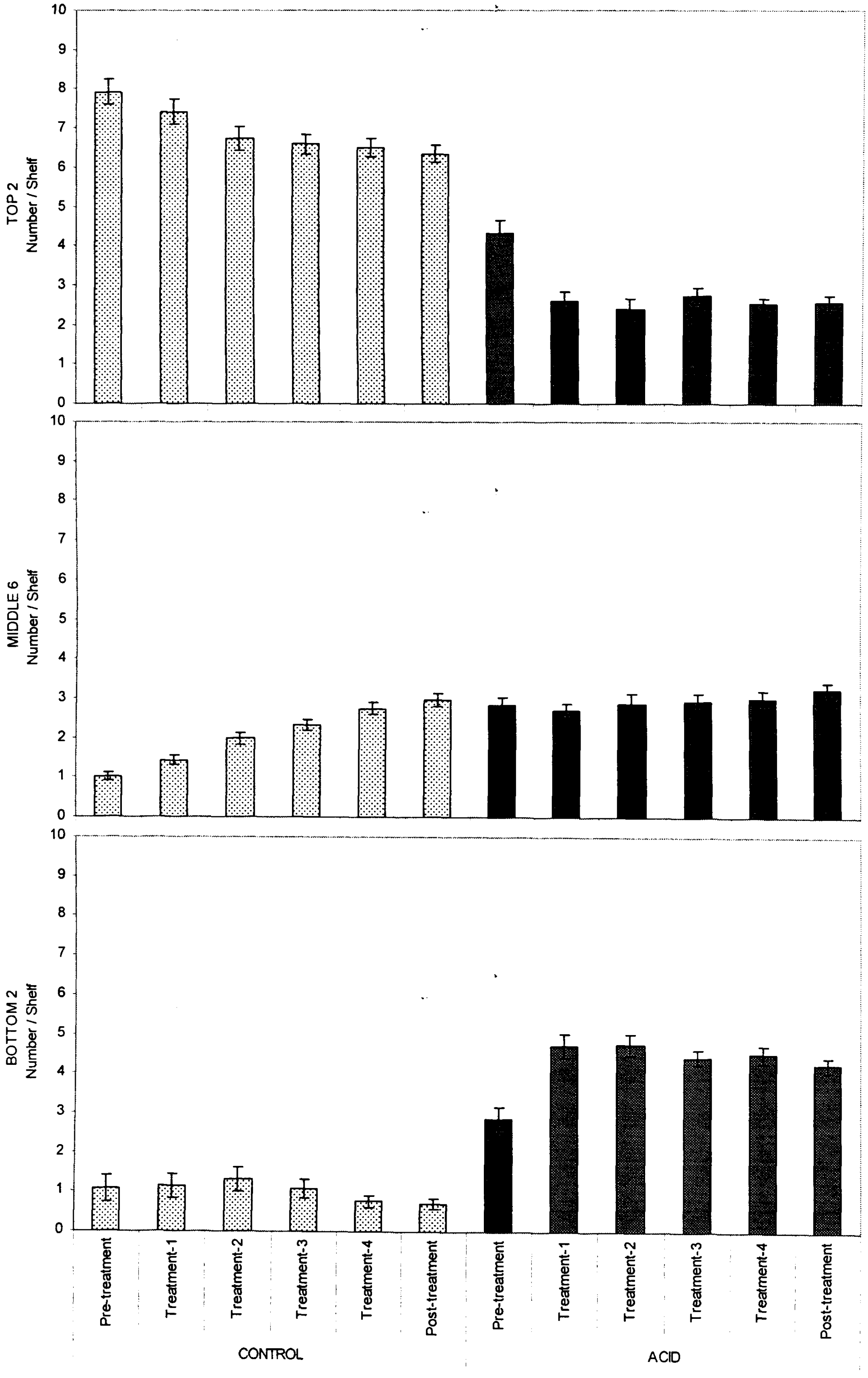


Figure 3.2: Number of alevins (mean ± SE) on the top two, middle six and bottom two shelves in the pre-treatment, treatment and post-treatment periods of the control group (stippled columns) and acid group (shaded columns) (total of *n* = 140 fish).

3.4.2 Opercular rate

Opercular rate (mean ± SE; Figure 3.3) varied significantly with sentinel fish position ($F_{1,1679} = 396.14$, $P < 0.005$), though this depended on treatment – opercular rate of the upper sentinel was significantly higher than the lower sentinel within the acid group, but not in the control group. Treatment type was significant ($F_{1,1679} = 2444.20$, $P < 0.005$); opercular rate of both the upper and lower sentinels was greatest in the acid group. Time period was significant ($F_{5,1679} = 13.53$, $P < 0.005$); opercular rate was higher throughout the treatment period in the acid group. The interaction between factors (shelf group, treatment type and time period) was also significant ($F_{5,1679} = 37.80$, $P < 0.001$).

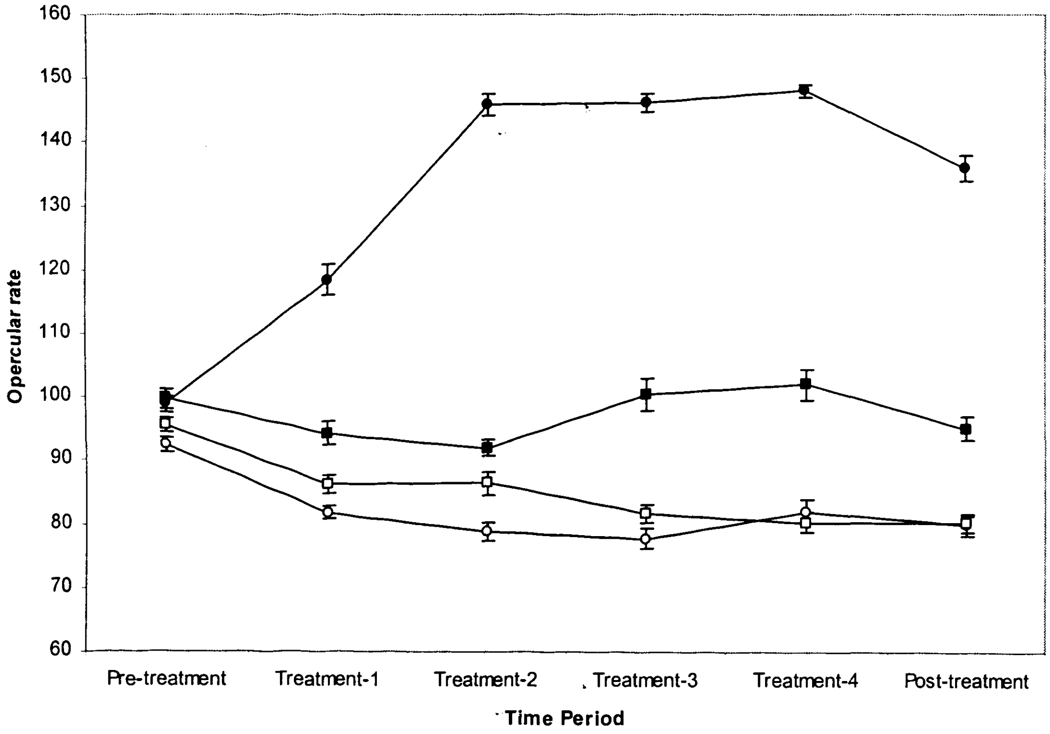


Figure 3.3: Opercular rate (mean ± SE) of sentinel fish (n = 7 in each case) in the pre-treatment, treatment (1 – 4) and post-treatment periods. Data are presented separately for the control (○ = upper sentinel; □ = lower sentinel) and acid (● = upper sentinel, ■ = lower sentinel) treatment groups.

A Tukey-Kramer *post-hoc* pairwise comparison of means revealed the following results:

(i) within treatment

In the control group, opercular rate was not significantly different between the upper or lower sentinel fish in the pre-treatment period (T = 3.394, P = 0.107). However, opercular rate was significantly different between the upper and lower sentinel fish in the treatment-1 – treatment-3 periods: opercular rate of the lower sentinel was significantly higher than that of the upper fish. The difference in opercular rate between the upper and lower sentinels in the treatment-4 and post-treatment periods was not significant, but there was a significant difference between the pre- and post-treatment periods for both the upper and lower sentinels (upper: T = -5.334, P = 0.0001; lower: T = -6.516, P = 0.0001); opercular rate was significantly lower at the end of the experiment than at the start for fish in the control group.

Opercular rate in the acid group was not significantly different between the upper or lower sentinel fish in the pre-treatment period (T = 0.333, P = 1.000); an initial decline in opercular rate was also observed in the lower sentinel as alevins acclimatised to their surroundings following transfer to the tank. Following exposure to acid water, opercular rate of the upper sentinel increased rapidly, and was significantly different to that of the lower sentinel throughout all remaining treatment periods (e.g. post-treatment: T = -17.34, P < 0.005). In the post-treatment period, opercular rate of the upper sentinel declined markedly; however, despite this decline, opercular rate was still significantly higher than in the pre-treatment period (T = 15.729, P = 0.0001). After the initial decline previously described, opercular rate also increased in the lower sentinel, though there was no significant difference in opercular rate between the pre-treatment and any of the treatment periods, nor between the pre- and post-treatment periods. Opercular rate fluctuated during the trial but remained similar to pre-treatment levels throughout.

(ii) between treatments

In the pre-treatment period, there was no significant difference in opercular rate of the lower sentinels between treatments, nor in the treatment-1 or treatment-2 periods though the difference was significant in the latter two treatment periods (treatment-3 and treatment-4) and in the post-treatment period (e.g. $T = 6.334$, $P < 0.005$). Opercular rate of the upper sentinels in the control and acid treatment groups was significantly different throughout the experiment, though the magnitude of the difference varied (e.g. pre-treatment: $T = 4.819$, $P < 0.005$; treatment-1: $T = 17.638$, $P < 0.005$; treatment-2: $T = 30.5488$, $P < 0.005$; treatment-3: $T = 31.1246$, $P < 0.005$; treatment-4: $T = 30.1548$, $P < 0.005$; post-treatment: $T = 25.882$, $P < 0.005$).

3.4.3 Mortality

The results of the one-sample *t*-test ($t = 2.79$, *d.f.* = 7, $P < 0.05$) show that there were more dead alevins (an average of 2.6 fish per replicate) in the acid group than the control group (where mortality was zero).

3.5 DISCUSSION

The primary objective of this experiment was to examine the extent to which Atlantic salmon alevins were able to detect and avoid pulses of acidified water of the type that would be encountered in real river systems during acid episodes. The hypothesis that alevins would move away from acidified water was supported: alevins significantly avoided exposure to acidified water at the top of the tank by moving into the area of neutral water at the bottom of the tank. The opercular rate hypothesis was also supported: alevins exposed to acid water in the top part of the tank in the acid treatment had a significantly higher opercular rate than those in the bottom part of the tank in the acid treatment group and in both parts of the tank in the control (neutral) group. These results illustrate that conditions consistent with acid stress were created during acid exposure.

The avoidance results are supported by Gunn and Noakes (1986): their study with brook charr alevins showed that the young fish appeared unaffected by water with pH > 5.0, but actively avoided water with pH < 5.0. However, in a study with lake charr embryos in a vertical substrate-filled tank (from which the design of the tank used in this experiment was developed), Gunn *et al.* (1987) observed no significant avoidance response when lake charr embryos were exposed to an acid pulse (pH 4.5). The lack of movement by some individuals was attributed to the strong preference for remaining in contact with solid objects, which in normal circumstances would be the gravel of the stream bed. This type of response is termed thigmotaxis, a taxis in which physical contact is the directive factor. Though the lake charr embryos did appear able to detect pulses of acidified water, thigmotaxis, combined with the poor swimming ability of the embryos studied, overrode any avoidance behaviour. A thigmotactic rather than avoidance response has also been observed in older fish when exposed to pulses of low pH (Jones *et al.*, 1985a, b). However, the alevin life stage is widely considered to be the most sensitive period due to the stresses involved in the transition to independent exogenous feeding and would perhaps be more likely than other life stages to attempt to avoid acidified water. It is also possible that the authors of the previous studies assumed the lack of movement observed in fish indicated a thigmotactic response, but the lack of movement could also be explained by differences in acid sensitivity between species. Atlantic salmon are one of the more acid-sensitive salmonid species (compared with, for example, brown trout, rainbow trout and brook charr) and avoidance behaviour is well documented in other life stages of the species.

Although alevins in the acid treatment group did appear to avoid the acidified water at the top of the tank, alevins in the control group also moved down through the tank in the during-treatment and post-treatment phases (Figure 3.2); however, the pattern of movement varied between groups – control alevins moved gradually and throughout the trial whereas alevins in the acid treatment group moved rapidly at the beginning and then tended to settle at a level for the duration of the trial. There are several possible explanations for this, for example (i) alevins moved quickly from the uppermost levels with the onset of acid application then remained settled on lower levels when no longer exposed to acid conditions; (ii) alevins initially moved away from acid but at some point became immobile, or (iii) alevins ceased moving to minimise ventilation caused by activity and thus the risk of further physiological damage. Another factor could be a thigmotactic response, though as stated previously, the observed 'thigmotactic' response in the study described above was probably due more to the poor swimming ability and negative buoyancy of embryos rather than a lack of avoidance. It is more likely in this study at least that the majority of alevins reached a point in the tank at which water chemistry was tolerable and remained there for the duration of the test period. The continued movement of alevins in the control trial may be explained by passive drift (though the upwards flow of water in the tank would perhaps prevent passive downwards movement) or the need for continued foraging and exploratory behaviour required to facilitate growth at this critical life stage.

Differences in movement between control fish and fish in the acidic treatment group suggest that alevins preferentially avoid acid water, and even when there is no avoidance response, increases in opercular rate indicate that fish are still affected by low pH. Opercular rate of the upper sentinel fish was significantly greater in the acid compared to control treatment groups (a whole-treatment average of approximately 132 vs. 82 beats per minute respectively), and significantly greater in the upper compared to the lower sentinel fish within the acid treatment group (132 vs. 97 beats per minute). These differences may be a subtle signal of stress since opercular rate is considered to be a useful and sensitive stress indicator (Ekweozer *et al.,* 2001; Close *et al.,* 2003; Hajek and Kłyszejko, 2004; Artigas *et al.,* 2005). Several studies have documented an increased opercular rate in stressed fish, though these are usually in relation to exposure to a predator model (Metcalfe *et al.*, 1987a, b; Hojesjo *et al.*, 1999; Johnsson *et al.*, 2001). Changes in opercular rhythm have also been documented as a sensitive indicator of physiological stress in fish subjected to sub-lethal levels of pollutants (Davis, 1973), in particular, fish exposed to acid water have showed signs of respiratory distress

(Laurent and Perry, 1991). Ekweozer *et al.* (2001) found opercular rate to indicate a significant effect of sublethal concentrations of chemicals on 3 species of commercial fish in Nigeria, though in this case it was nitrogen-based fertiliser effluents, not sulphuric acid that was being tested. It is also interesting to note that in this study, the opercular rate of the lower sentinel in the acid treatment group was significantly higher than that of the lower sentinel in the control treatment group, this is more likely to be a direct result of the increased activity involved in moving to the bottom of the tank. Although an increased opercular rate may be beneficial in preparation for increased activity, it also puts fish at a disadvantage for several reasons: (i) the physiological effects of acid water are mediated by way of the gills; (ii) metabolic costs of ventilating the gills are high and during ventilation may constitute 25% of the total metabolic rate of a fish (Hughes and Shelton, 1962); (iii) movements of the opercula might also make the fish more visible to potential predators.

In addition to the avoidance behaviour and observed increases in opercular rate, the increased mortality rate within the acid treatment group shows that alevins were also subjected to sufficient physiological stress (either as a result of acid exposure or the increased activity it induced) as to be fatal. These findings suggest that while avoidance reactions may be of a significant adaptive advantage to even the earliest free-swimming life stage of Atlantic salmon, if the already high mortality rate of salmon alevins in the wild is exacerbated by the physiological effects of episodic depressions in pH, there may be severe implications for the long-term survival of salmon populations.

CHAPTER 4

The influence of acid episodes on the sheltering behaviour of hatchery-reared juvenile Atlantic salmon

4.1 SUMMARY

Previous work has shown that water chemistry differs between surface water and the hyporheic zone during acid episodes. Atlantic salmon are known to shelter in spaces within the hyporheic zone, though it is not apparent if fish are sheltering to take advantages of beneficial chemical conditions in addition to avoiding predators. This study used a shelter-choice tank to test the following primary hypothesis: juvenile Atlantic salmon avoid pulses of acidified water and seek refuge in more chemically favourable conditions. Two additional hypotheses tested were that: (i) in order to seek shelter from pulses of acid, activity will increase; (ii) opercular rate will increase in fish under environmental and physiological stress at low pH. The results supported all the hypotheses: exposure to an acid pulse of pH 4 – 5 significantly affected shelter use ($P < 0.005$), though this result was driven more by the tendency of fish to actively avoid an acidified shelter rather than using a neutral shelter during an acid pulse. Activity of fish increased significantly when exposed to acidified water ($P < 0.005$), with an average increase of 1.7 movements per minute following acid exposure compared with an increase of 0.3 movements per minute in the control group. Opercular rate also increased significantly following exposure ($P < 0.005$) from an average of 62 beats per minute to 88 beats per minute, compared with an increase of only 4 beats per minute in the control group. These results suggest that hyporheic or benthic sheltering could play an important role in the survival of juvenile Atlantic salmon during acid episodes. Conversely,

the lack of suitable shelter, or the inability of fish to seek them out due to the physiological effects of acid exposure, may have a severe impact on the health of wild populations.

4.2 INTRODUCTION

The use of shelters is a behavioural trait commonly observed in animals; in particular, several species of fish are known to shelter (Chapter 1), though sheltering behaviour has perhaps been best described in terms of steam-dwelling salmonids (Rimmer et al., 1983; Cunjak, 1988; Fraser et al., 1993; Valdimarsson et al., 1997, 2000; Valdimarsson and Metcalfe, 1998; Metcalfe et al., 1999; Bremset, 2000; Orpwood et al., 2003, 2004). As water temperatures decline there is a switch in behaviour from the 24-hour foraging typically observed in summer to daytime sheltering and nocturnal foraging in winter, though there is considerable debate over the temperature at which sheltering is triggered, and what other reasons may influence the decision to shelter. Some authors suggest that the shift in behaviour is triggered at approximately 6 – 8°C (Valdimarsson *et al.*, 2000), though several species of salmonid are known to shelter at 10°C (Greenwood and Metcalfe, 1998), and other studies state that sheltering is not restricted to winter months, but also occurs during summer (Gries and Juanes, 1998).

As described in Chapter 1, three primary functions of shelter use have been suggested: predator avoidance, protection from ice formation and respite from stream currents. These advantages of sheltering behaviour are concerned with the use of physical refuges, i.e. interstitial spaces within the stream bed, or areas underneath larger rocks. However, the term 'shelter' could also be applied to those areas within a stream where water quality is locally more favourable (e.g. due to higher pH). In these cases, the use of refuges by salmonids could either reflect direct chemical cues, or physiological benefits may accrue incidentally from behaviour stimulated by other cues. One such instance involves conditions encountered during acid episodes in which marked differences can occur in acid conditions between surface water at low pH and the circumneutral hyporheic zone typically used by sheltering salmonids (Chapter 2). Although the movement of fish away from acid water and towards patches of higher pH within the main body of stream surface water is well documented, there are currently no studies that consider how these movement patterns are affected by the interaction between physical and chemical properties of shelters. The continuing emergence of studies on other aspects of sheltering behaviour in salmonids implies that there is still much to be discovered, particularly the reasons for sheltering at certain times of day/night. Importantly, acid episodes are just as likely during darkness as during daylight and will affect fish in the surface water at either time.

This study investigates how the sheltering behaviour of Atlantic salmon is affected by water chemistry differences between surface and hyporheic water observed in the natural environment, exploring the behavioural changes shown by Atlantic salmon when exposed to acid water. Specifically, three hypotheses were tested: (i) Atlantic salmon detect and avoid pulses of acid water (ii) activity levels in acid water will increase as fish are forced to seek shelter, and (iii) opercular rate, a well documented stress indicator (Ekweozer *et al.,* 2001; Close *et al.,* 2003; Hajek and Kłyszejko, 2004; Artigas *et al.,* 2005), will increase with decreasing pH.

4.3 METHODS

4.3.1 Preliminary experiments

Though field work was yet to be carried out, it was hypothesised that differences in stream chemistry would exist between the surface water and stream hyporheos within a river and that such differences would create separate and distinct habitats, both accessible by Atlantic salmon. The widely accepted theory that Atlantic salmon will avoid pulses of acidified water was therefore tested, with the additional caveat that in order to avoid such pulses, fish would make use of a neutral refuge on the 'stream bed', the condition expected in the natural environment. Early fieldwork carried out in Summer 2005 (i.e. between the preliminary laboratory experiment and the more detailed experiment below) then reinforced the idea of spatial as well as temporal variations in pH, further building on the findings of the preliminary experiment and modifying future methods. Preliminary methods and results are given in Appendix 4.

4.3.2 Experimental animals

Juvenile (0+; i.e. fish in their first year of life) Atlantic salmon were collected from the Environment Agency hatchery at Abercynrig (OS Explorer sheet 12, grid reference 064264), near Abergavenny, Wales in Autumn 2005. Fish were approximately 7 – 8 months old, size-sorted (between 7 – 8 cm at time of collection), and had previously been kept in a large batch-specific tank (i.e. from the same parental brood stock) with a constant supply of aerated Afon Cynrig river water (circum-neutral). As in the preliminary experiment, following transportation to the temperature controlled facilities at Cardiff University (12.3 ± 0.1°C, *n* = 54 [measurements taken from holding tank prior to and during experiment]), fish were housed in a tank of free running de-chlorinated mains water, and fed hatchery-standard protein mix daily, after dark to encourage nocturnal foraging. Once used in the experiment, fish were kept in a separate tank to avoid re-use. Following completion of the study, fish were humanely euthanased (Home Office PIL 30/6656).

4.3.3 Test arena

Fish were observed in a plastic tank (52 L x 33 W x 24 D cm), through which de-chlorinated mains water (pH 7) flowed. Water within the test tank did not cool to the same temperature as water in the holding tank due to the different volumes and flow rates in each tank, so temperature was controlled using a LEEC closed-circuit cooling device (12.1 ± 0.2°C, *n* = 36 [measurements taken during experiment in test arena]); the coolant pipe was attached around all bottom edges of the tank interior, though water did not come into direct contact with the coolant. It is possible that the cooling element may have resulted in a vertical temperature gradient within the tank. Though the low flow rate of water through the tank makes it unlikely that any such gradient would have developed, any influences of temperature would have been identical across treatment groups. Water entered the tank from the bottom at one side (termed 'upstream') and exited at the top of the tank on the opposite side (termed 'downstream'). The tank was divided into 24 zones (each zone measuring 13 L x 11 W x 8 D cm) indicated by lines on the tank exterior, though it is important to note that no physical barriers were present within the tank, and water flowed freely between all areas. Division of the tank created groups of zones as follows: 4 from the upstream end to the upstream end (coded 1 to 4, left to right), 3 from the front of the tank to the rear (coded front [F], middle [M] and back [B]) and 2 vertical layers (coded upper [U] and lower [L]). A shelter (consisting of a hollow pipe 10 cm long sealed at one end with an internal diameter of 6 cm) was positioned in the centre of the tank over L2M (lower 2 middle) and L3M (lower 3 middle), with the open end facing downstream towards L4M (Figure 4.1). Water supplied to the sealed end of the shelter originated from one of two header tanks, according to treatment type, as described below.

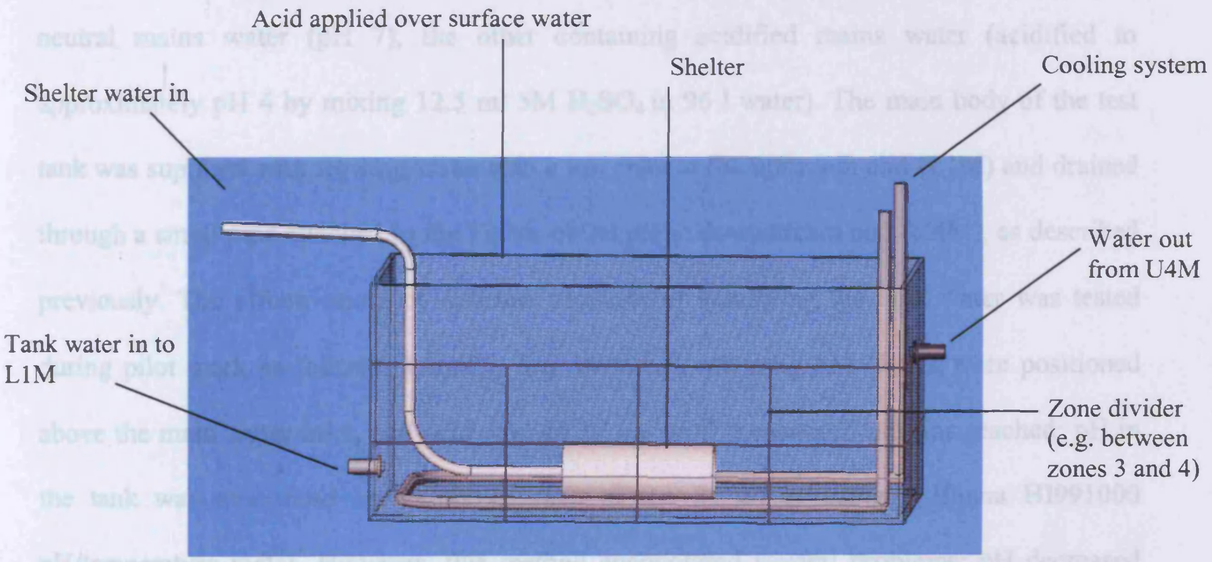


Figure 4.1: Representation of the test-tank, with downstream inlet, centrally positioned shelter, upstream outlet and self-contained cooling pipe around the tank perimeter.

4.3.4 Pilot work

Preliminary work showed that in preparation for the experiment, it was necessary to reach and maintain specific pH levels (either 'acid' or 'neutral') in both the main body of the test tank and in the shelter, and ensure that mixing of the water in these two areas was kept to a minimum. Several pilot trials were carried out in order to determine the necessary quantities of neutral and acidified mains water that must be applied to either the tank or the shelter to achieve the required pH.

For the purposes of this experiment, 'acid' was classed as any pH between 4 – 5. pH levels lower than 4 were avoided to prevent fish mortality (Daye and Garside, 1977) while pH levels above 5 were avoided to ensure that fish were exposed to naturalistic acid episode conditions (Chapter 2), as in previous laboratory experiments (e.g. Chapter 3). The term 'neutral' refers to the de-chlorinated mains water supplied to the aquarium room in which the experiments took place (pH 7). During these pilot trials (and in the experiment proper), the water supplies were as follows:

The shelter was supplied by water from one of two separate header tanks, one containing neutral mains water (pH 7), the other containing acidified mains water (acidified to approximately pH 4 by mixing 12.5 ml 5M H_2SO_4 in 96 l water). The main body of the test tank was supplied with flowing water into a low inlet at the upstream end (L1M) and drained through a small pipe attached to the higher outlet at the downstream end (U4M), as described previously. The effectiveness of different methods of acidifying the tank water was tested during pilot work as follows. Initially, four burettes containing 5M H_2SO_4 were positioned above the main water inlet, and acid applied *ad lib.* until the desired pH was reached; pH in the tank was monitored constantly in each of the 24 zones using a Hanna HI991000 pH/temperature meter. However, this method encountered several problems; pH decreased too quickly over time, and was inconsistent following application of the acid. This method was attempted again with two burettes, and then a single burette above the inlet, but with similar results. An Eppendorf Research hand pipette (100 – 1000 µl) was therefore used to acidify water in the main tank, since it allowed precise acid application and volume adjustment, thus an acid episode of pH 4 – 5 could therefore be created, accurately maintained over the exposure period, and replicated in each subsequent experiment.

Examples of the pilot work data sheets are given in Appendix 5. As a result of the acid addition pilot work, the sulphuric acid used in treatment groups 3 and 4 was added to the tank according to the time scale detailed in Table 4.1.

Where 4 applications are indicated, acid was applied onto the surface water in zones U1F, U1B, U4F and U4B, i.e. in the 4 corners of the tank. Where 2 applications are indicated, acid was applied onto the surface water in zones U1M and U4M.

Table 4.1: Number and quantity of acid applications during 40 minute treatment period in Acid tank/Neutral shelter and Acid tank/Acid shelter treatment groups.

Minutes into treatment period	Volume of acid added (μl)	Number of applications
0	300	4
2	300	4
4	300	4
6	300	4
8	80	4
14	70	4
18	70	2
25	90	2
27	80	4
30	70	2
35	70	2

4.3.5 Experimental protocol

A single Atlantic salmon was chosen at random by scooping a net through the water in the holding tank and using the first fish caught in the net. The fish was transferred to the test tank and allowed to acclimate overnight during the 12 hour dark period in the aquarium; lighting within the aquarium was controlled with a timer such that the light regime was identical for each fish observed. Observations were made in near-darkness (using a 15W red lamp) between 05:30 and 07:30 the following morning, by one of two observers. Each trial lasted 100 minutes, and consisted of three time periods: pre-treatment (an acclimation period of 30 minutes); treatment (40 minutes during which either acid or neutral water was applied to the test tank or shelter according to treatment, Table 4.2); post-treatment (30 minutes). During the first and last 30 minutes of each trial, the test tank and shelter were both supplied with neutral water. During the middle 40 minute treatment period, water conditions in the test tank and shelter were manipulated as in Table 4.2.

Table 4.2: pH conditions in the test-tank water and shelter water during the 40 minute treatment period; neutral pH = 7, acid pH = 4 – 5.

Group	Tank chemistry	Shelter chemistry	Group abbreviation
1	Neutral	Neutral	N/N
2	Neutral	Acid	N/A
3	Acid	Neutral	A/N
4	Acid	Acid	A/A

Though acid episodes in the natural environment can take place over the course of hours – weeks (depending on the driving climatic conditions), this time scale was chosen to allow observation of behavioural responses to, and avoidance of, episodic conditions, rather than an evaluation of the full ecotoxicological effect of prolonged exposure. The fish showed no obvious evidence of disturbance following the addition of acid water using hand pipettes held above the tank during the 40 minute treatment period in treatments 3 and 4 (A/N and A/A). Nevertheless, to discount the possibility that stress caused to the fish through movement of the observer may confound the results, hand pipettes were also used in treatments 1 and 2 (N/N and N/A respectively). In these treatments pipette water was pH neutral.

A total of nine replicate trials was carried out within each treatment group using a naïve fish in each trial (total *n* = 36 fish). Treatments were carried out at random by choosing a folded piece of paper indicating 'Neutral/Neutral', 'Neutral/Acid', 'Acid/Neutral' or 'Acid/Acid' from a box, until nine trials within each group had been completed. For each fish tested, three key behaviours were recorded: time spent in the shelter; the number of times a fish moved from one area of the tank to another (expressed as activity minute^{-1}); and opercular rate. Time spent in the shelter was recorded as a proportion of the corresponding test period (pre-treatment, treatment and post-treatment). Initial position (in one of 24 zones) was recorded at the start of the experiment, and every subsequent movement into another section, or the shelter, noted against the current time using a VisualBasic programme in Excel. Opercular rate was recorded every 5 minutes when the fish was visible by counting the number of gill openings over a 15 second period and multiplying by 4 to obtain a 1 minute count.

4.3.6 Statistical analysis

A General Linear Model was used to test the effects of treatment and time period (i.e. pre-treatment, treatment and post-treatment) on activity minute^{-1} and opercular rate. Results were investigated in more detail where possible, using the Tukey-Kramer *post hoc* method. Results for shelter use were not normally distributed and were therefore not suitable for ANOVA; the Sheirer-Ray-Hare test (a non-parametric ANOVA equivalent with replication) was performed on the arcsine transformed data instead. A significance level of $P < 0.05$ was used throughout the analyses. Mean values are shown ± standard error throughout the results discussion. Complete statistical results results are given in Appendix 6.

4.4 RESULTS

4.4.1 Shelter use

Treatment had a significant effect on the amount of time spent sheltering as a proportion of each period (Sheirer-Ray-Hare: $\chi^2_3 = 0.997$, $P < 0.005$) (Figure 4.2); test period also had a significant effect on sheltering behaviour ($\chi^2_2 = 0.990$, $P < 0.05$), though the interaction (treatment*time period) was not significant ($\chi^2_6 = 0.821$, $P = 0.179$).

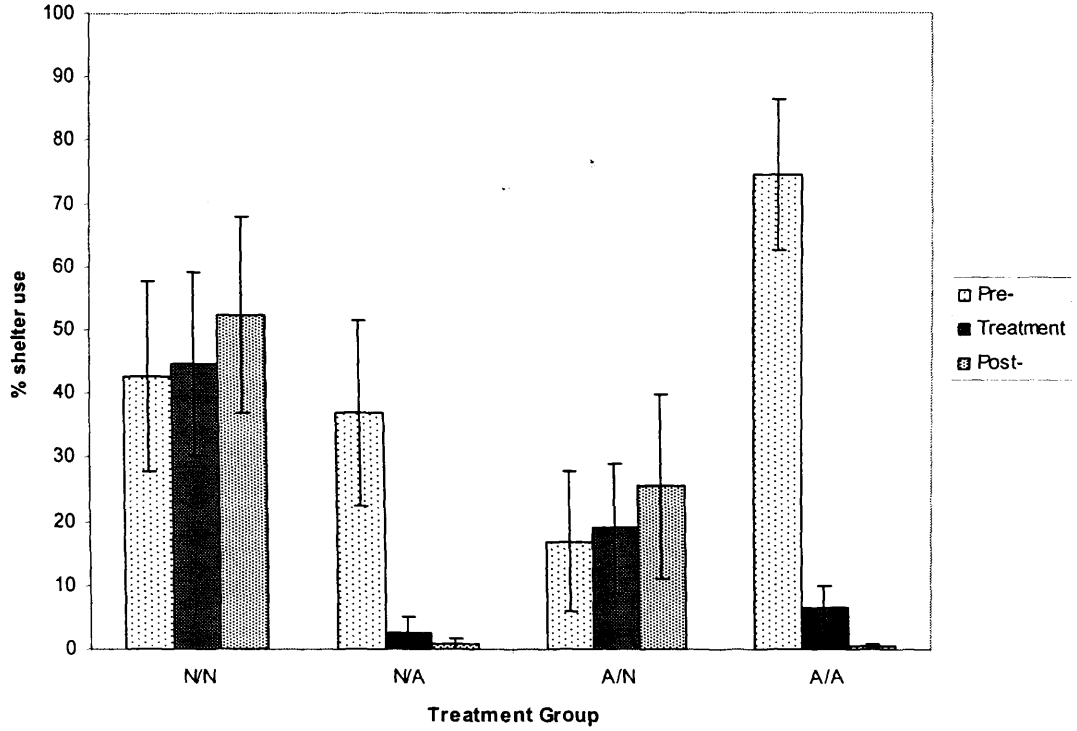


Figure 4.2: Mean shelter use as a proportion of corresponding test period ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (Neutral tank/Neutral shelter, Neutral tank /Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter).

Shelter use in control fish (i.e. those in the Neutral/Neutral group) remained broadly the same between test periods. In the Acid/Neutral group, fish appeared to preferentially use a neutral shelter when subjected to acidified tank water, though the difference in proportion of time spent sheltering between test periods is small (pre-treatment = 16.8 ± 10.9%; treatment = 18.9 ± 10.1%; post-treatment = 25.5 ± 14.4%), and the progressive increase in shelter use with time is not dissimilar to that observed in the Neutral/Neutral group. However, on average there was a marginally greater proportional increase in sheltering within the Acid/Neutral group between the pre-treatment/treatment and pre-treatment/post-treatment periods than in the Neutral/Neutral group (Table 4.3; figures shown were calculated using mean ± SE values from Figure 4.2 and not raw data, thus no statistical analysis is included). Additionally, fish actively avoided an acidified shelter (Neutral/Acid), spending 36.9 ± 14.4% of the pre-treatment period in the shelter, but only 2.7 ± 2.6% of the treatment period and 0.9 ± 0.9% of the post-treatment period in the shelter – sheltering was reduced in the treatment and post-treatment periods when compared with the pre-treatment period.

Table 4.3: Proportional increase in time spent sheltering (mean ± SE) between the pre-treatment/treatment and pre-treatment/post-treatment periods of the Neutral/Neutral and Acid/Neutral groups.

	Pre-treatment:treatment	Pre-treatment:post-treatment
Neutral/Neutral	0.841 ± 0.238	0.632 ± 0.175
Acid/Neutral	0.908 ± 0.8	0.655 ± 0.401

4.4.2 Activity

Activity minute^{-1} was significantly different between treatment groups ($F_{3,88} = 6.48$, $P < 0.005$), though the effect of test period was not significant ($F_{2,88} = 2.29$, $P = 0.108$), nor were any significant interaction effects between treatment group and test period observed ($F_{6,88} = 1.69$, $P = 0.186$). Activity minute^{-1} was highest in the treatment and post-treatment periods of the trials in which some acid exposure took place, i.e. Neutral/Acid, Acid/Neutral, and Acid/Acid (Figure 4.3). A Tukey-Kramer *post hoc* comparison showed that the most significant differences were observed between fish in Neutral/Neutral post-treatment vs. (i) Neutral/Acid post-treatment ($T = 3.850$, $P < 0.05$, (ii) Acid/Acid treatment ($T = 3.935$, $P < 0.05$) and (iii) Acid/Acid post-treatment ($T = 3.585$, $P < 0.05$). Near-significant differences were observed between fish in Neutral/Neutral treatment vs. (i) Neutral/Acid post-treatment ($T = 3.1403$, $P = 0.0916$), (ii) Acid/Acid treatment ($T = 3.2282$, $P = 0.0732$) and between fish in Neutral/Neutral post-treatment vs. Neutral/Acid treatment ($T = 3.352$, $P = 0.0527$). The lack of any significant interactions may be explained by the large SE values and overlapping error bars between some treatment groups/test periods (Figure 4.4).

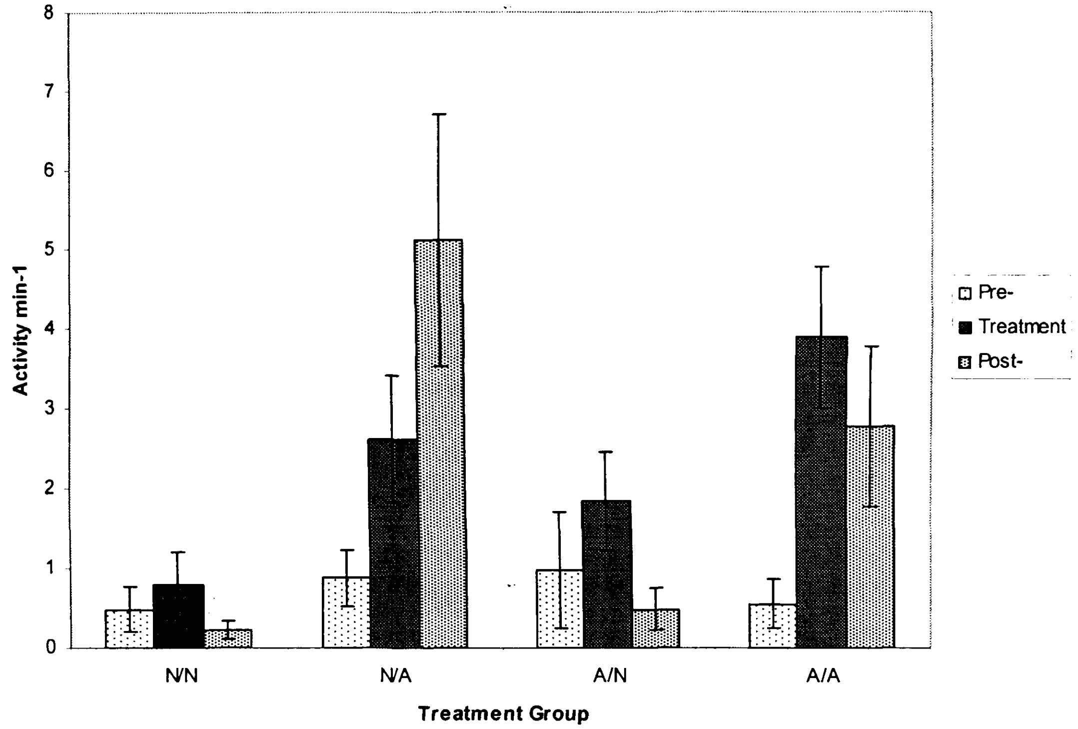


Figure 4.3: Mean activity minute^{-1} ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter).

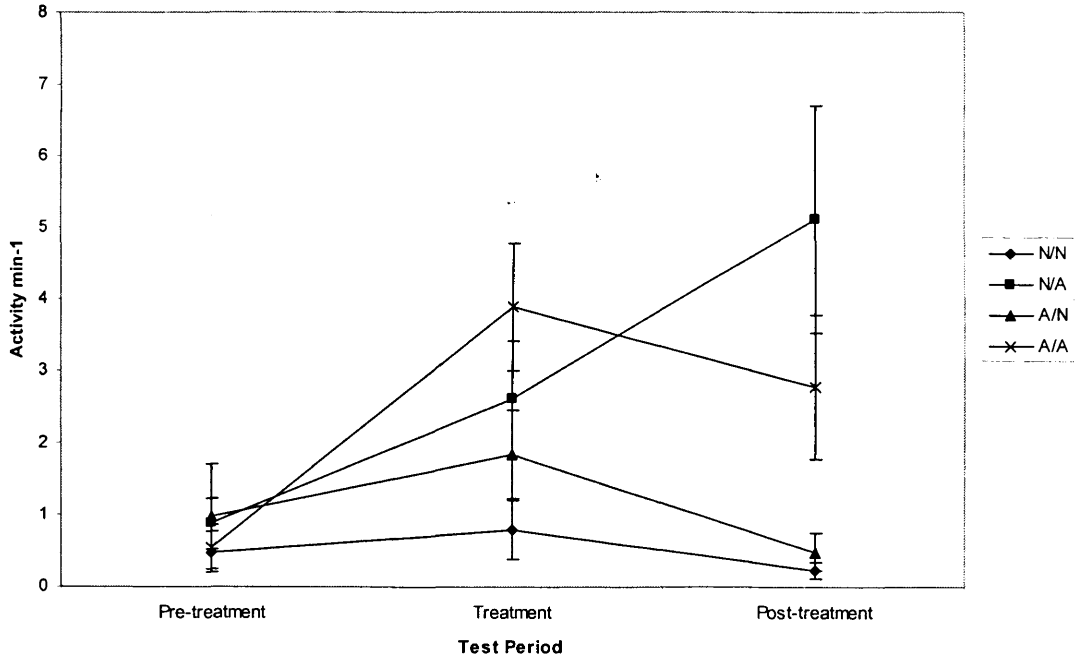


Figure 4.4: Interaction between treatment group (Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter) and test period (pre-treatment, treatment and post-treatment) (mean activity minute^{-1} ± SE).

4.4.3 Opercular Rate

The effect of treatment group was significant ($F_{3,88} = 16.54$, $P < 0.005$) with opercular rate being highest in those treatments when water was acidified (Figure 4.5). Test period also effected a significant change in opercular rate ($F_{2,88} = 17.12$, $P < 0.005$), with opercular rate highest in the treatment and post-treatment periods of the Acid/Acid, Acid/Neutral and Neutral/Acid treatments, i.e. those in which fish were subjected to acidified water of some type. The interaction effect between treatment and test period was also significant ($F_{6,88} = 2.41$, $P < 0.05$); Figure 4.5.

Multiple Tukey-Kramer *post hoc* comparisons revealed the following specific results: within treatments, only fish in the Acid/Acid group showed a significant effect of both treatment group and test period; there was a significant increase in opercular rate between the pre-treatment and treatment periods ($T = 5.277$, $P < 0.005$). The difference in opercular rate between the treatment and post-treatment periods was not significant ($T = -0.5197$, $P = 1.000$), suggesting that an increase in opercular rate during acid episodes is maintained even after neutral conditions are restored. Between groups, the interaction was significant for fish in the treatment period of the Neutral/Neutral and Acid/Neutral groups ($T = 4.5589$, $P < 0.005$) and the Neutral/Neutral and Acid/Acid groups ($T = 5.5496$, $P < 0.005$) and in the post-treatment period of the Neutral/Neutral and Acid/Neutral groups ($T = 3.6239$, $P < 0.05$) and the Neutral/Neutral and Acid/Acid groups ($T = 5.4615$, $P < 0.005$) – these are the treatments in which acid water was applied to the main body of the tank; the shelter was also acidified in the Acid/Acid group.

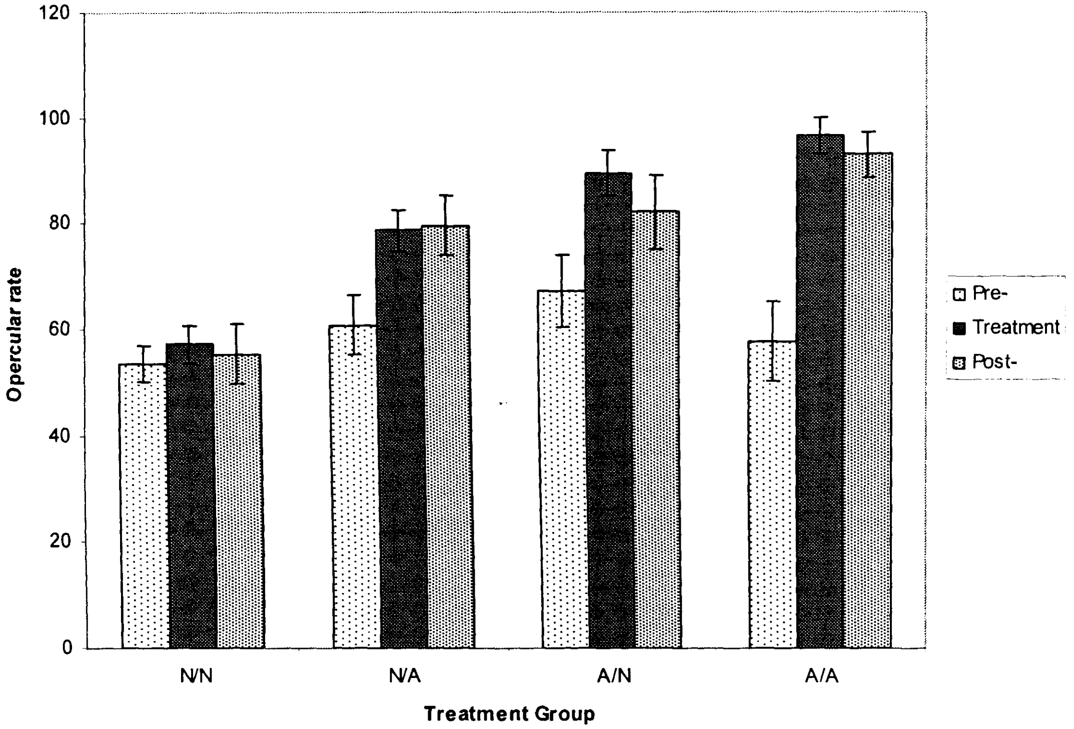


Figure 4.5: Mean opercular rate ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter).

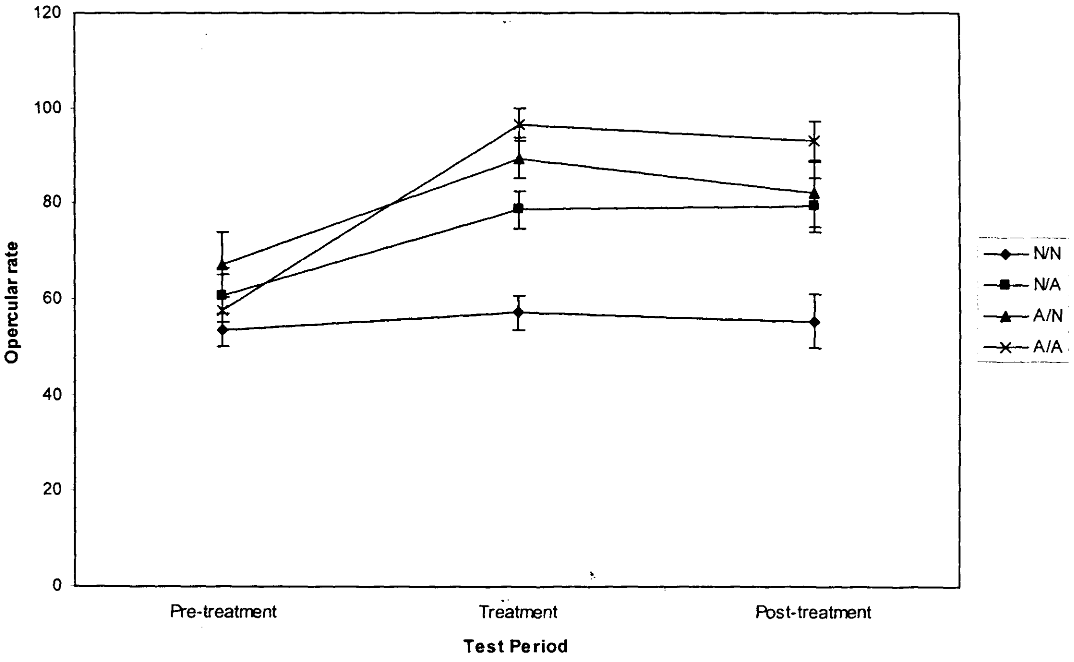


Figure 4.6: Interaction between treatment group (Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter) and test period (pre-treatment, treatment and post-treatment) (mean opercular rate ± SE).

4.5 DISCUSSION

The objective of this study was to examine the extent to which Atlantic salmon change refuge use behaviour, activity and opercular rate when exposed to pulses of acidified water. All the hypotheses were supported: sheltering was affected by an acid pulse – although fish did not appear to preferentially use a neutral shelter when tank water was acidified, they actively avoided using acidified shelters. Activity increased with exposure to an acid pulse (an average increase of 1.9 movements per minute was observed between the pre-treatment and treatment periods in groups 2, 3 and 4 compared with an increase of 0.3 movements per minute in the control group). Opercular rate also increased from an average of 62 beats per minute in the pre-treatment period to 88 beats per minute in the during treatment period compared with an increase of only 4 beats per minute in the control group, and remained elevated even after the neutral water supplies to the tank and shelter were resumed (on average 85 beats per minute across groups 2, 3, and 4).

Shelter use varied significantly between different treatment types and test periods, though the difference in percentage time spent sheltering between the pre-treatment and treatment periods within the Acid/Neutral group (in which the main tank was acidified and the shelter supplied with neutral mains water) was not significant when considered in isolation. Indeed, the observed increases in sheltering with time follow the changes in sheltering observed in the control trial (Neutral/Neutral), in which both the main tank and the shelter were neutral during the treatment period. However, on average there was a greater *proportional* increase in percentage time spent sheltering in the A/N treatment than in the N/N treatment (though this was not tested statistically); the change induced by the treatment period therefore had more of an impact within the A/N treatment and caused proportionally more sheltering in fish that were, in normal conditions, less inclined to shelter. In addition, the almost complete avoidance of an acidified shelter in the N/A treatment suggests that fish in this trial were able to detect and move away from acidified water. It is also interesting to note that differences were observed in the proportion of time spent sheltering prior to acid exposure, though the reason for this is unclear – it may be attributed to individual differences in behaviour between fish, and could be investigated with further experimental trials.

Although the results for shelter use are unexpected, they seem to be in general agreement with the theory that Atlantic salmon will try to avoid acidified water (Hoglund, 1961; Johnson and Webster, 1977; Jones *et al.*, 1985a; Gunn and Noakes, 1986; Nakamura, 1986; Atland and Barlaup, 1996; Atland, 1998; Ikuta *et al.*, 2001), and provide additional evidence that fish will at least attempt to exploit more favourable areas within their environment (as indicated by increased activity) even if they are not successful.

Activity is a behavioural stress indicator (Artigas *et al.,* 2005); 'fast swimming' and 'jumping' suggest increased stress levels while 'slow swimming' and 'standing still' are signs of a less stressed fish (Artigas *et al.,* 2005). Recording the type of movement is one way to record activity, another is to record the rate of movement, or activity rate, as in this study. Activity was greatest in the treatment groups in which fish were exposed to acid conditions of some type; control fish showed no significant changes in activity between test periods. The increase in activity in the acidified treatment groups (2, 3 and 4) in the treatment and post-treatment periods was not consistent between all fish; fish were generally very active and frequent bursts of 'fast swimming' were observed; however, a small proportion of fish remained stationary in a few areas of the tank. A reduction in activity following exposure to acid water has been observed in other trials with salmonids, for example brown trout (Atland, 1998). This might suggest that remaining stationary, or 'freezing' is a more effective way of mitigating the effects of acid episodes. However, it is likely that the tendency to remain passive in acid water is attributed more to the possible immobilisation of salmon at low pH (Peterson *et al.*, 1989), though as stated previously, this behaviour was not observed in all fish examined. Previous exposure experiments have also shown that brown trout are less sensitive to acid water than other species of salmonids (Grande *et al.*, 1987; Rosseland and Skogheim, 1984), in particular, Atlantic salmon, so the difference in acid sensitivity may account for the general increase in activity of fish in this study. It is interesting to note that although only the shelter was acidified during the Neutral/Acid treatment, activity was highest in this treatment – perhaps being forced out of a shelter due to the onset of an acid pulse is more stressful to an individual than not being able to find a shelter at all.

Changes in activity, either an increase or a decrease, may both be costly for fish in the wild. Increases in activity may make a fish more noticeable to predators and thus more vulnerable (Rahel and Stein, 1987); however, a reduction in activity means that less time is spent actively foraging. Additionally, choosing not to avoid acid water in favour of remaining stationary on the tank bottom/stream bed will make fish more vulnerable to the physiological changes that take place following acid exposure.

Opercular rate was also significantly different between treatments; while this may be a direct result of increased activity, it may also be a subtle indicator of stress. As described in Chapter 3, opercular rate has been used in other studies as a stress indicator in fish, though there are few studies that focus on the effect of acidified water on opercular rhythms in Atlantic salmon. However, it is widely accepted that exposure to sub-lethal concentrations of pollutants, including acid water, will lead to respiratory distress and an increased opercular rate (Davis, 1973; Laurent and Perry, 1991; Ekweozer *et al.,* 2001; Close *et al.*, 2003; Hajek and Kłyszejko, 2004; Artigas *et al.,* 2005), which supports the observations made in this study.

The results of this study have highlighted several points that may have implications for fish in the wild. Field data have shown that there are significant differences in pH (and other variables) between surface water and in the hyporheic zone, with water in the stream bed much closer to the growth and survival optima required by juvenile Atlantic salmon (Chapter 2). Most of the fish in this trial appeared able to avoid pulses of acidified water, or at least increase their activity enough to facilitate some avoidance behaviour, which further supports the idea that behavioural avoidance of acid waters could increase the probability of survival during acid episodes (Breck *et al.*, 1988). More importantly, if these results generalised to other times and locations, the use of shelters by salmonids during acid episodes could maintain populations in locations that were otherwise marginal for population persistence.

CHAPTER 5

Effects of rearing environment on acid avoidance in juvenile Atlantic salmon

5.1 SUMMARY

Differences in behaviour from wild fish may have implications for the post-release survival of fish released as part of re-stocking programmes, for example due to their ability to exploit refuges. Previous work has indicated that hatchery-reared fish increased activity to avoid acidified water, but they did not always shelter in ways that could be important in avoiding surface water acidification. However, this behaviour may develop following repeated exposure to acid episodes, and some studies have shown that differences between wild and hatchery-reared fish can be overcome by a period of 'training' under conditions that mimic the natural environment. This study aimed to determine the extent to which hatchery-reared fish can be trained to avoid acid episodes by comparing the acid avoidance abilities of fish reared either in a simulated wild environment with repeated exposure to an acid pulse or in a conventional hatchery environment. Specifically, the following hypotheses were tested: (i) fish with previous experience of acid episodes reared in a simulated wild environment will spend a greater proportion of time in a neutral shelter than hatchery-reared fish during acid episodes; (ii) activity will be lower in fish with experience of acid episodes (as a direct result of increased sheltering) and (iii) opercular rate will be lower in experienced fish, since they are better able to exploit more favourable areas of their environment and will not be subject to the same physiological stresses as hatchery-reared fish that do not shelter. The sheltering hypothesis (i) was supported ($P < 0.005$), with fish reared in an enriched environment spending almost twice as much time (44%) in shelters during acid episodes as hatchery-reared fish (23%). The activity hypothesis (ii) was also supported ($P < 0.005$), with fish reared in an

enriched environment showing a lower level of activity during episodes (as a result of increased sheltering) than hatchery-reared fish. The opercular rate hypothesis was not supported, fish reared in an enriched environment showed a similar opercular rate to hatchery-reared fish despite sheltering more. These results show that potential differences between hatchery-reared and wild-reared fish can be overcome, and inclusion of such ‘training’ periods in hatchery procedures prior to release could enhance the success of re-stocking programmes.

5.2 INTRODUCTION

Recent work has shown that juvenile salmon reared in the wild actively defend refuges within gravel on the stream bed to the extent that the availability of refuges can limit population size (Armstrong and Griffiths, 2001). Shelter use is strongly density-dependent, with the proportion of fish using shelters decreasing as population size increases (Armstrong and Griffiths, 2001). Wild fish rarely share shelters (Cunjak, 1988; Armstrong and Griffiths, 2001; Griffiths and Armstrong, 2002; Harwood *et al.*, 2002), though there is no conclusive evidence to explain why (Griffiths and Armstrong, 2002). However, shelter sharing has been widely observed in hatchery-reared fish (Burns *et al.*, 1997; Metcalfe *et al.*, 1999; Griffiths and Armstrong, 2002) as a result of being reared in high densities. When introduced into an environment in which wild fish are also present, however, wild fish generally out-compete hatchery-reared fish if the number of refuges is limited (Orpwood *et al.*, 2004).

While refuge availability may restrict population growth, the ability of fish to exploit such refuges may also influence survival. Work presented in Chapter 4 showed that hatchery-reared fish increased activity in an attempt to avoid acidified water, but did not always shelter. One inference is that sheltering behaviour is affected by the rearing environment, and this may have implications for fisheries management. Other behaviours can also be affected by domestication (e.g. Álvarez and Nicieza, 2003). In the wild, for example, juvenile brown trout reacted to the presence of piscivorous brown trout by increasing the use of refuges. In contrast, second-generation hatchery fish and the offspring of wild fish raised under hatchery conditions were insensitive to predation risk (Álvarez and Nicieza, 2003). Activity patterns also differed between wild and hatchery-reared brown trout: second-generation hatchery fish were predominantly active during daytime regardless of risk levels, but wild fish showed a shift towards nocturnal activity in the presence of predators (Álvarez and Nicieza, 2003). This study highlights the possible role of domestication and hatchery-rearing programmes in weakening adaptive behaviours, and supports the idea that behavioural differences between wild and hatchery-reared individuals can arise from a process of direct or indirect selection on reduced responsiveness to risk, or as a lack of previous experience.

A further key feature of hatchery rearing is that behavioural differences that arise as a result of rearing environment may be overcome by a period of 'training'. For example, Berejikian *et al.* (2000) reared steelhead trout in two environments, enriched and conventional hatchery, and examined competitive ability and habitat use. Juveniles reared in the enriched tanks socially dominated size-matched fish from the conventional hatchery tanks, and when introduced into a simulated stream environment, the enriched fish also grew at a higher rate than the hatchery fish.

The benefits of training could also be applied to to the avoidance of acid episodes through sheltering behaviour. Sheltering within the stream bed may be an important behaviour type in avoiding surface water acidification (Breck *et al.*, 1988), but it is possible that this behaviour may only develop following repeated exposure to acid episodes. Equally, the lack of exposure to acid conditions in hatchery-reared fish may influence survival rates once released into acid-sensitive streams since their ability to exploit spatial variability in habitat quality will be reduced. Could exposure to acid episodes in the hatchery environment allow fish to develop the adaptive behaviours that will enhance survival in the wild? This study compared the behaviour of conventionally reared hatchery fish with fish reared in a simulated wild environment to determine if the low levels of sheltering observed in Chapter 4 can be overcome by training, and enable fish to develop adaptive behaviours.

5.3 METHODS

5.3.1 Experimental animals

Juvenile (0+; i.e. fish in their first year of life) Atlantic salmon were collected from the Environment Agency hatchery at Abercynrig (OS Explorer sheet 12, grid reference 064264), near Abergavenny, Wales in Summer 2006. Fish were approximately 4 months old, size-sorted (between 5 – 6 cm at time of collection), and had previously been kept in a large batch-specific tank (i.e. from the same parental brood stock) with a constant supply of aerated Afon Cynrig river water (circum-neutral). Following transportation to the temperature-controlled facilities at Cardiff University (11.2 ± 0.2°C, $n = 88$ [measurements taken from holding tank prior to and during experiment]), fish were split into two groups (described below). Both groups were fed a hatchery-standard protein mix daily, after dark, to encourage nocturnal foraging. Once used in the experiment, fish were kept in a separate tank to avoid re-use. Following completion of the study, fish were humanely euthanased (Home Office PIL 30/6656).

5.3.2 Rearing conditions

Fish were split equally and randomly into two tanks respectively with basic 'hatchery' conditions and a simulated 'wild' environment. Both groups were supplied with the same free-running, de-chlorinated mains-water, and exposed to the same lighting regime. Tanks were covered with an opaque plastic sheet during daylight to exclude light and avoid disturbing the fish. Sheets were removed after dusk prior to feeding then replaced the following morning. The wild rearing tank was supplemented with a gravel bed, embedded plant-pot shelters, and aquatic plants to provide further refuge and shade. In addition, the wild rearing-tank was periodically dosed with an artificial acid episode, to allow the fish to experience conditions they may be subject to in the natural environment. Episodes of pH 4 – 5 lasting 2 – 3 hours were induced at random intervals in the period September to December, with increasing frequency as autumn progressed from one per week at the start of September to a maximum of three per week in the last week of the training period, after which the experiment started. This approximated the increasing risk of episodic acidification in temperate western Britian (Kowalik and Ormerod, 2006). The episode originated from the same header tank filled with acidified water used in previous experiments, through a series of several additions over the course of a few hours. During the acid episode, the main supply of neutral mains water to the rearing tank was switched off. The plant pot shelters were each separately supplied with de-chlorinated neutral mains water, to mimic the conditions fish would soon experience in the test tank and those observed in the field (i.e. acidified surface water flowing over a circum-neutral hyporheic zone). After the simulated episode, the neutral supply to the main body of the rearing tank was resumed and the acidified header tank supply switched off. Fish that remained in the wild rearing-tank after the experiment commenced continued to be exposed to acid episodes (three per week) through to completion.

5.3.3 Test arena

Fish were observed in the same plastic tank described in Chapter 4 (52 L x 33 W x 24 D cm) and supplied with de-chlorinated mains water cooled with a LEEC closed-circuit cooling device (11.9 ± 0.2°C, n = 44 [measurements taken during experiment in test arena]). It is possible that the cooling element may have resulted in a vertical temperature gradient within the tank. Though the low flow rate of water through the tank makes it unlikely that any such gradient would have developed, any influences of temperature would have been identical across treatment groups. Water entered the tank at the bottom of the upstream end (L1M) and exited at the top of the downstream end (U4M). The tank was divided into 24 zones (13 L x 11 W x 8 D cm) as before: 4 from the upstream end to the upstream end (coded 1 to 4, left to right), 3 from the front of the tank to the rear (coded front [F], middle [M] and back [B]) and 2 vertical layers (coded upper [U] and lower [L]). A shelter (consisting of a hollow pipe 10 cm long sealed at one end with an internal diameter of 6 cm) was positioned in the centre of the tank over L2M (lower 2 middle) and L3M (lower 3 middle), with the open end facing downstream towards L4M.

5.3.4 Experimental protocol

A single Atlantic salmon was chosen at random by scooping a net through the water in either the hatchery or wild rearing tank and using the first fish caught in the net. The fish was transferred to the test tank and allowed to acclimate overnight during the 12 hour dark period in the aquarium; lighting within the aquarium was controlled with a timer such that the light regime was identical for each fish observed. Observations were made in near-darkness (using a 15W red lamp) between 05:30 and 07:30 the following morning. Each trial lasted 100 minutes, and consisted of three time periods: pre-treatment (an acclimation period of 30 minutes); treatment (40 minutes over which time either acid or neutral water was applied to the test tank or shelter according to treatment) and post-treatment (30 minutes). During the first and last 30 minutes of each trial, the test tank and shelter were both supplied with neutral water. During the middle 40 minute period, water conditions in the test tank were manipulated to give conditions as shown in Table 5.1.

Table 5.1: pH conditions in the test-tank water and shelter water during the 40 minute treatment period; neutral pH = 7, acid pH = 4 – 5.

Group	Tank chemistry	Shelter chemistry	Group abbreviation
1. Hatchery 1	Neutral	Neutral	H1
2. Hatchery 2	Acid	Neutral	H2
3. Wild 1	Neutral	Neutral	W1
4. Wild 2	Acid	Neutral	W2

As in Chapters 3 and 4, this time scale was chosen to allow observation of behavioural responses to, and avoidance of, episodic conditions, rather than an evaluation of the full ecotoxicological effect of prolonged exposure. Water supplied to the sealed end of the shelter originated from a neutral header-tank. Water in the main body of the test tank was supplied directly from a tap and acidified using a hand pipette to apply H_2SO_4 over the surface of the tank (according to treatment), following the timescale established in Chapter 4: Pilot work, shown in Table 5.2 (details given in Appendix 4). Treatments 1 and 3 (H1 and W1) acted as controls; during the 40 minute treatment period, neutral water was applied to the main body of water using the hand pipette to mimic conditions in the trials in which sulphuric acid was added to the main tank (treatments 2 and 4, H2 and W2 respectively).

Table 5.2: Number and quantity of acid applications during 40 minute treatment period in the Hatchery Acid (H2) and Wild Acid (W2) groups.

Minutes into treatment period	Volume of acid added (μl)	Number of applications
0	300	4
2	300	4
4	300	4
6	300	4
8	80	4
14	70	4
18	70	2
25	90	2
27	80	4
30	70	2
35	70	2

Where 4 applications are indicated, acid was applied onto the surface water in zones U1F, U1B, U4F and U4B, i.e. in the 4 corners of the tank. Where 2 applications are indicated, acid was applied onto the surface water in zones U1M and U4M.

Eleven replicate trials were carried out within each treatment group using a new fish in each trial (total *n* = 44 fish). Treatments were carried out at random by choosing a folded piece of paper indicating 'H1', 'H2', 'W1' or 'W2' from a box, until 11 trials within each group had been completed. For each fish tested, three behaviours were recorded:

1. Shelter use: time spent in the shelter as a proportion of the corresponding test period;
2. Activity minute^{-1}: the number of times a fish moved from one area of the tank to another;
3. Opercular rate: recorded every five minutes when the fish was visible by counting the number of gill openings over a 15 second period and multiplying by 4 to obtain a 1 minute count; mean values from each period were then used in analyses.

5.3.5 Statistical analysis

A General Linear Model was used to assess the effects of treatment and time period (i.e. pre-treatment, treatment and post-treatment) on activity minute^{-1} and opercular rate (using transformations to normalise data where necessary; activity minute^{-1} data were $\log_{10}$ transformed). Pairwise differences were assessed, where possible, using the Tukey-Kramer *post hoc* method. Data for shelter use were not normally distributed and were therefore not suitable for ANOVA; the Sheirer-Ray-Hare test (a non-parametric ANOVA equivalent with replication) was performed on the arcsine-square root transformed data. A significance level of $P < 0.05$ was used throughout the analyses. Mean values are shown ± standard error throughout the results discussion. Complete statistical results are given in Appendix 7.

5.4 RESULTS

5.4.1 Shelter use

Treatment had a significant effect on the amount of time spent sheltering as a proportion of the corresponding time period (Sheirer-Ray-Hare: $\chi^2_3 = 0.964$, $P < 0.005$) (Figure 5.1). There was no significant effect of pre-treatment/treatment/post-treatment period ($\chi^2_2 = 0.739$, $P = 0.260$), nor was the interaction (treatment*time period) significant ($\chi^2_6 = 0.0.025$, $P = 0.975$).

The amount of time spent sheltering by both hatchery-reared and wild fish was similar between test periods, particularly within the H1 and W1 treatment groups (control groups). However, increases in sheltering behaviour were observed in all treatment groups between the pre-treatment and treatment periods, most notably within the W2 (wild acid) group. Fish spent on average 24.7 ± 8.1% of the pre-treatment period within the shelter, and 44.4 ± 7.4% of the treatment period within the shelter. By contrast, fish in the H2 (hatchery acid) group spent 16.3 ± 4.6% of the pre-treatment period in the shelter, increasing to 23.6 ± 7.2% in the treatment period – an increase of approximately 7%, compared with an increase of 20% in the W2 group; thus fish reared in a simulated wild environment did shelter more when exposed to an acid episode.

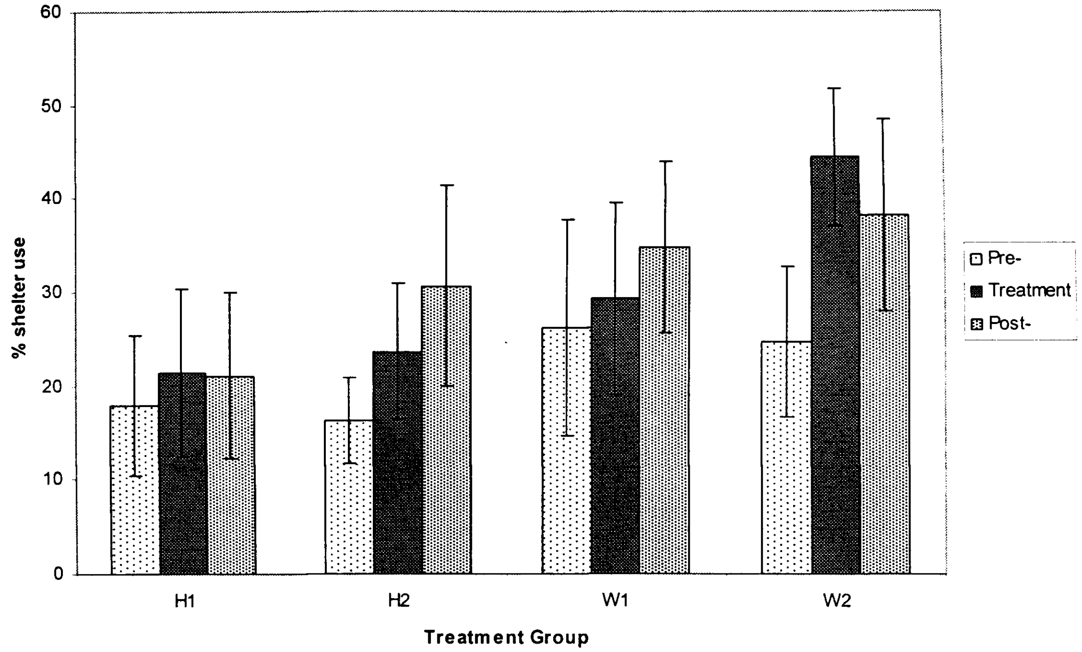


Figure 5.1: Mean shelter use as a proportion of corresponding test period ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (H1: Hatchery Control; H2 = Hatchery Acid; W1 = Wild Control; W2 = Wild Acid).

Activity

Activity minute^{-1} was significantly different between treatment groups ($F_{3,108} = 7.49$, $P < 0.005$), activity was greatest in the hatchery acid (H2) and wild acid (W2) groups, i.e. those in which fish were exposed to an acid episode. Test period was also significant ($F_{2,108} = 7.27$, $P < 0.005$), activity was generally greatest in the treatment and post-treatment periods, particularly in both acid treatment groups (Figure 5.2). The interaction between treatment group and time period was not significant ($F_{6,108} = 0.90$, $P = 0.500$).

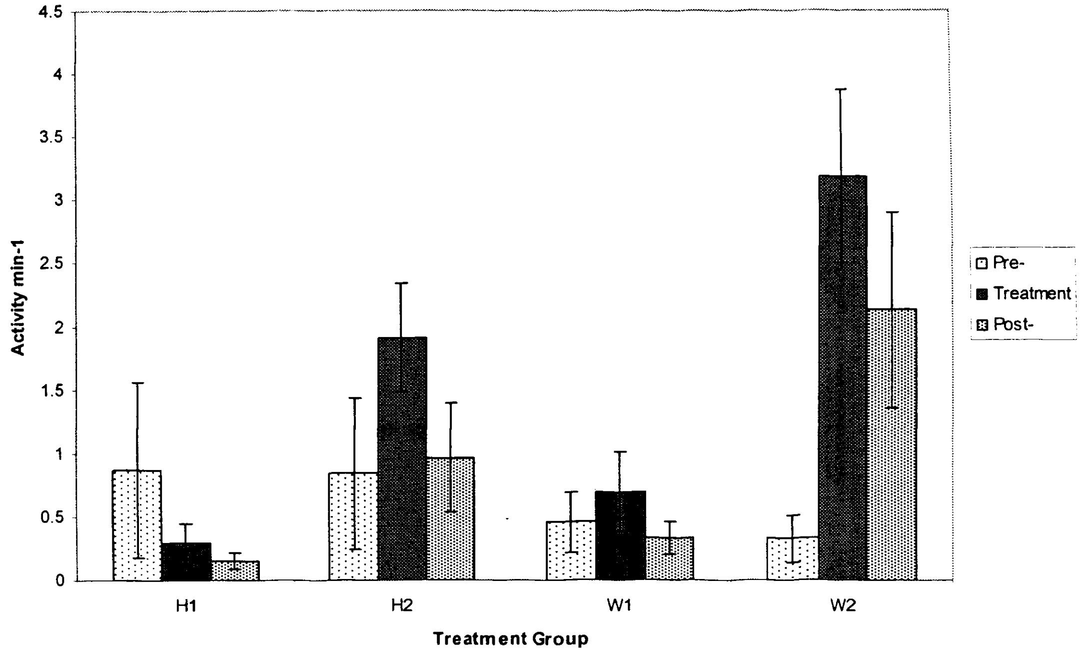


Figure 5.2: Mean activity minute^{-1} ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (H1: Hatchery Control; H2 = Hatchery Acid; W1 = Wild Control; W2 = Wild Acid).

Tukey-Kramer *post hoc* pairwise comparison showed that activity minute^{-1} increased significantly or near-significantly between the pre-treatment and treatment periods in the hatchery acid group ($T = 2.276$, $P < 0.05$) and in the wild acid group ($T = 3.312$, $P < 0.055$). Interestingly, there was no significant difference in activity minute^{-1} between the treatment periods of the hatchery acid and wild acid groups; activity minute^{-1} increased such that the rate of movement was similar between groups.

Opercular rate

The effect of treatment group on opercular rate was significant ($F_{3,108} = 51.44$, $P < 0.005$); opercular rates were greatest in acidified water regardless of the rearing environment (Figure 5.3). Test period also significantly affected opercular rate ($F_{2,108} = 49.61$, $P < 0.005$), with opercular rate highest in the treatment and post-treatment periods of both the acid treatments. The interaction effect between treatment and test period was also significant ($F_{6,88} = 11.29$, $P < 0.05$) (Figure 5.4).

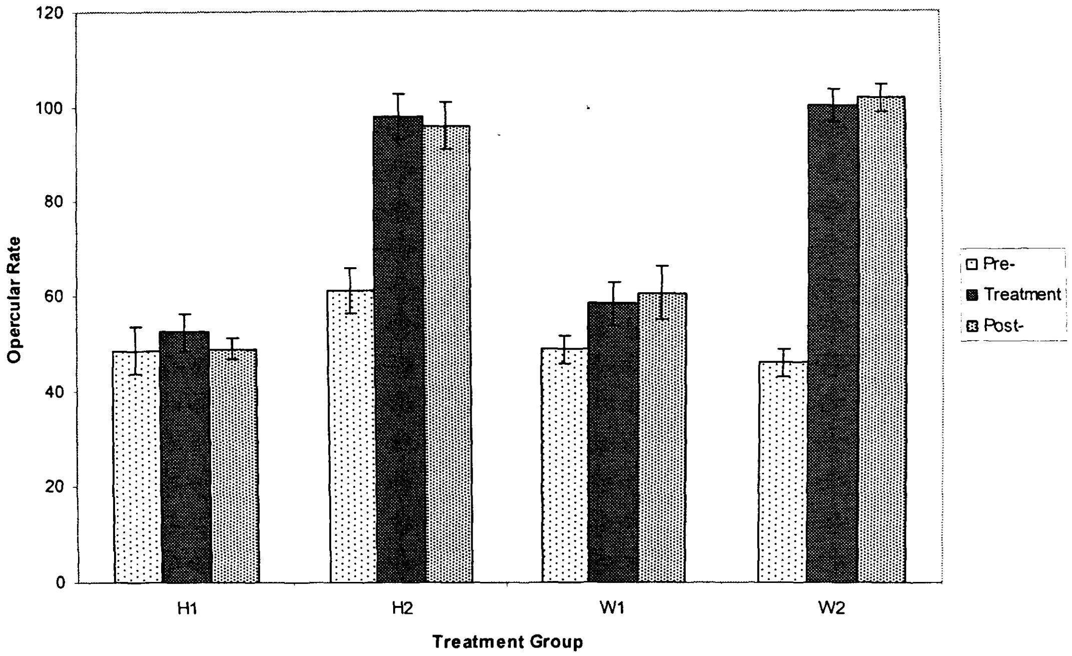


Figure 5.3: Mean opercular rate ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups (H1: Hatchery Control; H2 = Hatchery Acid; W1 = Wild Control; W2 = Wild Acid).

Multiple Tukey-Kramer *post hoc* comparisons revealed a significant increase in opercular rate between the pre-treatment and treatment/post-treatment periods for both the H2 and W2 groups (H2: pre-treatment vs. during treatment T = 5.327, $P < 0.0055$; pre-treatment vs. post-treatment T = 4.889, $P < 0.005$; W2: pre-treatment vs. during treatment T = 9.311, $P < 0.005$; pre-treatment vs. post-treatment T = 9.99, $P < 0.005$). Opercular rate did not return to pre-treatment levels in either group even after a neutral water supply to the tank was resumed. Differences between test periods within the H1 and W1 groups were not significant, thus opercular rate remained broadly the same throughout the experiment when fish were not exposed to an acid episode. There was no significant difference between the treatment periods in the H2 and W2 groups (T = 0.523, P = 1.000), which shows that opercular rate increased to a similar level in both groups.

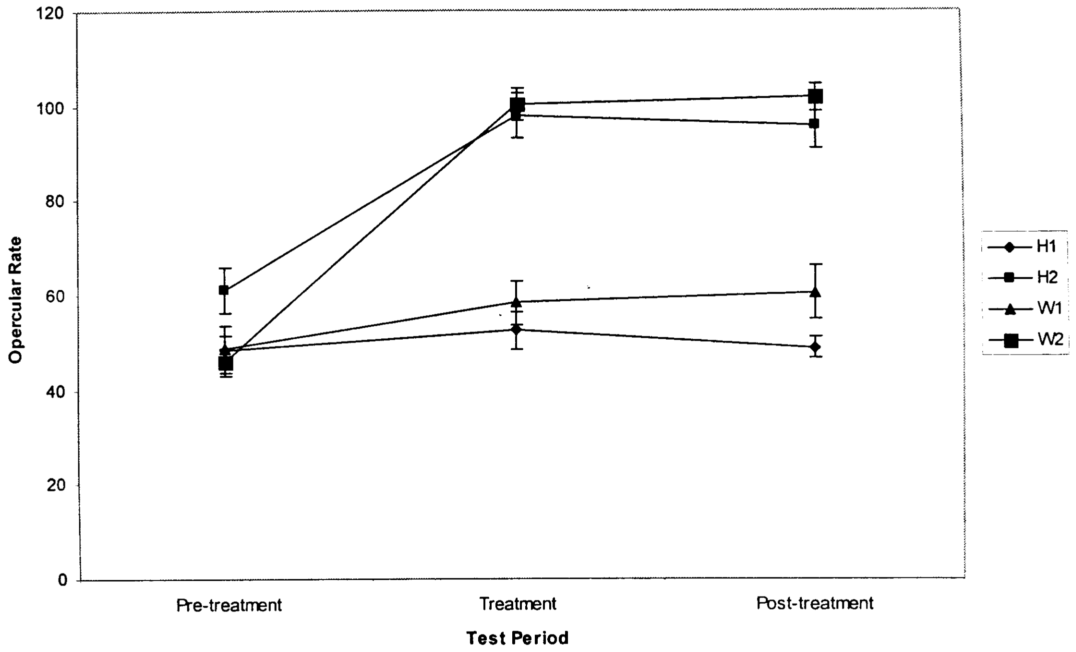


Figure 5.4: Interaction between treatment group (H1: Hatchery Control; H2 = Hatchery Acid; W1 = Wild Control; W2 = Wild Acid) and test period (mean opercular rate ± SE).

5.5 DISCUSSION

The objective of this study was primarily to examine the differences in sheltering behaviour during an acid episode that may arise as a result of rearing environment. The data provide some support for the hypothesis that fish reared in a simulated wild environment will be better able than fish reared in a conventional hatchery environment to detect and avoid an acid episode by using a neutral shelter. Treatment significantly affected the amount of time spent sheltering as a proportion of each test period, though examination of the data showed that this may have been due to increases in sheltering in both groups subjected to an acid pulse rather than an increase just in the wild group. Additionally, even without an acid pulse, on average wild control fish sheltered more in all periods than hatchery control fish did. However, exposure to an acid pulse resulted in a greater proportional increase in sheltering behaviour of wild fish than hatchery-reared fish. In spite of the increase in sheltering with time, test period was not significant, possibly due to the similarities in time spent sheltering by fish between time periods within each control group.

It was also hypothesised that activity minute^{-1} and opercular rate would be lower in wild fish than in hatchery-reared fish when subjected to an acid episode, since wild fish would spend more time in the neutral shelter, thus avoiding the potentially damaging behavioural and physiological effects of an acid episode (see Chapter 4).

The activity hypothesis was supported: activity minute^{-1} was significantly different between treatments, though higher levels of activity were observed in both acid groups compared to the control groups, not just the hatchery acid group. However, activity was higher in the treatment and post-treatment periods of the hatchery acid group than in the wild acid group, possibly due to the greater proportion of time spent sheltering in the treatment period of the wild acid group. This sort of behavioural difference has been previously observed in studies with other salmonids, for example, hatchery-reared brown trout introduced into an experimental stream with wild brown trout fed less, moved more, and spent less time in and around the streambed than the wild fish (Bachman, 1984).

Opercular rate was also significantly different between treatments, and between test periods. However, as with activity, the significant difference between treatments was due to higher opercular rates in the treatment and post-treatment periods of both the acid groups, not just the hatchery acid group. In fact, opercular rate was higher in the wild acid group than in the hatchery acid group. The hypothesis that the opercular rate of wild fish subjected to an acid episode would be lower than hatchery-reared in the same conditions due to increased sheltering was therefore not supported. Additionally, opercular rate remained elevated in both groups within the post-treatment period, after a neutral water supply was resumed to both the tank and shelter, which might indicated the longer-term physiological effects of acid episodes.

These results suggest that rearing environment does affect the activity of fish during exposure to an acid episode; additionally, it seems even a brief acid exposure is enough to elicit changes in opercular rate similar to those shown by non-sheltering hatchery-reared fish. However, increasing opercular rate may be an adaptive behaviour (e.g. to facilitate the observed increases in activity) that serves to enhance survival in the wild, and will be observed regardless of time spent in a neutral shelter vs. an acidified tank. Differences in similar behaviours have been observed in trials with other salmonids. For instance, sensitivity to a perceived predation risk (as indicated by behavioural and heart rate responses) has been widely reported to be greater in wild fish than in hatchery-reared fish (Johnsson and Abrahams, 1991; Berejikian, 1995; Johnsson *et al.*, 1996; Einum and Fleming, 1997; Fleming and Einum, 1997). In a comparison of farmed Atlantic salmon and wild fish from the same principal population (Johnsson *et al.*, 2001), wild fish were found to have higher standard heart rates and showed a more pronounced flight and heart rate response to a simulated predator attack than farmed salmon. Importantly, the effects of domestication increased and adaptive behaviours in older wild fish weakened with time in captivity.

The theory that hatchery-reared salmonids are of inferior quality and have lower smolt-to-adult survival compared to naturally-reared salmon (Larsen *et al.*, 1992) is supported by a number of studies reporting low post-release survival of hatchery-reared fish (e.g. Miller, 1953; Flick and Webster, 1964), and is widely attributed to hatchery fish populations genetically adapting to the unnatural conditions of the hatchery environment at the expense of adaptations necessary for living in natural streams (Reisenbichler, 1994). Gradually declining fitness and ultimate reductions in survival of hatchery-reared fish (Reisenbichler, 1996) mean that the use of supplementation to enhance populations should be carefully considered (Jonsson and Fleming, 1993). Culture may impose new selection pressures that adapt fish to confinement (such as sheltering sharing), but it is important to take this into account when releasing hatchery-reared fish into streams with wild fish (Johnsson *et al.*, 2001). For example, differences in dispersal and habitat use within a novel environment have been observed between hatchery-reared and wild Atlantic salmon (Symons, 1969), with post-release hatchery fish largely remaining within the release site while wild fish moved downstream. Reduced dispersal might have important consequences for hatchery-reared fish released into a wild stream vulnerable to acid episodes. If fish are concentrated within an area where shelter availability is constant the number of refuges per fish will be reduced, and even with the shelter-sharing tendencies observed in hatchery-reared fish some fish may still be excluded from shelters. This may also occur if hatchery-reared fish are out-competed from a shelter by a more aggressive wild fish (Orpwood *et al.*, 2004); in either case, fish will be more vulnerable to an episode in the surface water.

The conventional hatchery environment has been implicated in reducing diet variability in released hatchery fish: when the stomach contents of hatchery-reared Atlantic salmon captured three months after release were compared with wild parr from the same streams, wild fish were found to include a wider range of invertebrate species in their diet than hatchery-reared fish, and also showed fuller stomachs – these differences were attributed primarily to the effects of feeding experience and habitat use (Sosiak *et al.*, 1979), and might mean that wild fish either forage more efficiently, or spend more time feeding. This theory is supported by an earlier study which showed that the high stocking densities within the hatchery environment cause fish to become aggressive and engage in more social interactions, at the expense of time available for foraging (Fenderson *et al.*, 1968). When released in wild streams, the high mortality of hatchery-reared salmon may therefore be due to a loss of feeding time, excessive use of energy, and increased exposure to predators. Thus, there may be important survival consequences for hatchery-reared fish released into a wild stream that arise due to lack of experience of wild conditions, or because typical selection pressures within the hatchery are reduced and new limits imposed. In the wild, sheltering behaviour may hold a significant adaptive advantage in fish exposed to acid episodes (Breck, 1988), but the lack of exposure to acid episodes in hatchery-reared fish clearly leads to a reduction in their ability to expoit variation in habitat quality (Chapter 4). However, this experiment has shown that it might be possible to subject fish in the hatchery environment to conditions mimicking those they are likely to encounter following release, and thus allow them to develop the behaviours necessary to avoid potentially damaging environments.

CHAPTER 6

Discussion

Acidic deposition is considered to be one of the main threats to base-poor fresh waters throughout the industrialised world. Although commonly termed 'acid rain', acidic deposition can take many forms, and has caused the widespread acidification of soil and water. Acid water has affected microscopic algae, zooplankton, insects, birds and mammals. Fish, in particular, have been severely impacted by acidification, with examples of whole populations being eliminated from some ecosystems (Dillon *et al.*, 1984; Muniz, 1984; Baker *et al.*, 1990). Fish play pivotal roles in almost all freshwater ecosystems as well as being economically valuable to national and local economies; for example, rod-caught Atlantic salmon are worth an estimated £350 million to the Scottish economy each year (Drumlean, 2003). Additionally, whilst acidification has been responsible for the loss of many fish populations, salmonids are particularly sensitive to low pH, and were often the first to disappear as acidification problems developed. Salmonids are more physiologically susceptible than other fish, and juveniles occur mainly in upland areas where soils and rocks are most sensitive to acid deposition and adults use headwaters disproportionately during spawning. The combination of high susceptibility and economic value make salmonids, particularly Atlantic salmon, a focus for conservation and management efforts.

As a result of increasing environmental concern and a reduction in the intensity of heavy industry, the last 30 years have seen a decline in emissions responsible for acidification, and some freshwaters have responded, for example through increased average pH. Although responses are slow, changes have made it possible for fisheries managers to attempt to rehabilitate populations affected by acidification, and up to ten million juvenile salmon are released into British rivers every year. Unfortunately, such re-stocking programmes are not always successful, and even where acid tolerant strains are reared and released, fisheries managers report that populations have not returned in the numbers expected despite the apparent improvements in various indices of water quality. One possible explanation is that average measurements of pH mask short-term fluctuations linked to acid episodes. Such episodes occur regularly in upland streams during rainstorms and snowmelt, and are now seen as a major management issue in upland areas throughout Europe. Predictions of recolonisation based on improvements in average stream pH may not therefore have taken into account the damaging effect of these short-term acid events on population survival or recovery.

Although the large-scale chemical recovery of surface waters has been widely reported, indications of biological recovery are fewer (Skjelkvåle, *et al.*, 2003). Lime (Calcium carbonate) has been used previously to mitigate the biological effects of surface water acidification, and while some positive biological responses have been recorded, few ecosystems have been returned to pre-acidification status (Weatherley, 1988). The continued occurrence of acid episodes still adversely affects some species, offsetting biological recovery (Skjelkvåle, *et al.*, 2003; Kowalik *et al.*, 2007). Acid-sensitive salmonids such as brown trout are now returning to some previously impacted waters in the UK, but "recovery" in most cases is modest and very gradual (Monteith *et al.*, 2005). One key question is why are supplemented salmonid populations responding so poorly if some wild populations are recovering? One fundamental explanation may lie in the physiological and behavioural responses to acid episodes of wild populations and those reared in a hatchery environment.

The main focus of this project was to therefore examine the extent to which acid episodes (as characterised by increased discharge and short-term pH fluctuations) affect the behaviour of Atlantic salmon as a possible explanation for the relatively poor establishment and survival of fish populations supplemented with hatchery-reared fish in natural streams. More specifically, the emphasis was to consider the likely success and the problems that might be encountered of using hatchery-reared fish to rehabilitate damaged populations if those fish were exposed to an acid-stressful environment following release and lacked the behavioural adaptations necessary to cope with such stress. Although *in situ* behavioural investigations were not possible, water chemistry data collected in the field were used to design environmentally relevant laboratory-based experiments in which the behaviour of juvenile Atlantic salmon in varying life stages during acid episodes could be studied. The main findings of the project are summarised and discussed below, together with comments on the applied significance of these findings and suggestions for future work.

The first requirement was to obtain an overview of the chemical changes that take place in streams following a prolonged period of rainfall. Preliminary investigations were carried out in 2005 (Appendix 1), and the detailed study in September and December 2006 compared the impact of increased discharge on the surface water and hyporheos in streams with different sensitivities to acid episodes. The expectation was that surface and hyporheic water would show different chemistry, and that increased discharge would have a greater effect on the surface water, whereas low flow velocity and longer residence times of water within the hyporheos would limit dilution of acid-neutralising base cations in this zone to create a more stable and favourable habitat for fish. In addition to assessing the viability of methods to sample water and site suitability, the preliminary study conducted in 2005 showed that there were differences in chemistry between the surface and hyporheos in terms of pH, temperature and conductivity. Differences were not consistent between stream types, though certain trends were observed (such as a general decline in pH, temperature and conductivity as discharge increased), suggesting that the changes observed were part of a wider pattern. The 2006 field study expanded on these findings to further investigate the difference in chemistry between the surface and hyporheos, and how this difference varies with seasonally-related differences in discharge. Sampling was restricted to extremes of flow with streams surveyed at low (base) and at high (episodic) flow, following a prolonged period of rainfall. The field data indicated that there were clear differences in chemistry between the surface and hyporheos, creating spatially distinct habitats of different water quality; furthermore, this difference became more pronounced following increases in discharge, causing the hyporheos to become the more favourable of the two environments. Similar results have been obtained in other studies in terms of temperature and dissolved oxygen (Malcolm *et al.*, 2003a, b, 2004a, b, 2005; Youngson *et al.*, 2004; Soulsby *et al.*, 2005), but this study is one of the first to document spatial differences in pH which in combination with temporal variations (i.e. following an acid episode), may have important consequences for salmonid behaviour and survival, especially for newly released hatchery-reared fish.

Linked to the occurrence of acid episodes and observed differences between the surface and hyporheos is the idea that fish will avoid acidified water and move to areas of more favourable chemistry, and as part of this strategy, would utilise neutral refuges/shelters within the hyporheos, as long as they were available and the fish could recognise their presence. To investigate these ideas, a series of laboratory experiments was designed to test whether fish in different life stages are able to detect and avoid acid episodes, either by moving into an area of more favourable pH, or utilising a shelter.

Atlantic salmon alevins were tested in a vertical substrate-filled tank (Chapter 3). Fish were tested in two groups: a control group in which the entire tank was neutral, and a treatment group in which the lower portion was kept neutral by an upwelling supply of de-chlorinated mains water while the upper portion was acidified with the addition of acid over the top of the tank. It was expected that with the onset of the acid pulse at the top of the tank, alevins would move downwards to areas of more favourable pH, whereas in the neutral control treatment, alevins would move more randomly up and down the tank. Additionally, opercular rate of alevins exposed to an acid pulse (i.e. in the acidified portion of the tank) was predicted to be higher than those in neutral water in the lower portion of the tank and in the control group. The initial predictions were supported – alevins moved away from the acid pulse at the top of the tank into neutral areas, whereas alevins in the control group were more evenly distributed throughout the test tank. Also, opercular rate was higher in alevins exposed to an acid pulse than those in the control group, and within the treatment group, was also higher in the top (acid) portion of the tank than in the bottom (neutral) portion. Importantly, mortality was also higher in the treatment group. This experiment therefore showed that the alevin stage could detect and move away from water with a low pH, and that the investigated range of pH caused physiological stress (as indicated by the higher opercular rate), and death. The avoidance behaviour observed in this study has been reported by other authors (e.g. brook charr alevins; Gunn and Noakes, 1986) but with some exceptions to this general trend (e.g. lake charr embryos; Gunn *et al.*, 1987); the contradictory results obtained in some studies may be explained by differences in acid sensitivity between species (e.g. Turnpenny, 1989), increased susceptibility to acid water at certain life cycle stages or ages (Sayer *et al.*, 1993), or the reduced ability of very young fish to take directional avoidance measures (Gunn *et al.*, 1987). Current studies suggest that the most widely exhibited behavioural response to increased acidity is avoidance behaviour of some type, and that when given an opportunity, fish will move away from water of low pH. In streams susceptible to acid episodes where alevins might be especially vulnerable due to the timing of emergence (Magee *et al.*, 2003) it is possible that behavioural avoidance of acid waters may be a significant adaptive advantage and could increase survival during episodes (Breck *et al.*, 1988). However, if the already high mortality rate of salmon alevins in the wild (Fay *et al.*, 2006) is exacerbated by the physiological effects of episodic depressions in pH, there may be severe implications for the long-term survival of salmon populations.

The acid avoidance behaviour of juvenile Atlantic salmon was investigated using a tank containing a shelter choice arena, in which the test tank and shelter were separately supplied with water of different pH in several combinations (Chapter 4). Preliminary work conducted in 2004 (Appendix 4) showed that fish did not preferentially seek refuge in a neutral shelter during an acid episode, but that other behaviours such as activity and opercular rate were affected, suggesting that acid episodes do lead to changes in behaviour that might affect survival in the wild. The preliminary study was also valuable in refining the methods to be used in the main study conducted in 2006, which used a similar tank and shelter choice arena.

The main hypothesis tested in this experiment was that fish would spend more time in a neutral shelter during an acid episode than under normal (control) conditions; also, it was expected that activity and opercular rate would be highest in those fish exposed to an episode, particularly when no neutral shelter was available (i.e. when both tank and shelter were exposed to the acid pulse). In this study, clear evidence was obtained to show that fish can avoid pulses of acidified water, and in doing so, make use of a neutral shelter. Also, fish actively avoided an acidified shelter proving that use of the neutral shelter during an acid episode was not a chance occurrence, but a behavioural response directly linked to acid exposure. This behaviour is supported by many other studies, all of which conclude that the most widely observed response following exposure to acid water is avoidance behaviour of some type (Johnson and Webster, 1977; Jones *et al.*, 1985a; Gunn and Noakes, 1986; Nakamura, 1986; Atland and Barlaup, 1996; Atland, 1998; Ikuta *et al.*, 2001). Other behavioural responses (activity and opercular rate) observed in the preliminary experiment were also recorded in the main experiment. Activity increased following exposure to an acid pulse, and was highest in the group in which no neutral shelter was provided. Opercular rate also increased, was highest in the treatment without a favourable shelter, and remained elevated even after the acid pulse. In both the alevin (Chapter 3) and parr (Chapter 4) experiments, the observed increases in activity and opercular rate may have implications for fish survival in the wild, including increased metabolic costs and energy losses (Hughes and Shelton, 1962), increased visibility and vulnerability to predators (Rahel and Stein, 1987), and higher susceptibility to the physiological effects of acidified water (Daye and Garside, 1977; Laurent and Perry, 1991).

An important aspect of the acid avoidance experiment with hatchery-reared parr was the idea that differences in behaviour are governed, at least in part, by the previous experience of the fish, or of their rearing environment – as has been observed with many other behaviours, e.g. predator avoidance (Álvarez and Nicieza, 2003), feeding (Sosiak *et al.*, 1979), shelter use (Armstrong and Griffiths, 2001), movement (Bachman, 1984) and habitat use (Symons, 1969) – and that this might affect the way in which fish respond to an acid episode. The hatchery-reared parr did not use the neutral shelter during an acid episode as much as was expected, and shelter use was similar between fish in a neutral environment and those exposed to an acid episode.

These observations led to the hypothesis that shelter utilisation might be higher if fish were exposed to acid conditions during rearing, and prior to experiencing such events after release into streams and rivers, since similar 'training' experiments with other species have reported improvements in potentially adaptive behaviours (e.g. Berejikian *et al.*, 2000). To test this idea, hatchery parr were split into two groups (Chapter 5). One group was held in a conventional hatchery environment (i.e. with no habitat enrichment and a constant water supply); the other group was held in a simulated wild tank, which contained a gravel bed and aquatic plants, and was periodically dosed with an acid episode, during which a refuge was provided by pH neutral plant-pot shelters.

Fish were reared in these two environments for several months to allow for 'conditioning', after which their behaviour was observed in the tank and shelter choice arena previously used for the acid-naïve parr (Chapter 4). It was expected that as a result of experience, wild fish would be better able to take avoidance measures during an episode, and would spend a higher proportion of the treatment period in the neutral shelter. As a direct result of the increased sheltering time, it was also expected that activity and opercular rate would be lower than in hatchery-reared fish that did not shelter. The results confirmed the hypothesis that rearing environment affects fish behaviour during acid episodes – fish reared in the simulated wild environment preferentially used a neutral shelter more than fish from the conventional hatchery environment. However, despite the increased sheltering observed in wild fish, activity and opercular rate were broadly similar between fish from each rearing environment, so even brief acid exposure may still be harmful.

The results obtained in this project are in general agreement with other studies on the same and on other fish species, but have also highlighted areas of new knowledge, particularly in terms of what is known about the effect of increased discharge on stream chemistry, the impact of acid water on the behaviour of Atlantic salmon, and the way in which rearing environment can affect this behaviour. Thus, although differences between surface and hyporheic/groundwater chemistry have been observed in other studies, to date there are none that have considered the effect of increased discharge during an episodic event on chemistry within these zones. Field data showed that differences in chemistry between the surface and hyporheos are increased by high flow, creating a preferable environment within the hyporheos that could be exploited as a refuge. Similarly, although streambed sheltering and acid avoidance in salmonid fish are both well documented, this is the first study that has suggested a link between these behaviours as a direct result of differing surface and hyporheic chemistry. Laboratory-based behavioural experiments showed that Atlantic salmon alevins could detect and avoid acid pulses (by moving to an area of neutral water) but suffered high mortality under acid conditions. Also, Atlantic salmon parr avoided an acid pulse by using a neutral shelter, but this response was much enhanced in 'wild' fish with previous experience of acid episodes.

General conclusions

Acidification of soil and water is a long-term problem, and despite recent declines in acidifying emissions and improvements in water quality, is clearly still impacting freshwater systems. Episodic acidification is most common during seasons of high precipitation and is least common in summer; however, one of the features of climate warming is not only a gradual increase in average temperatures, but also greater frequency of unpredictable extreme climatic events. For example, flooding in England and Wales in 2007 occurred in July, at a time of year when rainfall should have been lowest. If the conditions that give rise to acid episodes occur more often then it is probable that such episodes will continue to inhibit the survival of hatchery-reared fish, particularly those released into systems without any habituation to the conditions that might occur after release.

It is difficult to predict exactly what effect acid episodes might have on populations of Atlantic salmon, for a number of reasons. Differences in habitat structure and genetic variability between populations mean that a standard response probably does not exist. In addition, although acid episodes are considered a major management issue through upland areas of Europe, individual areas vary in their vulnerability to episodes. However, given the impact acid episodes clearly have on freshwater systems, and the likelihood that the frequency of episodes might increase, it is imperative that measures be taken to minimise the effects on fish populations in vulnerable areas.

First, longer-term monitoring of chemistry in acid-sensitive streams and rivers should be undertaken to obtain a year-round overview of conditions potentially damaging to fish populations. To further investigate behavioural avoidance of acid waters, a semi-natural environment (e.g. the stream channel at FRS Almondbank in Pitlochrie, Scotland) could be used to conduct behavioural experiments (similar to that in Chapter 4) with PIT-tagged fish to determine if they preferentially use more favourable hyporheic shelters, or if sheltering takes place regardless of water quality. Also, a more extensive training experiment could be performed. A genetically identical population of fish could be developed using eggs from a single wild male and female fish, fertilised and hatched under controlled conditions. Following fry emergence, the initial populations would be split into two, one batch to be reared in a conventional hatchery environment while the other would be released into a contained stretch of stream, for example beneath an impassable waterfall (Orpwood, 2005), from which other salmonids had been removed through electrofishing and relocation. At the parr stage (by which time wild fish should have experienced several episodes), acid avoidance behaviour could be tested in a semi-natural environment as described above to confirm variability between rearing environment. Furthermore, to test if such differences can be eradicted by experience/training, both populations could be released back into a confined stretch of water for episode exposure, then recaptured and retested. The differing responses of hatchery-reared and wild fish observed in Chapter 5 suggest that it might be possible to improve the survival of hatchery fish after release; new procedures could be introduced into standard hatchery-rearing protocol so that fish are exposed to more naturalistic conditions prior to release. Additionally, since mortality of wild fish is strongly density dependent and intrinsically linked to refuge availability (Armstrong and Griffiths, 2001), development of mitigation measures within streams, such as chemically manipulated artificial shelters, would ensure that refuge availability does not limit population growth.

References

Ailbone, M. R. and Fair, P. (1981). The effects of low pH on the respiration of *Daphnia magna* (Straus). *Hydrobiologica* **85**: 185-188.

Alexander, G. R. (1979). Predators of fish in coldwater streams. In *Predator-Prey Systems in Fisheries Management* (Stroud, H. R. and Clepper, H., eds.) pp. 153-170. Washington DC.

Allard, M. and Moreau, G. (1985). Short-term effect on the metabolism of lotic benthic communities following experimental acidification. *Canadian Journal of Fisheries and Aquatic Sciences* **42** (10): 1676-1680.

Allard, M. and Moreau, G. (1987). Effects of acidification on a lotic macroinvertebrate community. *Hydrobiologia* **144**: 37-49.

Almer, B. (1972). Effects of acidification on fish populations in lakes on the Swedish west coast. *Institute of Freshwater Research Drottningholm, Information* **12**: 1-47.

Almer, B., Dickson, W., Ekstrom, E. and Miller, U. (1974). Effects of acidification on Swedish lakes. *Ambio* **3**: 30-36.

Almer, B., Dickson, W. T., Ekström, C. and Hörnström, E. (1978). Sulphur pollution and the aquatic environment. In *Sulphur in the Environment* (Nriagu, J. O., ed.) pp. 272-311. New York: J. O. Wiley and Sons.

Alvarez, D. and Nicieza, A. G. (2003). Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. *Journal of Fish Biology* **63**: 1565-1577.

Alvo, R., Hussell, D. J. T. and Berrill, M. (1988). The breeding success of common loons (*Gavia immer*) in relation to alkalinity and other lake characteristics in Ontario. *Canadian Journal of Zoology* **66**: 746-752.

Andelman, J. B. and Miller, J. R. (1986). Impact of acid rain on aluminium species in streams. *Water Quality Bulletin* **11**: 19-25.

Andersson, G. (1985). Decomposition of alder leaves in acid lake waters. *Ecological Bulletin* **37**: 1154-1162.

Andrén, C., Henriksen, L., Olsson, M. and Nilson, G. (1988). Effects of pH and aluminium on embryonic and early larval stages of Swedish brown frogs *Rana arvalis*, *R. temporaria* and *R. dalmatina. Holartic Ecology* **11**: 127-135.

Armstrong, J. D. and Griffiths, S. W. (2001). Density dependent refuge use among over-wintering wild Atlantic salmon juveniles. *Journal of Fish Biology* **58**: 1524-1530.

Arnold, D. E., Bender, P. M., Hale, A. B. and Light, R. W. (1981). Studies on infertile, acidic Pennsylvania streams and their benthic communities. In *Effects of Acidic Precipitation on Benthos* (Singer, R., ed.). North American Benthological Society.

Artigas, M. L., Skjæraasen, J. E., Utne-Palm, A. C. and Nilsen, T. (2005). Recovery from handling stress in *Gadus morhua. Journal of Fish Biology* **67**: 384-391

Atchison, G. J., Henry, M. G. and Sandheinrich, M. B. (1987). Effects of metals on fish behaviour: a review. *Environmental Biology of Fishes* **18**: 11-25.

Atland, A. (1998). Behavioural responses of brown trout, *Salmo trutta*, juveniles in concentration gradients of pH and Al - a laboratory study. *Environmental Biology of Fishes* **53** (3): 331-345.

Atland, A. and Barlaup, B. T. (1995). Avoidance of toxic mixing zones by Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) in the limed River Audna, southern Norway. *Environmental Pollution* **90**: 203-208.

Bache, B W. (1984). Soil-water interactions. *Philosophical Transactions of the Royal Society of London Series B* **305**: 393-407.

Bachman, R. A. (1984). Foraging Behavior of Free-Ranging Wild and Hatchery Brown Trout in a Stream. *Transactions of the American Fisheries Society* **113**: 1-32.

Baker, J. P. and Schofield, C. L. (1982). Aluminium toxicity to fish in acidic waters. *Water, Air and Soil Pollution* **18**: 289-309.

Baker, J. P., Bernard, D. P., Christensen, S. W., Sale, M. J., Freda, J., Heltcher, K., Marmorek, D., Rowe, L., Scanlon, P., Suter, G., Warren-Hicks, W. and Welbourn, P. (1990). Biological effects of changes in surface water acid-base chemistry. In *National Acid Precipitation Assessment Program, Acid Deposition: State of the Science and Technology* (Baker, J. P., ed.) pp. 381. NAPAP Report 13, Volume 2.

Baker, J. P., Van Sickle, J., Gagen, C. J., DeWalle, D. R., Sharpe, W. E., Carline, R. F., Baldigo, B. P., Murdoch, P. S., Bath, D. W., Kretser, W. A., Simonin, H. A. and Wigington, P. J. (1996). Episodic acidification of small streams in the northeastern United States: Effects on fish populations. *Ecological Applications* **6** (2): 422-437.

Baldigo, B. P. and Murdoch, P. S. (1997). Effect of stream acidification and inorganic aluminium on mortality of brook trout (*Salvelinus fontinalis*) in the Catskill Mountains, New York. *Canadian Journal of Fisheries and Aquatic Sciences* **54**: 603-615.

Baldigo, B. P. and Lawrence, G. B. (2000). Composition of fish communities in relation to stream acidification and habitat in the Neversink River, New York. *Transactions of the American Fisheries Society* **129**: 60-76.

Baldigo, B. P. and Lawrence, G. B. (2001). Effects of stream acidification and habitat on fish populations of a North American river. *Aquatic Sciences* **63**: 196-222.

Bardonnet, A. and Bagliniere, J-L. (2000). Freshwater habitat of Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **57**: 497-506.

Barlaup, B. T. and Åtland, A. (1996a). Avoidance behaviour of Atlantic salmon (*Salmo salar* L.) fry in waters of low pH and elevated aluminium concentration: laboratory experiments. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 1827-1834.

Barlaup, B. T. and Åtland, A. (1996b). Episodic mortality of brown trout (*Salmo trutta* L.) caused by sea salt induced acidification in western Norway: effects on different life stages within three populations. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 1835-1843.

Battarbee, R. W. (1984). Diatom analysis and the acidification of lakes. *Philosophical Transactions of the Royal Society of London Series B* **305**: 451-477.

Beamish, R. J. (1970). Factors affecting the age and size of white suckers at maturity. PhD Thesis, University of Toronto, Canada.

Beamish, R. J. (1976). Acidification in lakes in Canada by acid precipitation and the resulting effects on fishes. *Ambio* **4**: 98-102.

Beamish, R. J. and Harvey, H. H. (1972). Acidification of the LaCloche Mountain Lakes, Onatario, and resulting fish mortalities. *Journal of Fisheries Research Board of Canada* **29**: 1131-1143.

Beamish, R. J., Lockhart, W. L., Van Loon, J. C. and Harvey, H. H. (1975). Long-term acidification of a lake and resulting effects on fishes. *Ambio* **4**: 98-102.

Beauchamp, J. J., Christensen, S. W. and Smith, E. P. (1992). Selection of factors affecting the presence of brook trout (*Salvelinus fontinalis*) in Adirondack lakes – A Case Study. *Canadian Journal of Fisheries and Aquatic Sciences* **49**: 597-608.

Beggs, G. L., Gunn, J. M. and Olver, C. H. (1985). The sensitivity of Ontario lake trout (*Salvelinus namaycush*) and lake trout lakes to acidification. *Ontario Fisheries Technical Report Series* **13**: 1-27.

Beggs, G. L. and Gunn, J. M. (1986). Response of lake trout (*Salvelinus namaycush*) and brook trout (*S. fontinalis*) to Surface Water Acidification in Ontario. *Water, Air and Soil Pollution* **30**: 711-717.

Bell, H. L. (1971). Effect of low pH on the survival and emergence of aquatic insects. *Water Resources Research* **5**: 313-319.

Berejikian, B.A. (1995). The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. *Canadian Journal of Fisheries and Aquatic Sciences* **52**: 2476- 2482.

Berejikian, B. A., Tezak, E. P., Flagg, T. A., LaRae, A. L., Kummerow, E. and Mahnken, C. V. W. (2000). Social dominance, growth, and habitat use of age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environments. *Canadian Journal of Fisheries and Aquatic Sciences* **57**: 628-636.

Bick, H. and Drews, E. F. (1973). Self purification and silicate communities in an acid milieu (model experiments). *Hydrobiologia* **42**: 393-402.

Binkley, D. and Sollins, P. (1990). Factors determining differences in soil pH in adjacent conifer and alder-conifer stands. *Soil Science Society of America Journal* **54**: 1427-1433.

Bradley, D. C., Ormerod, S. J. (2002). Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater Biology* **47**: 161–171.

Brakke, D. F., Baker, J. P. Bohmer, J., Hartmann, A., Havas, M., Jenkins, A., Keey, C., Ormerod, S. J., Paces, T., Putz, R., Rosseland, B. O., Schindler, D. W. and Segner, H. (1994). Group report: physiological and ecological effects of acidification on aquatic biota. In *Acidification of Freshwaters: Implications for the Future* (Steinberg, C. E. W. and Wright, R. F., eds.) pp. 275-312. Chichester: Wiley Publishers.

Breck, J. E., DeAngelis, D. L., van Winkle, W. and Christensen, S. W. (1988). The potential importance of spatial and temporal heterogeneity in pH, Al and Ca in allowing survival of a fish population: a model demonstration. *Ecological Modelling* **41** (1/2): 1-16.

Bremset, G. (2000). Seasonal and diel changes in behaviour, microhabitat use and preferences by young pool-dwelling Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*. *Environmental Biology of Fishes* **59**: 163-179.

Brett, J. R. (1958). Implications and assessments of environmental stress. In *Investigations of Fish-power Problems* (P. A. Larkin, ed.) pp. 69-83. H. R. MacMillan Lecture in Fisheries, University of British Columbia.

Brett, M.T. (1989). Zooplankton communities and acidification processes (a review). *Water, Air, and Soil Pollution* **44**: 387-414.

Brettum, P. (1996). Changes in the volume and composition of phytoplankton after experimental acidification of a humic lake. *Environment International* **22**: 619-628.

Brewin, P. A., Reynolds, B., Stevens, P.A., Gee, A.S., and Ormerod, S.J. (1996). The effects of sampling frequency on chemical parameters in acid sensitive streams. *Environmental Pollution* **93**: 147–157.

Brezonik P.L., Eaton J.G., Frost T.M., Garrison, P. J., Kratz, T. K., Mach, C. E., McCormick, J. H., Perry, J. A., Rose, W. A., Sampson, C. J., Shelley, B. C. L., Swenson, W. A. and Webster, K. E. (1993). Experimental acidification of Little Rock Lake, Wisconsin: chemical and biological changes over the pH range 6.1 to 4.7. *Canadian Journal of Fisheries and Aquatic Sciences* **50**: 1101-1121.

Brown, D. J. A. (1982). Influence of calcium on the survival of eggs and fry of brown trout (*Salmo trutta*) at pH 4.5. *Bulletin of Environmental Contamination and Toxicology* **28**: 664-668.

Brown, D. J. A. and Sadler, K. (1989). Fish survival in acid waters. In *Acid Toxicity and Aquatic Animals* (Morris, R., Taylor, E. W., Brown, D. J. A. and Brown, J. A., eds.) pp.31-44. Cambridge: Cambridge University Press.

Buckton, S. T., Brewin, P. A., Lewis, A., Stevens, P. and Ormerod, S. J. (1998). The distribution of dippers, *Cinclus cinclus* (L.), in the acid-sensitive region of Wales, 1984-95. *Freshwater Biology* **39**: 387-396.

Burns, J. C., Coy, J. S., Tervet, D. J., Harriman, R., Morrison, B. R. S. and Quine, C. P. (1984). The Loch Dee project: a study of the ecological effects of acid precipitation and forest management on an upland catchment in South-West Scotland. 1. Preliminary investigations. *Fisheries Management* **15** (4): 145-167.

Burns, M. D., Fraser, N. H. C and Metcalfe, N. B. (1997). An automated system for monitoring fish activity patterns. *Transactions of the American Fisheries Society* **126**: 1036-1040.

Burton, T. M., Stanford, R. M. and Allan, J. W. (1982). The effects of acidification on stream ecosystems. In *Acid Precipitation: Effects on Ecological Systems* (D'Itri, ed.) Ann Arbor, MI: Ann Arbor Science Publishers Inc.

Burton, T. M., Stanford, R. M. and Allan, J. W. (1985). Acidification effects on stream biota and organic matter processing. *Canadian Journal of Fisheries and Aquatic Sciences* **42**: 669-675.

Bustard, D. R. and Narver, D. W. (1975). Aspects of the winter ecology of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **32**: 667-680.

Camargo, J. A. (1995). Effect of body size on the interspecific tolerance of aquatic insects to low pH: a laboratory study. *Bulletin of Environmental Contamination and Toxicology* **54**: 403-408.

Cannon, W. B. (1935). Stresses and strains of homeostasis. *American Journal of Medical Science* **189**: 1-14.

Carline, R. F., DeWalle, D. R., Sharpe, W. E., Dempsey, B. A., Gagen, C. J. and Swistock, B. (1992). Water chemistry and fish community responses to episodic stream acidification in Pennsylvania, USA. *Environmental Pollution* **78**: 45-48.

Carr, R. S., Barrows, M. E., Reinchenbach, N. G., De Graeve, G. M., Pollock, T. L., Fava, J. A., and Glickman, A. H. (1990). Investigation of preference-avoidance responses to an oil refinery effluent with striped bass and steelhead trout. *Environmental Toxicology and Chemistry* **9**: 1513-1521.

Carrick, T. R. (1979). The effect of acid water on the hatching of salmonid eggs. *Journal of Fish Biology* **14**: 165-172

Carter, J. C. H., Taylor, W. D., Chengalath, R. and Scruton, D. A. (1986). Limnetic zooplankton assemblages in Atlantic Canada with special reference to acidification. *Canadian Journal of Fisheries and Aquatic Sciences* **43**: 444-456.

Chamier, A. C. (1987). The effects of pH on microbial degradation of leaf litter in seven streams in the English Lake District. *Oecologia* **71**: 491-500.

Clark, K. L. and Euler, D. (1982). The importance of pH and habitat disturbance in amphibian distributions in central Ontario. *Ontario Ministry of Natural Resources, Lakeshore Capacity Study Technical Report.* Toronto: O. M. N. R.

Cleveland, L., Little, E. E., Hamilton, S. J., Buckler, D. R. and Hunn, J. B. (1986). Interactive toxicity of aluminium and acidity to early life stages of brook trout. *Transactions of the American Fisheries Society* **115**: 610-620.

Cleveland, L., Little, E. E., Wiedmeyer, R. H. and Buckler, D. R. (1989). Chronic no-observed-effect concentrations of aluminium for brook trout exposed in low-calcium, dilute acidic water. In *Environmental Chemistry and Toxicology of Aluminium* (Lewis, T.E., ed.) pp. 229-245. Chelsea, Minnesota: Lewis Publishers.

Close, D. A., Fitzpatrick, M. S., Lorion, C. M., Li, H. W. and Schrek, C. B. (2003). Effects of introperitoneally implanted radio transmitters on the swimming performance and physiology and Pacific Lamprey. *North American Journal of Fisheries Management* **32**: 1184-1192

Clymo, R. S. (1984). Sphagnum-dominated peat bog: a naturally acid ecosystem. *Philosophical Transactions of the Royal Society of London Series B* **305**: 487-499.

Coble, D. W. (1961). Influence of water exchange and dissolved oxygen in redds on survival of steelhead trout embryos. *Transactions of the American Fisheries Society* **90**:469-474.

Collier, K. J. and Winterbourn, M. J. (1990). Structure of epilithon in some acidic and circumneutral streams in South Westland, New Zealand. *New Zealand Natural Sciences* **17**: 1-11.

Conway, H. L. and Hendrey, G. R. (1982). Ecological effects of acid precipitation on primary producers. In *Acid Precipitation: Effects on Ecological Systems* (D'Itri, ed.) Ann Arbor, MI: Ann Arbor Science Publishers Inc.

Cooper, D. M. and Jenkins, A. (2003). Response of acid lakes in the UK to reductions in atmospheric deposition of sulphur. *Science of the Total Environment* **313**: 91-100

Cumming, B. F., Smol, J. P. and Birks, H. J. B. (1992). Scaled Chrysophytes (Chrysophyceae and Synurophyceae) from Adirondack drainage lakes and their relationship to environmental variables. *Journal of Phycology* **28**: 162-178.

Cumming, B. F. and Smol, J. P. (1993). Development of diatom-based salinity models for palaeoclimatic research from lakes in British Columbia (Canada). *Hydrobiologia* **269/270**: 179-196.

Cunjak, R. A. (1988). Behaviour and microhabitat of young Atlantic salmon (*Salmo salar*) during winter. *Canadian Journal of Fisheries and Aquatic Sciences* **45**: 2156-2160.

Curry, R. A., Powles, P. M., Gunn, J. M. and Liimatainen, V. A. (1991). Emergence chronology of brook charr, *Salvelinus fontinalis*, alevins in an acidic stream. *Environmental Biology of Fishes* **31**: 25-31.

Dalziel, T. R. K. and Lynam, S. (1991). Survival and development of four strains of Norwegian trout and one strain of Scottish trout (*Salmo trutta*) exposed to different pH levels in the absence of aluminium. *PowerGen Technology and Research Department Report TR/91/23052/R*. 34 pp.

Davies, T. D., Tranter, M., Wigington, P. J. Jr and Eshleman, K. N. (1992). 'Acidic episodes' in surface waters in Europe. *Journal of Hydrology* **132**: 25–69.

Davis, J. C. (1973). Sublethal effects of bleached kraft pulp mill effluent on respiration and circulation in sockeye salmon (*Onchorhynchus nerka*). *Journal of the Fisheries Research Board of Canada* **30**: 369-377.

Davis R. B., Smith, M. O., Bailey, J. H. and Norton, S. A. (1978). Acidification of Maine (USA) lakes by acidic preciptation. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **20**: 532-537.

Daye, P. G. and Garside, E. T. (1977). Lower lethal levels of pH for embryos and alevins of Atlantic salmon, *Salmo salar* L. *Canadian Journal of Zoology* **55**: 1504-1508.

Daye, P. G. and Garside, E. T. (1979). Development and survival of embryos and alevins of the Atlantic salmon, *Salmo salar* L., continuously exposed to acidic levels of pH, from fertilization. *Canadian Journal of Zoology* **57**: 1713-1718.

Daye, P. G. and Garside, E. T. (1980). Structural alterations in embryos and alevins of the Atlantic salmon, *Salmo salar* L. induced by continuous or short-term exposure of acidic levels of pH. *Canadian Journal of Zoology* **58**: 27-43.

Dietrich, D. and Schlatter, C. (1989). Aluminium toxicity to rainbow trout at low pH. *Aquatic Toxicology* **15** (3): 197-212.

Dillon, P. J., Yan, N. D. and Harvey, H. H. (1984). Acidic deposition: effects on aquatic ecosystems. *CRC Critical Reviews in Environmental Control* **13**; 167-194.

Dixit, A. S., Dixit, S. S. and Smol, J. P. (1992). Long-term trends in lake water pH and metal concentrations inferred from diatoms and chrysophytes in three lakes near Sudbury, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* **49** (Supplement 1): 17-24.

Dixit, S. S, Smol, J. P., Charles, D. F., Hughes, R. M., Paulsen, S. G. and Collins, G. B. (1998). Assessing water quality changes in the lakes of the north-eastern United States using sediment diatoms. *Canadian Journal of Fisheries and Aquatic Sciences* **56**: 131-152.

Dockray, J. J., Morgan, I. J., Reid, S. D. and Wood, C. M. (1998). Responses of juvenile rainbow trout under food limitation, to chronic low pH and elevated summer temperatures, alone and in combination. *Journal of Fish Biology* **52** (1): 62-82.

Eddy, F. B. and Talbot, C. (1985). Sodium balance in eggs and dechlorinated embryos of the Atlantic salmon *Salmo salar* L. exposed to zinc, aluminium, and acid waters. *Comparative Biochemistry and Physiology* **81C**: 259-266.

Edmunds, W. and Coe, K. (1986). Geochemical aspects of acid rain. *Journal of the Geological Society of London*. **143**: 619-620.

Einum, S. and Fleming, I.A. (1997). Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. *Journal of Fish Biology* **50**: 634-651.

Elder, F. C. (1992). Acid deposition – acidification of the environment. *Acs Symposium Series* **483**: 36-63.

Ekweozor, I. K. E., Bobmanual, N. O. K. and Gabriel, U. U. (2001). Sublethal effects of Ammoniacal fertiliser effluents on 3 commercial fish species from Niger Delta area, Nigeria. *Journal of Applied Sciences and Environmental Management* **5**: 63-68.

Elliott, J. M. (1994). *Quantitaive Biology and the Brown Trout*. Oxford University Press, Oxford.

Elliott, J.M. (1997). Variable effects of droughts on the density of a sea-trout *Salmo trutta* population over 30 years. *Journal of Applied Ecology* **34**: 1229-1238.

Elwood, J.W. and Waters, T.F. (1969). Effects of floods on food consumption and production rates of a stream brook trout population. *Transactions of the American Fisheries Society* **98**: 253–262.

Engblom, E. and Lindell, P. (1984). The mapping of short-term acidification with the help of biological pH indicators. *Institute of Freshwater Research Drottningholm, Report* **61**: 60-68.

Environment Canada (1990). Long range transport of Airborne Pollutants, Research and Coordinating Committee, Canada LRTAP Assessment, Atmospheric Environment Service. Environment Canada, 7 Volumes.

Eriksson, M. O. G. (1984). Acidification of lakes: Effects on waterbirds in Sweden. *Ambio* **13**: 260-262.

Esch G. W., Gibbons, J. W. and Bourque, J. E. (1975). An analysis of the relationship between stress and parasitism. *American Midland Naturalist* **93**: 339-353.

Fangmeier, A., Hadwiger-Fangmeier, A., Van der Eerden and Jager, H-J. (1995). Effects of atmospheric ammonia on vegetation – a review. *Environmental Pollution*. **86**: 43-82.

Fay, C., Barton, M., Craig, S., Hecht, A., Pruden, J., Saunders, R., Sheehan, T., and Trial, J. (2006). Status review for anadromous Atlantic salmon (*Salmo salar*) in the United States. Report to the National Marine Fisheries Service and U.S. Fish and Wildlife Service. 283 pp.

Feldman, R. S. and Connor, E. F. (1992). The relationship between pH and community structure of invertebrates in streams of the Shenandoah National Park, Virginia, USA. *Freshwater Biology* **27**: 261-276.

Fenderson, O. C., Everhart, W. H. and Muth, K. K. (1968). Comparative agonistic and feeding behaviour of hatchery-reared and wild salmon in aquaria. *Journal of the Fisheries Research Board of Canada* **25**: 1-14.

Findlay, D. L. and Saesura, G. (1980). Effects on phytoplankton biomass, succession and composition in Lake 223 as a result of lowering pH levels from 7.0 to 5.6. Data from 1974-1979. *Can. MS Rep. No. 1585, Fisheries and Aquatic Sciences, Western Region*. Wiinipeg: Department of Fisheries and Oceans. 16 pp.

Findlay, D. L. and Kasian, S. E. M. (1990). Phytoplankton communities of lakes experimentally acidified with sulphuric and nitric acids. *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 1378-1386.

Fleming I. A., and Einum, S. (1997). Experimental test of genetic divergence of farmed from wild Atlantic salmon due to domestication. *ICES Journal of Marine Science* **54**: 1051-1063.

Flick, W. A., and Webster, D. A. (1964). Comparative first year survival and production in wild and domestic strains of brook trout, *Salvelinus fontinalis*. *Transactions of the American Fisheries Society* **93**: 58–69.

Flower, R. J. and Battarbee, R. W. (1983). Diatom evidence for recent acidification of two Scottish lochs. *Nature* **305**: 130-133.

Flower, R. J., Battarbee, R. W. and Appleby, P. G. (1987). The recent palaeolimnology of acid lakes in Galloway, south-west Scotland: diatom analysis, pH trends, and the role of afforestation. *Journal of Ecology* **75**: 797-824.

France, R. L. and Wellbourn, P. M. (1992). Influence of lake pH and macrograzers on the distribution and abundance of nuisance epiphytic algae in Ontario, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **49**: 185-195.

Fraser, N. H. C., Metcalfe, N. B. and Thorpe, J. E. (1993). Temperature-dependent switch between diurnal and nocturnal foraging in salmon. *Proceedings of the Royal Society of London Series B* **252**: 135-139.

Fraser, N. H. C., Heggenes, J., Metcalfe, N. B. and Thorpe, J. E. (1995). Low summer temperatures cause juvenile Atlantic salmon to become nocturnal. *Canadian Journal of Zoology* **73**: 446-451.

Freda, J. (1986). The influence of acidic pond water on amphibians. A review. *Water, Air and Soil Pollution* **30**: 439-450.

Frenette, J.-J. and Dodson, J. J. (1984). Brook trout (*Salvelinus fontinalis*) population structure in acicified Lac Tantare, Quebec. *Canadian Journal of Fisheries and Aquatic Sciences* **41**: 865-877.

Frick, K. G. and Hermann, J. (1990). Aluminium and pH effects on sodium ion regulation in mayflies. In *The Surface Waters Acidification Programme* (Mason, B. J., ed.) pp. 409-412. Cambridge: Cambridge University Press.

Fricke, H., Hissman, K., Schauer, J., Reinicke, O., Kasang, L. and Plante, R. (1991). Habitat and population size of the living coelacanth, *Latimeria chalumnae*, at Grande Comore. *Environmental Biology of Fishes* **32**: 287-300.

Friedland, K. D., Hansen, L. P. and Dunkley, D. A. (1998). Marine temperature experienced by post-smolts and survival of Atlantic salmon, *Salmo salar* L., in the North Sea area. *Fisheries Oceanography* **7**: 22-34.

Gagen, C. J., Sharpe, W. E., DeWalle, D. R. and Kimmel, W. G. (1989). Pumping alkaline groundwater to restore a put-and-take trout fishery in a stream acidified by atmospheric deposition. *North American Journal of Fisheries Management* **9**: 92-100.

Gagen, C. J., Sharpe, W. E. and Carline, R. F. (1994). Downstream movement and mortality of brook trout (*Salvelinus fontinalis*) exposed to acidic episodes in streams. *Canadian Journal of Fisheries and Aquatic Sciences* **51**: 1620-1628.

Galloway, J. N., Hendrey, G. R., Schofield, C. L., Peters, N. E. and Johannes, A. H. (1987). Processes and causes of lake acidification during spring snowmelt in the west-central Adirondack Mountains, New York. *Canadian Journal of Fisheries and Aquatic Sciences* **44**: 1595-1602.

Gerhardt, A. (1993). Review of impact of heavy metals on stream invertebrates with special emphasis on acid conditions. *Water, Air and Soil Pollution* **66**: 289-314.

Gee, A. S. and Stoner, J. H. (1988). The effects of afforestation and acid deposition on the water quality of upland Wales. In *Ecological Change in the Uplands* (Usher, M. B. and Thompson, D. B. A., eds.) pp. 237-287. Oxford: Blackwell Publishers.

Grahn, O. (1980). Fish kills in two moderately acid lakes due to high aluminium concentration. In *Proceedings of an International Conference on the Ecological Impact of Acid Precipitation* (Drablos, D. and Tollan, A., eds.) pp. 310-311. Sandefjord, Norway.

Grahn, O. (1986). Vegetation structure and primary production in acidified lakes in south-western Sweden. *Experientia* **42**: 465-470.

Grahn, O., Hultberg, H. and Landner, L. (1974). Oligotrophication – a self-accelerating process in lakes subjected to excessive supply of acid substances. *Ambio* **3**: 93-94.

Grande, M., Muniz, I. P. and Andersen, S. (1978). Relative tolerance of some salmonids to acid waters. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **20**: 2076-2084.

Gray, R. H. (1983). Behavioural response of fish to altered water quality: a review of selected examples with emphasis on salmonids. *Environmental Impact Assessment Review* **4**: 84-96.

Gray, R. H. (1990). Fish behaviour and environmental assessment. *Environmental Toxicology and Chemistry* **9**: 53-67.

Greenwood, M. F. D. and Metcalfe, N. B. (1998). Minnows become nocturnal at low temperatures. *Journal of Fish Biology* **53**: 25-32.

Gries, G. and Juanes, F. (1998). Microhabitat use by juvenile Atlantic salmon (*Salmo salar*) sheltering during the day in summer. *Canadian Journal of Zoology* **76**: 1441-1449.

Griffiths, S. W. and Armstrong, J. D. (2002). Rearing conditions influence refuge use among over-wintering Atlantic salmon juveniles. *Journal of Fish Biology* **60**: 363-369.

Gunn, J. M. (1986). Behaviour and ecology of salmonid fishes exposed to episodic pH depression. *Environmental Biology of Fishes* **17**: 241-252.

Gunn, J. M. and Noakes, D. L. G. (1986). Avoidance of low pH and elevated Al concentration by brook charr (*Salvelinus fontinalis*) alevins in laboratory tests. *Water, Air and Soil Pollution* **30**: 497-503.

Gunn, J. M., Noakes, D. L. G. and Westlake, G. F. (1987). Behavioural responses of lake charr (*Salvelinus namaycush*) embryos to simulated acidic runoff conditions. *Canadian Journal of Zoology* **65**: 2786-2792.

Gustafson-Marjanen, K. I and Moring, J. M. (1984). Construction of artificial redds for evaluating survival of Atlantic salmon eggs and alevins. *North American Journal of Fisheries Management* **4**: 455-456.

Hajek, G. J. and Klyszejko, B. (2004). The effects of proposcin (etomidae) on the behaviour, heart rate, and ventilation of common carp *Cyprinus carpio* L. *Acta Ichthyologica et piscatoria* **34(2)**: 129-143.

Hall, R. J., Likens, G. E., Fiance, S. B. and Hendrey, G. R. (1980). Experimental acidification of a stream in the Hubbard Brook Experimental Forest, New Hampshire. *Ecology* **61**: 976-989.

Hall, R. J. and Ide, F. P. (1987). Evidence of acidification effects on stream insect communities in central Ontario between 1937 and 1985. *Canadian Journal of Fisheries and Aquatic Sciences* **44**: 1652-1657

Hann, B. J. and Turner, M. A. (2000). Littoral microcrustacea in Lake 302S in the Experimental Lakes Area of Canada: acidification and recovery. *Freshwater Biology* **43**: 133-146.

Harriman, R. and Morrison, B. R. S. (1982). Ecology of streams draining forested and non-forested catchments in an area of central Scotland subject to acid precipitation. *Hydrobiologia* **88**: 251-263.

Harriman, R. and Wells, D. E. (1985). Causes and effects of surface water acidification in Scotland. *Water Pollution Control* **84**: 215-224.

Harriman, R., Morrison, B. R. S., Caines, L. A., Cullen, P. and Watt, A. W. (1987). Long-term changes in fish populations of acid streams and lochs in Galloway, south west Scotland. *Water, Air and Soil Pollution* **32**: 89-112.

Harvey, H. H. (1982). Population responses of fish in acidified waters. In *Acid Rain/Fisheries* (Johnson, R. E., ed.) pp. 227-242. Bethesda: American Fisheries Society.

Harvey, H. H. and Lee, C. (1980). Fishes of the LaCloche Mountain Lakes of Ontario 1965-1980. *Ontario Ministry of Natural Resources Report.* Toronto: O. M. N. R. pp. 1-89.

Harvey, H. H. and Lee, C. (1982). Historical fisheries changes related to surface water pH changes in Canada. In *Acid/Rain Fisheries, Symposium on Acidic Precipitation and Fishery Impacts in North-Eastern North America* (Johnson, R. E., ed.) pp. 45-55. Bethesda: American Fisheries Society.

Harvey, H. H., Dillon, P. J., Kramer, J. R., Pierce, R. C. and Welpdale, D. M. (1981). Acidification in the Canadian Environment. Scientific criteria for and assessment of the effects of acidic deposition on aquatic ecosystems. *National Research Council of Canada Publications* **18475**: 1-369.

Harwood, A. J., Metcalfe, N. B., Griffiths, S. W. and Armstrong, J. D. (2002). Intra- and inter-specific competition for winter concealment habitat in juvenile salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* **59**: 1515-1523.

Havas, M. and Likens, G. E. (1985a). Changes in 22Na influx in *Daphnia magna* (Straus) as a function of elevated Al concentrations in soft water at low pH. *Proceedings of the National Academy of Sciences of the United States of America* **82**: 7345-7349.

Havas, M. and Likens, G. E. (1985b). Toxicity of aluminium and hydrogen ions to *Daphnia magna*, *Holopedium gibberum*, *Chaoborus punctipennis* and *Chironomus antrocinus* from Mirror Lake, New Hampshire. *Canadian Journal of Zoology* **63**: 1114-1119.

Havens, K.E. and J. DeCosta. (1985). The effects of acidification on the biomass and population size structure of *Bosmina longirostris* in a circum-neutral lake. *Hydrobiologia* **122**: 153-158.

Haya, K., Waiwood, B. A., Burridge, L. E. and Van Eeckhaute, L. (1985). Liver energy metabolism and gill ATPase activity of *Salmo salar* juveniles after exposure to sublethal levels of phenols. In *Marine Pollution and Physiology: Recent Advances* (Vernberg, F. J., Thurberg, F. P., Calabrese, A. and Vernberg, W., eds.) 521-535.

Heard, R. M., Sharpe, W. E., Carline, R. F. and Kimmel, W. G. (1997). Episodic acidification and changes in fish diversity in Pennsylvania headwater streams. *Transactions of the American Fisheries Society* **126**: 977-984.

Heggenes, J. and Borgstrøm, R. (1988). Effect of mink, *Mustela vison* Schreber, predation on cohorts of juvenile Atlantic salmon, *Salmo salar* L., and brown trout, *S. trutta* L., in three small streams. *Journal of Fish Biology* **33**: 885-894.

Heggenes, J. and Traaen, T. (1988). Downstream migration and critical water velocities in stream channels for fry of four salmonid species. *Journal of Fish Biology* **32**: 717–727.

Heggenes, J., Krog, O. M. W., Lindås, O. R., Dokk, J. G. and Bremnes, T. (1993). Homeostatic behavioural responses in a changing environment: brown trout (*Salmo trutta*) become nocturnal during winter. *Journal of Animal Ecology* **62**: 295-308.

Helliwell, R. C., Ferrier, R. C., Johnston, L., Goodwin, J. and Doughty, R. (2001). Land use influences on acidification and recovery of freshwaters in Galloway, south-west Scotland. *Hydrology and Earth System Sciences* **5** (3): 451-458.

Hendershot, W. H., Warfvinge, P., Courchesne, F. and Sverdrup, H. U. (1991). The Mobile Anion Concept – Time for a Reappraisal? *Journal of Environmental Quality* **20**: 505-509.

Hendrey, G. R. (1976). Effects of pH on the growth of periphytic algae in artificial stream channels. *Research Report* **25/76**, SNSF Project, Oslo, Norway.

Hendrey, G. R. and Vertucci, F. (1980). Benthic plant communities in acid Lake Colden, New York: *Sphagnum* and the algal mat. In *Ecological Impact of Acid Precipitation* (Drablos, D. and Tolan, A., eds.) pp. 314-315. SNFC Project, Oslo, Norway.

Henriksen, A. (1989). Air pollution effects on aquatic ecosystems and their restoration. In *Ecological Assessment of Environmental Degradation, Pollution and Recovery* (Ravera, O., ed.) pp. 291-312. Amsterdam: Elsevier.

Henriksen, A., Skogheim, O. K. and Rosseland, B. O. (1984). Episodic changes in pH and aluminium-speciation kill fish in a Norwegian salmon river. *Vatten* **40**: 255-260.

Henriksen, A., Kämäri, J., Posch, M. and Wilander, A. (1992). Critical loads of acidity: Nordic surface waters. *Ambio* **21**: 356-363.

Hessen, D. O. and Lydersen, E. (1996). The zooplankton story of humic lake Skjervatjern during whole catchment acidification. *Environment International* **22**: 643-652.

Hoglund, L. B. (1961). The reactions of fish in concentration gradients: a comparative study based on fluvarium experiments with special reference to oxygen, acidity, carbon dioxide, and sulphite waste liquor (SWL). *Institute of Freshwater Research Drottningholm, Report* **43**: 1-147.

Höjesjö, J., Johnsson, J. I., and Axelsson, M. (1999). Behavioural and heart rate responses to food limitation and predation risk: an experimental study on rainbow trout. *Journal of Fish Biology* **55**: 1009-1019.

Hopkins, P. S., Kratz, K. W. and Cooper, S. D. (1989). Efects of an experimental acid pulse on invertebrates in a high altitude Sierra Nevada stream. *Hydrobiologia* **171**: 45-58.

Hornung, M. (1985). Acidification of soils by trees and forests. *Soil Use and Management* **1** (1): 24-28.

Howell, E. T. (1988). Ecology of periphyton associated with Isoetid plants in low alkalinity lakes. PhD Thesis, University of Toronto, Canada.

Howells, G. (1990). *Acid Rain and Acid Waters*. Chichester: Ellis Horwood. 215 pp.

Hughes, G. M. and Shelton, G. (1962). Respiratory mechanisms and their nervous control in fish. *Advances in Comparitive Physiology and Biochemistry* **1**: 275-364.

Hughes-Clarke, S. A. and Mason, C. J. (1992). Ecological development of field corner tree plantations on arable land. *Landscape Urban Planning* **22**: 59-72.

Hultberg, H. and Stenson, J. (1970). The effects of acidification on the fish fauna in two small lakes in Bohuslän County, Sweden. *Fauna och Flora* **1**: 11-19.

Hunn, J. B., Cleveland, L., and Little, E. E. (1987). Influence of pH and aluminium on developing brook trout in low calcium water. *Environmental Pollution* **43**: 63-73.

Hunter, M. L., Jones, J. J., Gibbs, K. W. and Moring, J. R. (1986). Duckling responses to lake acidification: Do black ducks and fish compete? *Oikos* **47**: 26-32.

Hunter, W. R. (1964). Physiological aspects of ecology of non-marine molluscs. In *Physiology of Molluscsa, Volume 2* (Wilbur, K. M. and Yonge, C. M., eds.) pp. 83-126. New York and London: Academic Press.

Hutchinson, N. J. and Sprague, J. B. (1986). Toxicity of trace metal mixtures to American flagfish (*Jordanella floridae*) in soft, acidic water and implications for cultural acidification. *Canadian Journal of Fisheries and Aquatic Sciences* **43**: 647-655.

Ikuta, K., Shikama, T., Oda, S. and Okumoto, N. (1992). Acid tolerance of eyed embryos and larvae in salmonid fishes. *Bulletin of the National Research Institute of Aquaculture* **21**: 39-45.

Ikuta, K. and Kitamura, S. (1995). Effects of low pH exposure of adult salmonids on gametogenesis and embryo development. *Water, Air and Soil Pollution* **85**: 327-332.

Ikuta, K., Munakata, A., Aida, K., Amano, M. and Kitamura, S. (2001). Effects of low pH on upstream migratory behaviour in land-locked sockeye salmon *Oncorhynchus nerka*. *Water, Air and Soil Pollution* **130**: 99-106.

Ingersoll, C. G., Mount, D. R., Gulley, D. D., LaPoint, T. W. and Bergman, H. L. (1990). Effects of pH, aluminium, and calcium on survival and growth of eggs and fry of brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 1580-1592.

Irwin, J. G. (1985). Acid deposition in the United Kingdom. *Environmental Health* **93** (8): 212-220.

Jackson, S. T. and Charles, D. R. (1988). Aquatic macrophytes in Adirondack (New York) lakes: Patterns of species composition in relation to environment. *Canadian Journal of Botany* **66**: 1449-60.

Jagoe, C. H., Haines, T. A. and Buckler, D. R. (1987). Abnormal gill development in Atlantic salmon (*Salmo salar*) fry exposed to aluminium at low pH. *Annales de la Societe Royale Zoologique de Belgique* **117** (Supplement 1): 375-386.

Jenkins, A., Helliwel, R. C., Swingewood, P. J., Sefton, C., Renshaw, M. and Ferrier, R. C. (1998). Will reduced sulphur emissions under the Second Sulphur Protocol lead to recovery of acid sensitive sites in UK? *Environmental Pollution* **99**: 309-318.

Jensen, K. W. and Snekvik, E. (1972). Low pH levels wipe out salmon and trout populations in southern Norway. *Ambio* **1**: 223-225.

Jensen, A. J. and Johnsen, B. O. (1999). The functional relationship between peak spring floods and survival and growth of juvenile Atlantic Salmon (*Salmo salar*) and Brown Trout (*Salmo trutta*). *Functional Ecology* **13 (6)**: 778–785.

Johnson, D. W. and Webster, D. A. (1977). Avoidance of low pH in selection of spawning sites by brook trout (*Salvelinus fontinalis*). *Journal of the Fisheries Research Board of Canada* **34**: 2215-2218.

Johnson, E. O., Kamilaris, T. C., Chrousos, G. P. and Gold, P. W. (1992). Mechanisms of stress: a dynamic overview of hormonal and behavioural homeostasis. *Neuroscience and Biobehavioural Reviews* **16**: 115-130.

Johnsson, J. I. and Abrahams, M. V. (1991). Interbreeding with domestic strain increases foraging under threat of predation in juvenile steelhead trout (*Oncorhynchus mykiss*): an experimental study. *Canadian Journal of Fisheries and Aquatic Sciences* **48**: 243-247.

Johnsson, J. I., Petersson, E., Jonsson, E., Bjornsson, B. T., and Jarvi, T. (1996). Domestication and growth hormone alter antipredator behaviour and growth patterns in juvenile brown trout, Salmo trutta. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 1546-1554.

Johnsson, J. I., Hojesjo, J. and Fleming, A. (2001). Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **58**: 788-794.

Jones, K. A., Hara, T. S. and Scherer, E. (1985a). Behavioural modifications in Arctic charr (*Salvelinus alpinus*) chronically exposed to sublethal pH. *Physiological Zoology* **58** (4): 400-412.

Jones, K. A., Hara, T. J. and Scherer, E. (1985b). Locomotor response by Arctic charr (*Salvelinus alpinus*) to gradients of H^+ and CO_2. *Physiological Zoology* **58**: 413-420.

Jonsson, B., and Fleming, I. A. (1993). Enhancement of wild salmon populations. In *Human impact on self-recruiting populations, an international symposium.* (Sundnes, G., ed.). Kongsvoll, Norway, Tapit, Trondheim, Norway.

Jordahl, D. M. and Benson, A. (1987). Effect of low pH on survival of brook trout embryos and yolk-sac larvae in West Virginia streams. *Transactions of the American Fisheries Society* **116**: 807-816.

Junger, M. and Planas, D. (1993). Alteration of trophic interactions between periphyton and invertebrates in an acidified stream; a stable carbon isotope study. *Hydrobiologia* **262**: 97-107.

Kinross, J. H., Christofi, N., Read, P. A. and Harriman, R. (1993). Filamentous algae related to pH in streams in The Trossachs, Scotland. *Freshwater Biology* **30**: 301-317.

Kitamura, S. and Ikuta, K. (2000). Acidification severely suppresses spawning of hime salmon (land-locked sockeye salmon, *Oncorhynchus nerka*). *Aquatic Toxicology.* **51**: 107-113.

Kitamura, S. and Ikuta, K. (2001). Effects of acidification on salmonid spawning behaviour. *Water, Air and Soil Pollution* **130**: 875-880.

Kennedy, L. A. (1980). Teratogenesis in lake trout (*Salvelinus namaycush*) in a experimentally acidified lake. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 2355-2358.

Kowalik, R. A. and Ormerod, S. J. (2006). Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology* **51 (1)**: 180-191.

Kowalik, R. A., Cooper, D. M., Evans, C. D. and Ormerod, S. J. (2007). Acidic episodes retard the biological recovery of upland British streams from chronic acidification. *Global Change Biology* **13 (11)**: 2439-2452.

Krause, J., Loader, S. P., McDermott, J. & Ruxton, G. (1998). Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. *Proceedings of the Royal Society of London Series B* **265**: 2373-2379.

Krug, E. C. and Frink, C. R. (1983). Acid rain on acid soil: a new perspective. *Science* **221**: 520-525.

Krug, E. C., Isaacson, P. J. and Frink, C. R. (1985). Appraisal of some current hypotheses describing acidification of watersheds. *Journal of the Air Pollution Control Association* **35** (2): 109-114.

Kwain, W. and Rose, G. A. (1985). Growth of brook trout *Salvelinus fontinalis* subject to sudden reductions of pH during their early life history. *Transactions of the American Fisheries Society* **114**: 564-570.

Lacroix, G. L. (1985). Survival of eggs and alevins of Atlantic salmon (*Salmo salar*) in relation to the chemistry of interstitial water in redds in some acidic streams of Atlantic Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **42**: 292-299.

Larsen, D., Beckman, B. and Dickhoff, W. (1992). Physiological Assessment of Wild and Hatchery Juvenile Salmonids. Bonneville Power Administration Report DOE/BP-00005943-1; Project No. 1992-02200, 130pp.

Laurent, P. and Perry, S. F. (1991). Environmental effects on gill morphology. *Physiological Zoology* **64** (1): 4-25.

Lawrence, G. B. (2002). Persistent episodic acidification of streams linked to acid rain effects on soil. *Atmospheric Environment* **36**: 1589-1598.

LaZerte, B. D. and Dillon, P. J. (1984). Relative importance of anthropogenic versus natural sources of acidity in lakes and streams of Central Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* **41**: 1664-1677.

Lee, D. S. and Longhurst, J. W. S. (1992). Estimates of emissions of SO_2, NO_x, HCl and NH_3 from a densely populated region of the UK. *Environmental Pollution* **79** (1): 37-44.

Lees, F. M. (1995). Have emission reductions led to improvements in water quality at Loch Dee in Galloway. *Water, Air and Soil Pollution* **85**: 695-700.

Lepori, F., Barbieri, A. and Ormerod, S. J. (2003). Causes of episodic acidification in Alpine streams. *Freshwater Biology* **48**: 175-189.

Leivestad, H. and Muniz, I. P. (1976). Fish kill at low pH in a Norwegian River. *Nature, London* **259**: 391-392.

Likens, G. E. and Bormann, F. H. (1974). Acid rain: a serious regional environmental problem. *Science* **184**: 1176-1179.

Logie, J. W. (1995). Effects of stream acidity on non-breeding dippers *Cinclus cinclus* in the south-central highlands of Scotland. *Aquatic Conservation* **5**: 25-35.

Lynch, J. A. and Corbett, E. S. (1989). Hydrological control of sulphate mobility in a forested watershed. *Water Resources Research* **25**: 1695-1703.

Mackenzie, C. and Moring, J. R. (1988). Estimating survival of Atlantic salmon during the intragravel period. *North American Journal of Fisheries Management* **8**: 45-49.

Magee, J. A., Obedzinski, M. McCormick, S. D. and Kocik, J. F. (2003). Effects of episodic acidification on Atlantic salmon (*Salmo salar*) smolts. *Canadian Journal of Fisheries and Aquatic Sciences* **60**: 214-221.

Malcolm I. A., Youngson, A. F. and Soulsby, C. (2003a). Survival of salmonid eggs in a degraded gravel-bed stream: effects of groundwater-surface water interactions. *River Research and Applications* **19**: 303-316.

Malcolm, I. A., Soulsby, C., Youngson, A. F. and Petry, J. (2003b). Heterogeneity in ground water-surface interactions in the hyporheic zone of a salmonid spawning stream. *Hydrological Processes* **17**: 601-617.

Malcolm, I. A., Soulsby, C., Youngson, A. F. and Hannah, D. M. (2004a). Spatial and temporal variability of groundwater-surface water in an upland salmon-spawning stream: implications for survival. *Hydrology: Science and Practice for the 21ST Century, Proceedings for the British Hydrological International Symposium, London, Volume 2; 130-138.*

Malcolm, I, A., Soulsby, C., Youngson, A. F., Hannah, D. M., McLaren, I. S. and Thorne, A. (2004b). Hydrological influences on hyporheic water quality: implications for salmon egg survival. *Hydrological Processes* **18**: 1543-1560.

Malcolm, I. A., Soulsby, C., Youngson, A. F. and Hannah, D. M. (2005). Catchment scale controls on groundwater-surface water interactions in the hyporheic zone: implications for salmon embryo survival. *River Research and Applications* **21**: 1-13.

Malley, D. F. (1980). Decreased survival and calcium uptake by the Crayfish *Orconectes virilis* in low pH. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 364-372.

Marker, A. F. H. and Willoughby, L. G. (1988). Epilithic and epiphytic algae in streams of contrasting pH and hardness. *Algae and the Aquatic Environment* (Round, F. E., ed.). Bristol: Biopress Publishers.

Mason, B. J. (1991). *The Surface Water Acidification Programme*. Cambridge University Press.

Mason, C. F. (2002). *Biology of Freshwater Pollution, 4th Edition*. Pearson Education Limited.

Mason, C. F. and Macdonald, S. M. (1987). Acidification and otter (*Lutra lutra*) distribution on a British river. *Mammalia* **51**: 81-87.

Mason, C. F. and Macdonald, S. M. (1989). Acidification and otter (*Lutra lutra*) distribution in Scotland. *Water, Air and Soil Pollution* **43**: 365-374.

Matthias, U. (1983). Der Einfluss der Versauerung auf die Zusammed setzung von Bergbachbiozönosen. *Archive für Hydrobiologie Supplement* **65**: 405-483.

Matuszek, J. E. amd Beggs, G. L. (1988). Fish species richness in relation to lake area, pH, and other abiotic factors in Ontario lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **45**: 1931-1941.

Maurice, C. G., Lowe, R. L., Burton, T. M. and Stanford, R. M. (1987). Biomass and compositional changes in the periphytic community of an artificial stream in response to lowered pH. *Water, Air and Soil Pollution* **33**: 165-177.

Mazeaud M. M., Mazeaud, F. and Donaldson, E. M. (1977). Primary and secondary effects of stress in fish: some new data with a general review. *Transactions of the American Fisheries Society* **106**: 201-212.

McAvoy, D. C. (1989). Episodic response of aluminium chemistry in an acid-sensitive Massachusetts catchment. *Water Resources Research* **25**: 233-240.

McColl, J. G. and Cole, D. W. (1968). A mechanism of cation transport in a forest soil. *Northwest Science* **42**: 134-140.

McCormick, J. H., Stokes, G. N. and Hermanutz, R. O. (1989). Oocyte atresia and reproductive success in fathead minnows (*Pimephales promelas*) exposed to acidified hardwater environments. *Archives of Environmental Contamination and Toxicology* **18**: 207-214.

McDonald, D. G. (1983). The effects of H+ on the gills of freshwater fish. *Canadian Journal of Zoology* **61**: 691-703.

McMahon, B. R. and Stuart, S. A. (1989). The physiological problems of crayfish in acid waters. In *Acid Toxicity and Aquatic Animals* (Morris, R., Taylor, E. W., Brown, D. J. A. and Brown, J. A., eds.) pp. 171-199. Cambridge: Cambridge University Press.

McWilliams, P. G. and Shephard, K. L. (1991). Water quality during egg incubation influences yolk-sac fry sodium kinetics in Atlantic salmon, *Salmo salar* L.: a possible mechanism of adaptation to acid water. *Journal of Fish Biology* **39**: 469-483.

Menendez, R. (1976). Chronic effects of reduced pH on brook trout. *Journal of the Fisheries Research Board of Canada* **33**: 118-123.

Metcalfe, N. B., Huntingford, F. A. and Thorpe, J. E. (1987a). The influence of predation risk on the feeding motivation and foraging strategy of juvenile Atlantic salmon. *Animal Behaviour* **35**: 901-911.

Metcalfe, N. B., Huntingford, F. A. and Thorpe, J. E. (1987a). Predation risk impairs diet selection in juvenile salmon. *Animal Behaviour* **35**: 931-933.

Metcalfe, N. B., Fraser, N. H. C. and Burns, M. D. (1999). Food availability and the nocturnal vs diurnal foraging trade-off in juvenile salmon. *Journal of Animal Ecology* **68**: 371-381.

Mierle, G., Clark, K., and France, R. (1986). The impact of acidification on aquatic biota in North America: a comparison of field and laboratory results. *Water, Air and Soil Pollution* **31**: 593-604.

Miller, R. B. (1953). Comparative survival of wild and hatchery-reared cutthroat trout in a stream. *Transactions of the American Fisheries Society* **83**: 120-130.

Miller, H. G. (1983). Studies of proton flux in forests and heaths in Scotland. In *Effects of accumulation of air pollutants in forest ecosystems* (Ulrich, B. and Pankrath, J., eds.) pp. 183-183. Dordrecht, Holland: D. Riedel Publishers.

Mills, K. H., Chalanchuk, S. M., Mohr, L. C. and Davies, I. J. (1987). Responses of fish populations in Lake 223 to 8 years of experimental acidification. *Canadian Journal of Fisheries and Aquatic Sciences* **44** (1): 114-125.

Mills, K. H., Chalanchuk, S. M. and Allan, D. J. (2000). Recovery of fish populations in Lake 223 from experimental acidification. *Canadian Journal of Fisheries and Aquatic Sciences* **57**: 192-204.

Milner, N. J. and Varallo, P. V. (1990). Effects of acidification on fish and fisheries in Wales. In *Acid Waters in Wales* (Edwards, R.W., Gee, A.S. and Stoner, J.H., eds). Dordrecht: Kluwer.

Monteith, D. T., Hildrew, A. G., Flower, R. J., Raven, P. J., Beaumont, W. R. C., Collen, P., Kreiser, A. M.Shilland, E. M. and Winterbottom, J. H. (2005). Biological responses to the chemical recovery of acidified fresh waters in the UK. *Environmental Pollution* **137**: 83-101.

Mount, D. R., Ingersoll, C. G., Gulley, D. D., Fernandez, J. D., LaPoint, T. W. and Bergman, H. L. (1988). Effect of long-term exposure to acid, aluminium, and low calcium on adult brook trout (*Salvelinus fontinalis*). 1. Survival, growth, fecundity and progeny survival. *Canadian Journal of Fisheries and Aquatic Sciences* **45**: 1623-1632.

Mulholland, P. J., Elwood, J. W., Palumbo, A. V. and Stephenson, R. J. (1986). Effect of stream acidification on periphyton composition, chlorophyll, and productivity. *Canadian Journal of Fisheries and Aquatic Sciences* **43**: 1846-1858.

Muniz, I. P. (1984). The effects of acidification on Scandinavian freshwater fish fauna. *Philosophical Transactions of the Royal Society of London Series B* **305**: 517-528.

Muniz, I. P. (1991). Freshwater acidification: its effects on species and communities of freshwater microbes, plants and animals. *Proceedings of the Royal Society of Edinburgh* **978**: 227-254.

Muniz, I. P. and Leivestad, H. (1981). Toxic effects of aluminium on the brown trout, *Salmo trutta* L. In *Proceedings of the International Conference on the Ecological Impact of Acid Precipitation* (Drablos, D. and Tollan, A., eds.) pp. 320-321. SNFS Project, Oslo, Norway.

Nilsson, S. I., Miller, H. G. and Miller, J. D. (1982). Forest Growth as a Possible Cause of Soil and Water Acidification: An Examination of the Concepts. *Oikos* **39**: 40-49.

Nakamura, F. (1986). Avoidance behaviour and swimming activity of fish to detect pH changes. *Bulletin of Environmental Contamination and Toxicology* **37**: 808-815.

Nelson, J. A. (1982). Physiological observations on developing rainbow trout, *Salmo gairdneri* (Richardson), exposed to low pH and varied calcium ion concentrations. *Journal of Fish Biology* **20**: 359-372.

Økland, J. (1983). Factors regulating the distribution of freshwater snails (Gastropoda) in Norway. *Malacologia* **24**: 227-288.

Økland, J. (1990). *Lakes and Snails, Environment and Gastropoda in 1500 Norwegian lakes, ponds and rivers* (Backhuys, W. ed.). Oegstgeest, The Netherlands: Universal Book Services.

Økland, J. and Økland, K. A. (1986). Effects of acid deposition on benthic animals in lakes and streams. *Experientia* **42**: 471-486.

Økland, K. A. and Økland, J. (1985). Factor interaction influencing the distribution of the freshwater shrimp Gammarus. *Oecologia* **66**: 364-367.

Olaveson, M. M. and Nalewajko, C. (1994). Effects of acid rain on freshwater algae (Chapter 4). In *Phycological Perspective of Water Pollution* (Rai, L. C. and Gaur, J. P., eds.) pp. 99-123. Schweizerbart'sche Verlagbuchhandlung.

Ormerod, S. J., Allinson, N., Hudson, D. and Tyler, S. J. (1986). The distribution of breeding dippers (*Cinclus cinclus* (L.); Aves) in relation to stream acidity in upland Wales. *Freshwater Biology* **16**: 501-507.

Ormerod, S. J. and Tyler, S. J. (1987). Dippers (*Cinclus cinclus*) and grey wagtails (*Motacilla cinerea*) as indicators of stream acidity in upland Wales. In *The Value of Birds, ICBP Technical Publications No. 6* (Diamon, A. W. and Filion, F. L., eds.) pp. 191-208. Cambridge: International Council for Bird Preservation.

Ormerod, S. J., Donald, A. P. and Brown, S. J. (1989). The influence of plantation forestry on the pH and aluminium concentration of upland Welsh streams: a re-examination. *Environmental Pollution* **62**: 47-62.

Ormerod, S. J. and Wade, K. R. (1990). The role of acidity in the ecology of Welsh lakes and streams. In *Acid Waters in Wales* (Edwards, R. W., Gee, A. S. and Stoner, J. H., eds.) pp. 93-119. Dordrecht: Kluwer.

Ormerod, S. J. and Tyler, S. J. (1993). Birds as indicators if change in water quality. In *Birds as Monitors of Environmental Change* (Furness, R. W. and Greenwood, J. J. D., eds.) pp. 179-216. London: Chapman and Hall.

Orpwood, J. E., Griffiths, S. W. and Armstrong, J. D. (2003). Effects of body size on sympatric shelter use in over-wintering juvenile salmonids. *Journal of Fish Biology* **63** (Supplement 1): 166-173.

Orpwood, J.E., Griffiths, S.W. and Armstrong, J.D. (2004). Effect of density on competition between wild and hatchery-reared salmon for shelter in winter. *Journal of Fish Biology.* **65**: 201-209, Supplement A.

Osgood, M. P. and Boylen, C. W. (1990). Seasonal variations in bacterial communities in Adirondack streams exhibiting pH gradients. *Microbial Ecology* **20**: 211-230.

Palumbo, A. V., Bogle, M. A., Turner, R. R., Elwood, J. W. and Mulholland, P. J. (1987a). Bacterial communities in acidic and circumneutral streams. *Applied Environmental Microbiology* **53**: 337-344.

Palumbo, A. V., Bogle, M. A., Turner, R. R., Elwood, J. W. and Mulholland, P. J. (1987b). Microbial communities on leaf material protected from macroinvertebrate grazing in acidic and circumneutral streams. *Canadian Journal of Fisheries and Aquatic Sciences* **44**: 1064-1070.

Parent, L., Allard, M., Planas, D. and Moreau, G. (1986). The effects of short-term and continuous experimental acidification on biomass and productivity of running water periphytic algae. In *Impact of Acid Rain and Deposition on Aquatic Biological Systems* (Isom, B. G. and Bates, J. M., eds.) pp.28-41. Philadelphia: American Society for Testing and Materials.

Parker, D. B. and McKeown, B. A. (1987). The effects of low pH on egg and alevin survival of kokanee and sockeye salmon, *Oncorhynchus nerka*. *Comparative Biochemistry and Physiology* **87C**: 259-268.

Pauwels, S. J. and Haines, T. A. (1994). Survival, Hatching, and Emergence Success of Atlantic Salmon Eggs Planted in Three Maine Streams. *North American Journal of Fisheries Management* **14 (1)**: 125-130.

Pedder, S. C. J. and Maly, E. J. (1986). The avoidance response of groups of juvenile brook trout, *Salvelinus fontinalis* to varying levels of acidity. *Aquatic Toxicology* **8**: 111-119.

Peterson, R. H., Daye, P. G., Lacroix, G. L. and Garside, E. T. (1982). Reproduction of fish experiencing acid and metal stress. In *Acid Rain/Fisheries* (R. E. Johnson, ed.) pp. 177-196. Bethesda: American Fisheries Society.

Peterson, R. H. and Martin-Robichaud, D. J. (1982). Water uptake by Atlantic salmon ova as affected by low pH. *Transactions of the American Fisheries Society* **111**: 772-774.

Peterson, R. H. and Martin-Robichaud, D. J. (1986). Growth and major cation budgets of Atlantic salmon alevins at three ambient acidities. *Transactions of the American Fisheries Society* **115**: 220-226.

Peterson, R. H., Coombs, K., Power, J. and Paim, U. (1989). Responses of several fish species to pH gradients. *Canadian Journal of Zoology* **67**: 1566-1572.

Peverly, R. H. (1984). Macrophytes and wetland plants. In *The Acidic Deposition Phenomenon and its Effects: Critical Assessment Review Paper Vol. 2* (Altshuller, A. P. and Linthurst, R. A., eds.) pp. 37-43. Washington DC: US Environmental Protection Agency.

Phillips, R. W., Lantz, R. L., Claire, E. W. and Moring, J. R. (1975). Some effects of gravel mixtures on emergence of coho salmon and steelhead trout fry. *Transactions of the American Fisheries Society* **104(3)**: 461-466.

Pickering, A. D. (1981). Introduction: the concept of biological stress. In *Stress and Fish* (A.D. Pickering, ed.) pp. 1-9. London and New York: Academic Press.

Pickering, A. D., Pottinger, T. G. and Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time course study. *Journal of Fish Biology* **20**: 229-244.

Pinder, M. J. and Morgan, R. P. (1995). Interactions of pH and Habitat on Cyprinid Distributions in Appalachian Streams of Maryland. *Transactions of the American Fisheries Society* **124**: 94-102.

Poleo, A. B. S., Ostbyte, K., Oxnevad, S. A., Andersen, R. A., Heibo, E. and Vollestad, L. A. (1997). Toxicity of acid aluminium-rich water to seven freshwater fish species; a comparative laboratory study. *Environmental Pollution* **96**: 129-139.

Potts, W. T. W. and Fryer, G. (1979). The effects of pH and salt content on sodium balance in *Daphnia magna* and *Acantholeberis curvirostris* (Crustacea: Cladocera). *Journal of Comparative Physiology* **129**: 289-294.

Pough, F. H. (1976). Acid precipitation and embryonic mortality of spotted salamanders, *Ambystoma maculatum*. *Science* **192**: 68-70.

Pough, F.H. and Wilson, R. E. (1977). Acid precipitation and reproductive success of *Ambystoma* salamanders. *Water, Air and Soil Pollution* **7**: 307-316.

Puhr, C. B., Donoghue, D. N. M., Stephen, A. B., Tervet, D. J. and Sinclair, C. (2000). Regional patterns of stream water acidity and catchment afforestation in Galloway, SW Scotland. *Water, Air and Soil Pollution* **120**: 47-70.

Raddum, G. G. and Fjellheim, A. (1984). Acidification and early warning organisms in freshwater western Norway. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* **22**: 1973-1980.

Rahel, F.J. and Stein, R. A. (1987). Complex predator-prey interactions and predator intimidation among crayfish, piscivorous fish, and small benthic fish. *Oecologia* **75**: 94-98.

Rask, M. (1983). The effect of low pH on perch, *Perca fluviatilis* L. 1. Effects of low pH on the development of eggs of perch. *Annales Zoologici Fennici* **20**: 73-76.

Rask, M. (1992). Effects of acidification and liming on fish populations in Finland. *Finnish Fisheries Research* **13**: 107-117.

Reader, J. P., Dalziel, T. R. K. and Morris, R. (1988). Growth, mineral uptake and skeletal calcium deposition in brown trout, *Salmo trutta* L., yolk-sac fry exposed to aluminium and manganese in soft acid water. *Journal of Fish Biology* **32**: 607-624.

Reader, J. P., Everall, N. C., Sayer, M. D. L. and Morris, R. (1989). The effects of eight trace metals in acid soft water on survival, mineral uptake and skeletal calcium deposition in yolk-sac fry of brown trout, *Salmo truuta* L. *Journal of Fish Biology* **35**: 187-198.

Rees, R. M. and Ribbens, J. C. H. (1995). Relationships between afforestation, water chemistry and fish stocks in an upland catchment in south-west Scotland. *Water, Air and Soil Pollution* **85**: 303-308.

Reisenbichler, R. R. (1994). Genetic factors contributing to declines of anadromous salmonids in the Pacific Northwest. In *Pacific salmon and their ecosystems* (Stouder, D., Bisson, P and Naiman, R., eds.). Chapman Hall, Inc.

Reisenbichler, R R. (1996). The risks of hatchery supplementation. *The Osprey. Issue 27.*

Renberg, I. and Hedberg, T. (1982). The pH history of lakes in south-western Sweden as calculated from the subfossil diatom flora of the sediments. *Ambio* **11**: 30-33.

Rimmer, D. M., Paim, U. and Saunders, R. L. (1983). Autumnal habitat shift of juvenile Atlantic salmon (*Salmo salar*) in a small river. *Canadian Journal of Fisheries and Aquatic Sciences* **40**: 671-680.

Roberge, J. and Plamondon, A. P. (1987). Snowmelt runoff pathways in a boreal forest hill slope; the role of pipe throughflow. *Journal of Hydrology* **95**: 39-54.

Roberts, D. A. (1984). Macrophytes. In *Biological Field Survey of Northeastern Acidified Lakes, Final Report* (Singer, R. and Boylen, C. W., eds.) pp. 33-38. New York: US Environmental Protection Agency.

Rosemond, A. D., Reice, S. R., Elwood, J. W. and Mulholland, P. J. (1992). The effects of stream acidity on benthic invertebrate communities in the south-eastern United States. *Freshwater Biology* **27**: 193-209.

Rosenqvist, T. (1978). Alternative sources of acidification of river water in Norway. *Science of the Total Environment* **10**: 39-49.

Rosenqvist, T., Jorgensen, P. and Rueslåtten, H. (1980). The importance of natural H+ production for acidity in soil and water. In *Proceedings of the International Conference on the Impact of Acid Precipitation* (Drablos, D. and Tollan, A., eds.). SNFS Project, Oslo, Norway.

Rosseland, B. O. (1986). Ecological effects of acidification on tertiary consumers: fish population responses. *Water, Air and Soil Pollution* **30**: 451-460.

Rosseland, B. O., Sevaldrud, I., Svalastog, D. and Muniz, I. P. (1980). Studies of freshwater fish populations – Effects on reproduction, population structure, growth and food selection. In *Proceedings of the International Conference on the Ecological Impact of Acid Precipitation* (Drablos, D. and Tollan, A., eds.) pp. 348-349. SNFS Project, Oslo, Norway.

Rosseland, B. O. and Skogheim, O. K. (1984). A comparative study on salmonid fish species in acid aluminium-rich water. II. Physiological stress and mortality of one- and two-year-old fish. *Institute of Freshwater Research Drottningholm, Report* **61**: 186-194.

Runn, P. and Sohtell, M. (1982). Sodium uptake and transepithelial potential in brown trout (*Salmo trutta*) embryos and yolk-sac fry at neutral and low pH. *Acta. Univ. Ups. Abstract of Uppsala Dissertations from the Faculty of Science* **64** (4): 1-20.

Runn, P., Johansson, N. and Millbrink, G. (1977). Some effects of low pH on the hatchability of eggs of perch, *Perca fluviatilis* L. *Zoon* **5**: 115-125.

Sadler, K. and Lynam, S. (1989). Results of a second year's experiments concerning survival and development of some Norwegian strains of brown trout when exposed to low pH and elevated aluminium concentrations. *National Power Technology and Environmental Centre Report ESTD/L/0053/R89*. 21 pp.

Sayer, M. D. J., Reader, J. P. and Morris, R. (1991a). Embryonic and larval development of brown trout, *Salmo trutta* L.: exposure to aluminium, copper, lead or zinc in soft, acid water. *Journal of Fish Biology* **38**: 431-455.

Sayer, M. D. J., Reader, J. P. and Morris, R. (1991b). Embryonic and larval development of brown trout, *Salmo trutta* L.: exposure to trace metal elements in soft water. *Journal of Fish Biology* **38**: 773-787.

Sayer, M. D. J., Reader, J. P. and Dalziel, T. R. K. (1993). Freshwater acidification: effects on the early life stages of fish. *Reviews in Fish Biology and Fishes* **3**: 95-132.

Schemenauer, R. S., Schuepp, P., Kermasha, S. and Cereceda, P. (1988). Acid Deposition Processes at High Elevation Sites. In *NATO Advanced Research Workshop* pp. 359-374. Dordrecht, Holland: D. Riedel Publishers.

Schindler, D. W., Mills, K. H., Malley, D. F. and Findlay, D. L. (1985). Long-term ecosystem stress: The effects of years of experimental acidification on a small lake. *Science* **228**: 1395-1401.

Schofield, C. L. and Driscoll, C. T. (1987). Fish species distribution in relation to water quality gradients in the North Branch of the Moose River Basin. *Biogeochemistry* **3**: 63-85.

Seip, H. M., Abrahemsen, G., Gjessing, E. T. and Stuanes, A. (1979). *Studies of Soil, Precipitation and Runoff Chemistry in Six Small Natural Plots (Mini-catchments). Research Report* **25/76**, SNSF Project, Oslo, Norway.

Seyle, H. (1936). A syndrome produced by diverse nocuous agents. *Nature, London* **138**: 32.

Seyle, H. (1946). The general adaptation syndrome and the diseases of adaptation. *Journal of Clinical Endocrinology and Metabolism* **6**: 117-230.

Seyle, H. (1950). Stress and the general adaptation syndrome. *British Medical Journal* **1**: 1383-1392.

Seyle, H. (1956). *The Stress of Life.* New York, Toronto, London: McGraw-Hill Book Co., Inc.

Shephard, K. L. (1987). The influence of water pH on the perivitelline fluid of perch (*Perca fluviatilis*) eggs. *Comparative Biochemistry and Physiology* **86C**: 383-386.

Siegfried, C. A., Bloomfield, J. A., and Sutherland, J. W. (1989a). Acidity status and phytoplankton species richness, standing crop and community composition in Adirondack, New York, USA lakes. *Hydrobiologia* **175**: 13-32.

Siegfried, C. A., Bloomfield, J. A., and Sutherland, J. W. (1989b). Planktonic rotifer community structure in Adirondack, New York, USA lakes in relation to acidity, trophic status and related water quality characteristics. *Hydrobiologia* **175**: 33-48.

Siewert, H. F. and Buck, J. P. (1991). Effects of low pH on survival of Crayfish (*Orconectes virilis*). *Journal of Freshwater Ecology* **6** (1): 87-91.

Simonin, H. A., Kretser, W. A., Bath, D. W., Olson, M. and Gallagher, J. (1993). In situ Bioassays of Brook Trout (*Salvelinus fontinalis*) and Blacknose Dace (*Rhinichthys atratulus*) in Adirondack Streams Affected by Episodic Acidification. *Canadian Journal of Fisheries and Aquatic Sciences* **50**: 902-912.

Simpson, K.W., Bode, R. W. and Colquhoun, J. R. (1985). The Macroinvertebrate Fauna of an Acid-Stressed Headwater Stream in the Adirondack Mountains, New York. *Freshwater Biology* **15**: 671-687.

Skjelkvåle B. L., Evans C., Larssen T., Hindar A., Raddum G. G. (2003). Recovery from Acidification in European Surface Waters: A View to the Future. *AMBIO: A Journal of the Human Environment* **32**: 170–175.

Skogheim, O. K., Rosseland, B. O. and Sevaldrud, I. H. (1984). Deaths of spawners of Atlantic salmon (*Salmo salar* L.) in river Ogna, S. W. Norway, caused by acidified aluminium rich water. *Institute of Freshwater Research Drottningholm, Report* **61**: 195-202.

Sosiak, A. J., Randall, R. G. and McKenzie, J. A. (1979). Feeding by hatchery-reared and wild Atlantic salmon (*Salmo salar*) parr in streams. *Canadian Journal of Fisheries and Aquatic Sciences* **36**: 1408-1412.

Soulsby, C., Malcolm, I. A., Youngson, A. F., Tetzlaff, D., Gibbins, C. N., Hannah, D. M. (2005). Groundwater-surface water interactions in upland Scottish rivers: hydrological, hydrochemical and ecological implications. *Scottish Journal of Geology* **41**: 39-49.

Stallsmith, B. W., Ebersole, J. P. and Hagar, W. G. (1996). The effects of acid episodes on Lepomis sunfish recruitment and growth in two ponds in Massachusetts, U.S.A. *Freshwater Biology* **36**: 731-744.

Steingraeber, M. T. and Gingerich, W. H. (1991). Hatching, growth, ion accumulation, and skeletal ossification of brook trout (*Salvelinus fontinalis*) alevins in acidic soft water. *Canadian Journal of Fisheries and Aquatic Sciences* **69**: 2266-2276.

Stenson, J. A. E. and Eriksson, M. O. G. (1989). Ecological mechanisms important for the biotic changes in acidified lakes in Scandinavia. *Archive for Environmental Contamination and Toxicology* **18**: 201-206.

Stephenson, M. and Mackie, G. L. (1986). Lake acidification as a limiting factor in the distribution of the freshwater amphipod *Hyalella azteca*. *Canadian Journal of Fisheries and Aquatic Sciences* **43**: 288-292.

Stevenson, R. J., Singer, R., Roberts, D. A. and Boylen, C. W. (1985). Patterns of benthic algae abundance with depth, trophic status, and acidity in poorly buffered New Hampshire lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **42**: 1501-1512.

Stoddard, J. L., Kahl, J. S., Deviney, F. A., DeWalle, D. R., Driscoll, C. T., Herlihy, A. T., Kellogg, J. H., Murdoch, J. R., Webb, J. R. and Webster, K. E. (2003). *Response of Surface Water Chemistry to the Clean Air Act Amendments of 1990.* Washington DC: US Environmental Protection Agency.

Stokes, P. M. (1986). Ecological effects of acidification on primary producers in aquatic systems. *Water, Air and Soil Pollution* **30**: 412-38.

Sullivan, T. J., Christophersen, N., Hooper, R. P., Seip, H. M., Muniz, I. P., Sullivan, P. D. and Vogt, R. D. (1987). Episodic variation in streamwater chemistry at Birkenes, southernmost Norway: evidence for the importance of water flow paths. In *Proceedings UNESCO-IHP Symposium on Acidification and Water Pathways* pp. 269-279. Bolkesjø, Norway.

Sullivan, T. J., Eilers, J. M., Cosby, B. J. and Vache, K. B. (2004). Increasing role of Nitrogen in the acidification of surface waters in the Adirondack mountains, New York. *Water, Air and Soil Pollution* **95**: 313-336.

Sutcliffe, D. W. and Carrick, T. R. (1973). Studies on mountain streams in the English Lake District. *Freshwater Biology* **3**: 437-462.

Symons, P. E. K. (1969). Greater dispersal of wild compared with hatchery-reared juvenile Atlantic salmon released in streams. *Journal of the Fisheries Research Board of Canada* **26**: 1867-1876.

Tam, W. H. and Payson, P. D. (1986). Effects of chronic exposure to sublethal pH on growth, egg production and ovulation in brook trout, *Salvelinus fontinalis*. *Canadian Journal of Fisheries and Aquatic Sciences* **43**: 275-280.

Tessier, A. J. and Horowitz, R. J. (1988). Analysis and interpretation of zooplankton samples collected during Phase II of the National Lake Survey. *Academy of Natural Sciences of Philadelphia Report* **88-18**: 1-313.

Thomsen, A., Korsgaard, B. and Joensen, J. (1988). Effect of aluminium and calcium ions on survival and physiology of rainbow trout *Salmo gairdneri* (Richardson) eggs and larvae exposed to acid stress. *Aquatic Toxicology* **12**: 291-300.

Traaen, T. S. (1980). The effects of acidity on decomposition of organic matter in aquatic environments. In *Proceedings of the International Conference on the Ecological Impacts of Acid Precipitation* (Drablos, D. and Tollan, A., eds.) pp. 340-341. SNFS Project, Oslo, Norway.

Tranter, M., Davies, T. D., Wigington, P. J. Jr., and Eshleman, K. N. (1994). Episodic acidification of freshwater in Canada. *Water, Air and Soil Pollution* **72**: 19-39/

Turner, M. A., Jackson, M. B., Findlay, D. L., Graham, R. W., DeBruyn, E. R. and Vandermeer, E. M. (1987). Early responses of periphyton to experimental lake acidification. *Canadian Journal of Fisheries and Aquatic Sciences* **44** (supp. 1): 135-149.

Turnpenny, A. W. H. (1989). Field studies on fisheries in acid waters in the United Kingdom. In *Acid Toxicity and Aquatic Animals* (Morris, R., Taylor, E. W., Brown, D. J. A. and Brown, J. A., eds.) pp. 45-65. Cambridge: Cambridge University Press.

Twitchen, J. B. (1990). The physiological bases of resistance to low pH among aquatic insect larvae. In *The Surface Waters Acidification Programme* (Mason, B. J., ed.) pp. 413-419. Cambridge: Cambridge University Press.

Tyler-Jones, R., Beattie, R. C. and Aston, R. J. (1989). The effects of acid water and aluminium on the embryonic development of the common frog, *Rana temporaria*. *Journal of Zoology* **219**: 355-372.

Ulrich, B. (1983). Soil acidity and its relations to acid deposition. In *Effects of Accumulation of Air Pollutants in Forest Ecosystems* (Ulrich, B. and Pankrath, J., eds.) pp. 127-146. Dordrecht, Holland: D. Riedel Publishers.

Urban-Jezierska, E. (2002). Effect of acidification in feeding in guppy fish (*Lebistes reticulatus* vel *Poecilia reticulata*). *Polish Journal of Ecology* **50** (1): 25-43.

Valdimarsson, S. K. and Metcalfe, N. B. (1998). Shelter selection in juvenile Atlantic salmon, or why do salmon seek shelter in winter? *Journal of Fish Biology* **52**: 42-49.

Valdimarsson, S. K., Metcalfe, N. B., Thorpe, J. E. and Huntingford, F. A. (1997). Seasonal changes in sheltering: effect of light and temperature on diel activity in juvenile salmon. *Animal Behaviour* **54**: 1405-1412.

Valdimarsson, S. K., Metcalfe, N. B. and Skulason, S. (2000). Experimental demonstration of differences in sheltering behaviour between Icelandic populations of Atlantic salmon (*Salmo salar*) and Arctic char (*Salvelinus alpinus*). *Canadian Journal of Fisheries and Aquatic Sciences* **57**: 719-724.

Valtonen, T. and Laitinen, M. (1988). Acid stress in respect to calcium and magnesium concentrations in the plasma of perch during maturation and spawning. *Environmental Biology of Fishes* **22**: 147-154.

Van Coilie, R., Thellen, C., Campbell, P. G. C. and Vegneault, Y. (1983). Effets toxique de l'aluminium chez les salmonidés en relation avec des conditions physico-chimiques acides. *Canadian Technical Report, Fisheries and Aquatic Sciences* **1237**, National Research Council of Canada, Otawa.

Vesely, J. (1994). Effects of acidification on trace metal transport in fresh waters. In *Acidification of freshwater ecosystems: implications for the future* (Steinberg, C. E. W. and Wright, R. F., eds.) pp. 141-151. Chichester: Wiley Publishers.

Vickery, J. A. (1991). Breeding density of dippers *Cinclus cinclus*, grey wagtails *Motacilla cinerea* and common sandpipers *Actitis hypoleucos* in relation to the acidity of streams in south-west Scotland. *Ibis* **133**: 18-23.

Voigt, G. K. (1980). Acid precipitation and soil buffering capacity. In *Proceedings of the International Conference on the Ecological Impact of Acid Precipitation* (Drablos, D. and Tollan, A., eds.) pp. 53-57. SNFS Project, Oslo, Norway.

Vuorinen, P. J. and Vuorinene, M. (1992). Acidification in Finland: a review of studies of fish physiology and toxicology. *Finnish Fisheries Research* **13**: 119-132.

Vuorinen, P. J., Vuorinen, M. and Peuranen, S. (1990). Long-term exposure of adult whitefish (*Coregonous watmanni*) to low pH/aluminium: effects on reproduction, growth, blood composition and gills. In *Acidification in Finland* (Kauppi, P., Kenttãmies, K. and Anttila, P., eds.) pp. 941-961. Berlin: Springer-Verlag.

Vuorinen, P. J., Vuorinen, M., Peuranen, S., Rask, M., Lappalainen, A. and Raitaniemi, J. (1992). Reproductive status, blood chemistry, gill histology and growth of perch (*Perca fluviatilis*) in three acidic lakes. *Environmental Pollution* **78**: 19-27.

Walker, I. R., Smol, J. P., Engstrom, D. R. and Birks, H. J. B. (1991). An assessment of Chironomidae as quantitative indicators of past climatic change. *Canadian Journal of Fisheries and Aquatic Sciences* **48**: 975-987.

Waring, C. P., Stagg, R. M. and Poxton, M. G. (1992). The effects of handling on flounder (*Platichtys flesus* L.) and Atlantic salmon (*Salmo salar* L.). *Journal of Fish Biology* **41**: 131-144.

Watt, W. D., Scott, C. D. and White, W. J. (1983). Evidence of acidification of some Nova Scotian rivers and its impact on Atlantic salmon, *Salmo salar*. *Canadian Journal of Fisheries and Aquatic Sciences* **40**: 462-473.

Weatherly, N. S. (1988). Liming to mitigate acification in freshwater ecosystems: A review of the biological onsequences. *Water, Air and Soil Pollution* **39**: 421-437.

Weatherly, N. S. and Ormerod, S. J. (1987). The impact of acidification on macroinvertebrate assemblages in Welsh streams: towards an empirical model. *Environmental Pollution* **46**: 223-240.

Wedemeyer, G. A. and McLeay, D. J. (1981). Methods for determining the tolerance of fishes to environmental stressors. In *Stress and Fish* (A. D. Pickering, ed.) pp. 247-275. London and New York: Academic Press.

Wetzel, R. G. (1964a). A comparative study of the primary productivity of higher plants, periphyton and phytoplankton in a large shallow lake. *Internationale Revue der Gesamten Hydrobiologie* **49**: 1-61.

Wetzel, R. G. (1964b). Primary productivity of aquatic macrophytes. *VerhandlungenInternationale Vereinigung für Theoretische und Angewandte Limnologie* **15**: 426-436.

Whalen, K. G., Parrish, D. L. and Mather, M. E. (1999). Effect of ice formation on selection of habitats and winter distribution of post-young-of-the-year Atlantic salmon parr. *Canadian Journal of Fisheries and Aquatic Sciences* **56**: 87-96.

Wigington, P. J. Jr., Davies, T. D., Tranter, M. and Eshleman, K. N. (1990). Episodic acidification of Surface Waters due to acidic deposition, NAPAP Report 12. In *Acidic Deposition: State of Science and Technology.* National Acid Precipitation Assessment Program, Washington DC, 200 pp.

Wigington, P. J. Jr., Davies, T. D., Tranter, M. and Eshleman, K. N. (1992). Comparison of episodic acidification in Canada, Europe and the United States. *Environmental Pollution* **78**: 29-35.

Winterbourn, M. J., Hildrew, A. G., Orton, S. (1992). Nutrients, algae and grazers in some British streams of contrasting pH. *Freshwater Biology* **28**: 173-182.

Wood, C. M. and McDonald, D. G. (1982). Physiological mechanisms of acid toxicity to fish. In *Acid/Rain Fisheries, Symposium on Acidic Precipitation and Fishery Impacts in North-Eastern North America* (Johnson, R. E., ed.) pp. 197-227. Bethesda: American Fisheries Society.

Wood, C. M., McDonald, D. G., Ingersoll, C. G., Mount, D. R., Johannsson, O. E., Landsberger, S. and Bergman, H. L. (1990a). Effects of water acidity, calcium, and aluminium on whole body ions of brook trout (*Salvelinus fontinalis*) continuously exposed from fertilisation to swim-up: a study by instrumental neutron activation analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 1593-1603.

Wood, C. M., McDonald, D. G., Ingersoll, C. G., Mount, D. R., Johannsson, O. E., Landsberger, S. and Bergman, H. L. (1990b). Whole body ions of brook trout (*Salvelinus fontinalis*) alevins: responses of yolk-sac and swim-up stages to water acidity, calcium, and aluminium, and recovery effects. *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 1604-1615.

Wren, C. D. and Stephenson, G. L. (1991). The effect of acidification and toxicity of metals to freshwater invertebrates. *Environmental Pollution* **71**: 205-241.

Wright, R. F., Cosby, B. J., Ferrier, R. C., Jenkins, A., Bulger, A. J. and Harriman, R. (1994). Changes in acidification of lochs in Galloway, southwestern Scotland, 1979-1988: The MAGIC model used to evaluate the role of afforestation, calculate critical loads, and predict fish status. *Journal of Hydrology* **161**: 257-285.

Yan, N. D. and Strus, R. (1980). Crustacean zooplankton communities of acidic, metal-contaminated lakes near Sudbury, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 2282-2293.

Yan, N. D., Miller, G. E., Wile, I. and Hitchen, G. G. (1985). Richness of aquatic macrophyte floras of soft water lakes of differing pH and metal content in Ontario, Canada. *Aquatic Botany* **23**: 27-40.

Youngson, A. F., Malcolm, I. A., Thorley, J. L., Bacon, P. J. and Soulsby, C. (2004). Long-residence groundwater effects on incubating salmonid eggs: low hyporehic oxygen impairs embryo development. *Canadian Journal of Fisheries and Aquatic Sciences* **61**: 2278-2287.

Appendices

APPENDIX 1. CHAPTER 2 – PRELIMINARY FIELD-WORK (SUMMER 2005)

Study site

The initial study was carried out in the upper reaches of the River Wye catchment, near Llangurig, Wales, over the period June – July 2005. Four stream types were surveyed within a 4 km radius, to include the following types: chronically acidified (n = 2), treated by catchment liming (n = 3), circumneutral (n = 3) and control (main river; n = 1) streams. The study was used to determine if differences in chemistry existed in streams that were likely to differ in short-term pH fluctuations with increased discharge (i.e. run-off) due to the surrounding geology and land use, and between the surface and hyporheic water within the same stream type.

Sampling procedure

Prior to sampling, three hyporheic extraction tubes (a 25 mm air-stone connected to a 500 mm length of polyethylene tubing with internal diameter 6 mm) were installed at each sampling site, and left undisturbed for 2 days before sampling commenced. Two samples were collected (surface and hyporheic water) from three locations at each site according to the position of the hyporheic tubes (termed upstream, middle and downstream, spaced approximately 2m apart). Following collection, three variables were measured in the field for each sample; pH, temperature (± 0.1°C) and conductivity (± 0.1 µS/cm) using a Hanna HI 9024 pH/temperature meter in combination with a Hanna HI 1053B low ionic strength pH electrode, and a Hanna HI 98308 conductivity meter. Samples were collected on average every 3 – 4 days over the course of a month. Daily discharge information (metres second^{-1}; recorded every 15 minutes from a sampling point on the main river) was later obtained from CEH.

Statistical Analysis

Variations in pH, temperature and conductivity between stream classes and depth (hyporheos versus surface water) were assessed using a GLM, with average daily discharge as a covariate. Any significant interactions between all combinations of these factors were also noted using multiple Tukey-Kramer *post-hoc* pairwise comparisons. A significance level of $P < 0.05$ was used throughout the analyses.

Results

pH differed significantly between stream types ($P < 0.001$), between the surface and hyporheic samples ($P < 0.001$), and with discharge ($P < 0.05$) (Figure A1.1). Although the results are in some respects inconclusive, some general comments can be made: pH was lowest in the chronically acidified streams, and highest in the circumneutral streams, hyporheic samples were less acidic than surface water samples, and pH decreased with increasing discharge, though there was considerable variation within the data. Temperature differed significantly between stream types ($P < 0.001$), between the surface and hyporheic samples ($P < 0.05$) and with discharge (Figure A1.2). In general, the main river site was warmest, and the chronically acidified streams were coolest; the limed streams and the circumneutral streams were broadly the same. Hyporheic samples were warmer than the surface water samples, and as with pH, temperature decreased with increasing discharge. Conductivity differed significantly between stream types ($P < 0.01$), between the surface and hyporheic samples ($P < 0.001$) and with discharge ($P < 0.001$) (Figure A1.3). The lowest conductivity measurement was recorded in the main river site, and the highest in the circumneutral streams, and higher values were recorded in the groundwater samples. Conductivity decreased as discharge increased, and as surface water became more diluted.

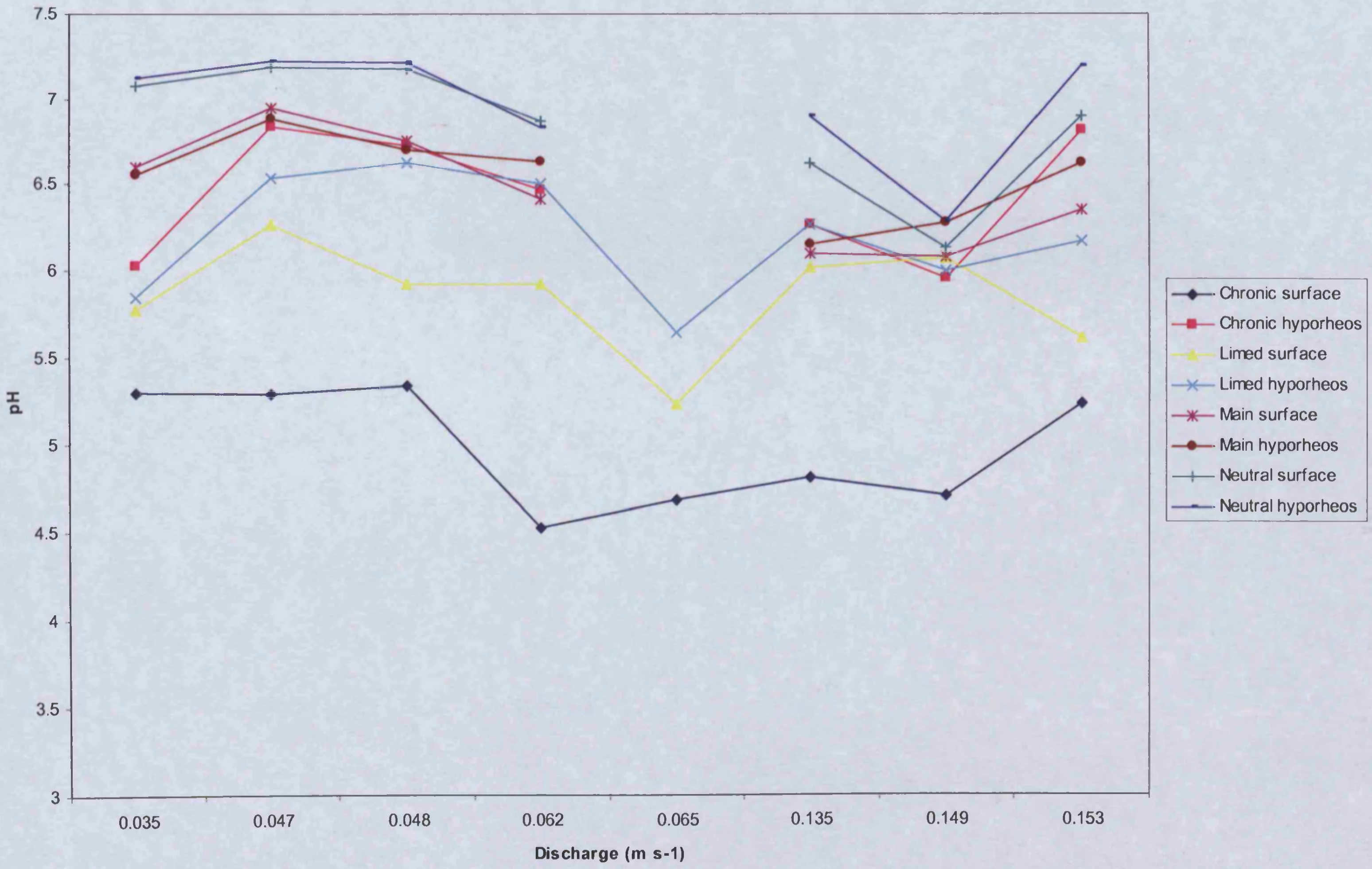


Figure A1.1: Mean pH in surface water and the hyporheic zone across four stream types with increasing discharge

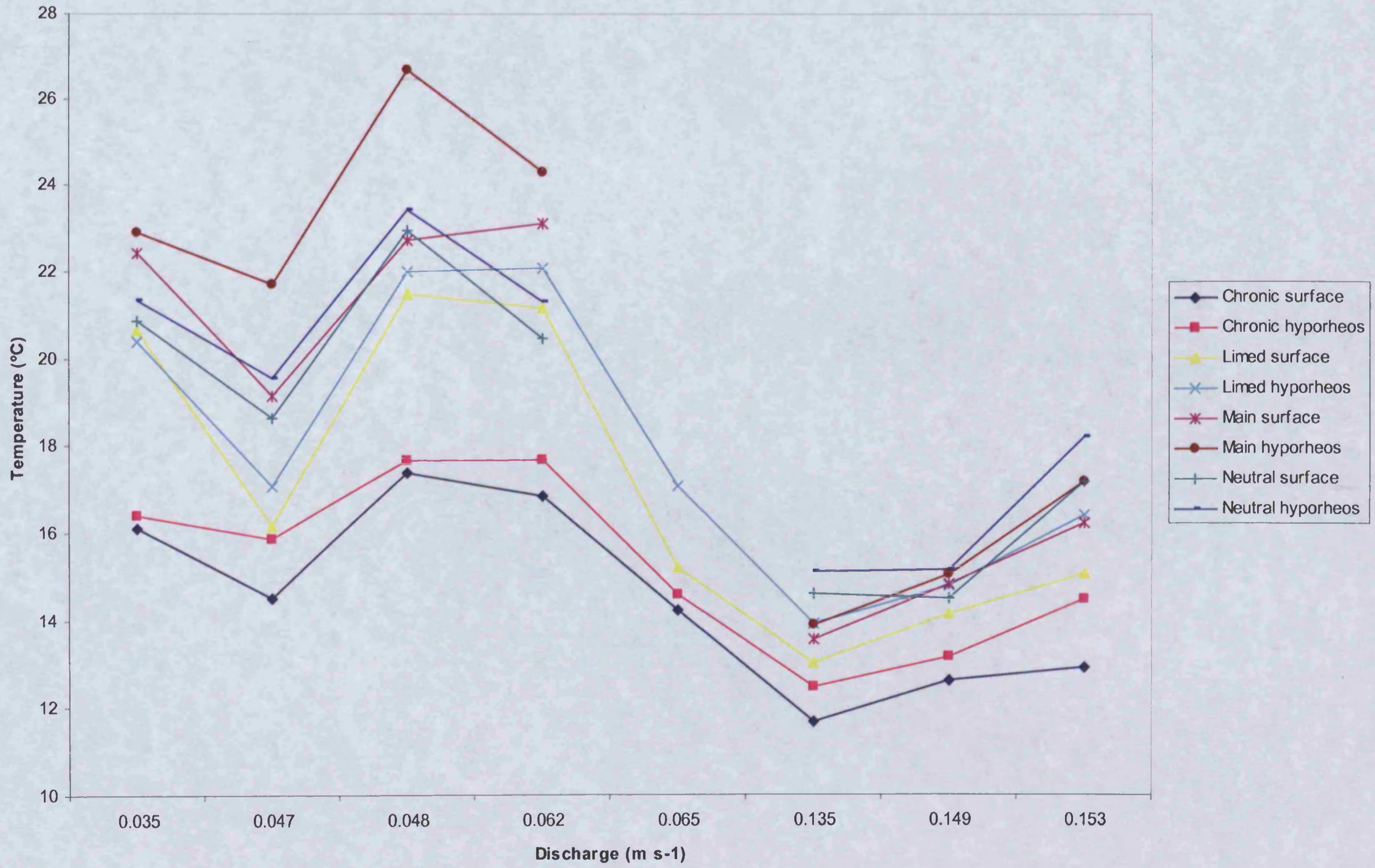


Figure A1.2: Mean temperature (ºC) in surface water and the hyporheic zone across four stream types with increasing discharge

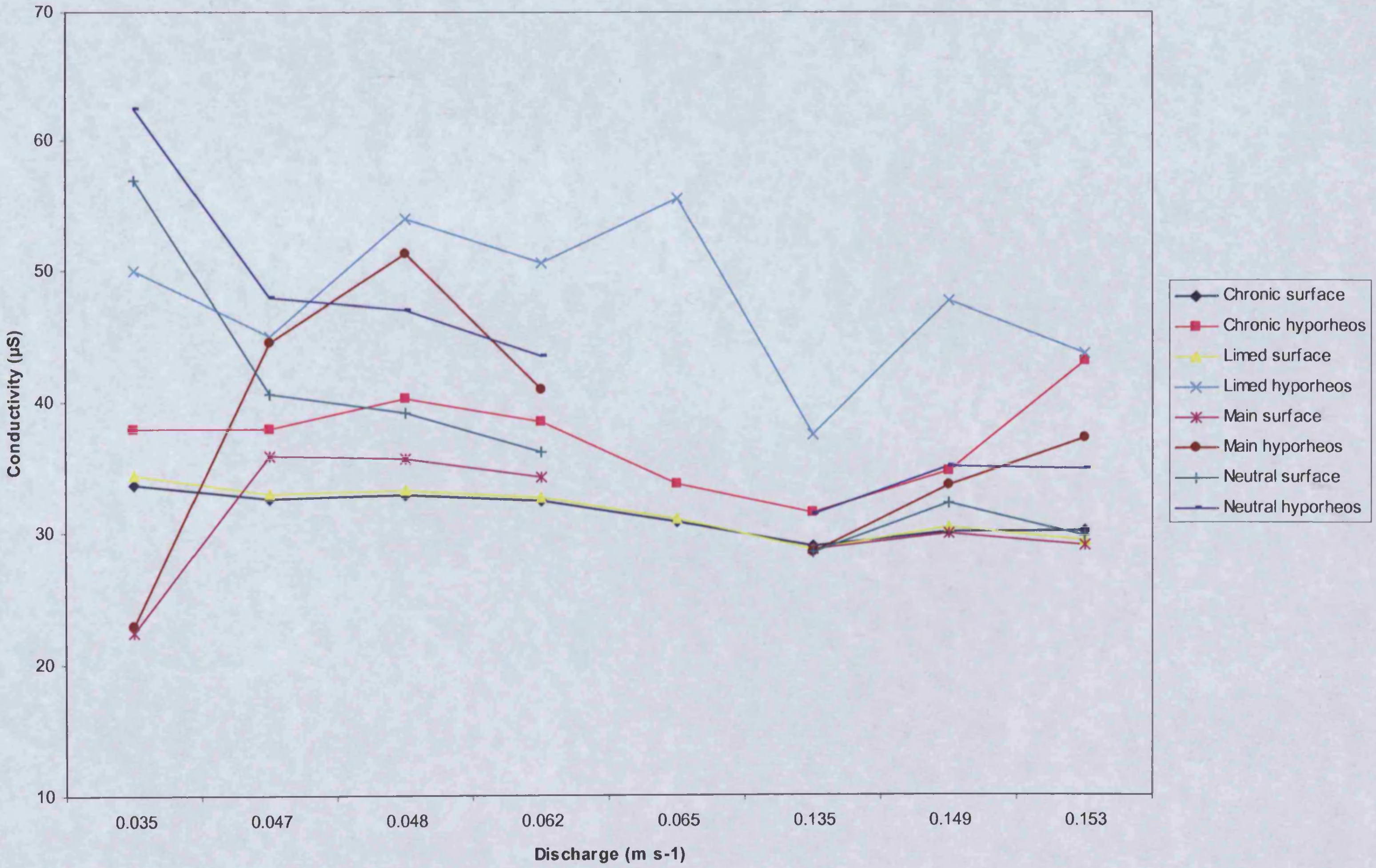


Figure A1.3: Mean conductivity (µS) in surface water and the hyporheic zone across four stream types with increasing discharge

The results of this preliminary experiment indicated that conditions differed between the surface water and the stream hyporheos. The three variables studied – pH, temperature and conductivity all differed significantly between surface water and groundwater, with consistent trends between variables. Also, these differences are found in each of the four stream types, which are (unrelated) tributaries, suggesting that the differences must be part of a wider pattern, and that streams within a large area are similarly affected. In general, all variables decreased with increasing discharge, though the relationship was more obvious for some parameters than others.

The preliminary study also highlighted some aspects of the methods used that could be refined to improve the accuracy and ecological relevance of the results. For example, sampling was carried out at regular intervals over a 4-week period but without knowledge of discharge levels at the time of sampling; discharge data were obtained retrospectively from CEH. Additionally, the 2005 sampling took place in mid-summer when it was unlikely sites would be impacted by increased discharge due to low rainfall (hence the low variability between the surface and hyporheic samples). It was planned therefore that future field sampling would focus more on differences between low and high (episodic) flow and thus be conducted in August (expected low flow) and then again in December when there was a greater likelihood of high flow.

A further problem encountered was that the hyporheic sampling tubes became clogged with silt in one of the chronically acidified streams, resulting in incomplete data sets. This stream was replaced in the 2006 study with a stream of similar chemistry but a more suitable stream bed substrate. The preliminary study in 2005 was therefore valuable in identifying which sites would be suitable for repeated sampling and the methodological issues that needed to be resolved to ensure as far as was possible complete data sets.

However, and notwithstanding these issues, the observed differences in pH between surface water and the hyporheic zone suggested that these habitats are largely independent, and possibly supplied by two different water sources, this idea is also supported by the differences in temperature and conductivity. Further field sampling was therefore planned to confirm the differences observed in the 2005 study, with particular reference to low and high (episodic) flows, rather than the relatively minor variation in discharge observed in the preliminary study. A second study was carried out in 2006, with methods modified as in Chapter 2.

APPENDIX 2. CHAPTER 2 – STATISTICS

General Linear Model: PH versus Flow_1, Type_1, Depth_1

```
Factor        Type          Levels        Values
Flow          fixed         2             High, Low
Type          fixed         4             Chronic, Limed, Main, Neutral
Depth         fixed         2             Ground, Surface

Analysis of Variance for PH, using Adjusted SS for Tests

Source                  DF     Seq SS     Adj SS     Adj MS       F      P
Flow_1                   1     6.6263     5.1269     5.1269   23.65  0.000
Type_1                   3    11.1784    10.9260     3.6420   16.80  0.000
Depth_1                  1     0.2224     0.4725     0.4725    2.18  0.144
Flow_1*Type_1            3     3.3896     3.9851     1.3284    6.13  0.001
Flow_1*Depth_1           1     3.3533     3.8152     3.8152   17.60  0.000
Type_1*Depth_1           3     1.1688     1.3149     0.4383    2.02  0.118
Flow_1*Type_1*Depth_1    3     0.7136     0.7136     0.2379    1.10  0.356
Error                   74    16.0405    16.0405     0.2168
Total                   89    42.6930
```

General Linear Model: Temperature versus Flow_1, Type_1, Depth_1

```
Factor        Type          Levels        Values
Flow          fixed         2             High, Low
Type          fixed         4             Chronic, Limed, Main, Neutral
Depth         fixed         2             Ground, Surface

Analysis of Variance for Temperature, using Adjusted SS for Tests

Source                  DF     Seq SS     Adj SS     Adj MS        F      P
Flow_1                   1    768.379    685.746    685.746  1717.67  0.000
Type_1                   3     23.483     36.321     12.107    30.33  0.000
Depth_1                  1     15.098     20.865     20.865    52.26  0.000
Flow_1*Type_1            3      8.557     11.227      3.742     9.37  0.000
Flow_1*Depth_1           1      6.673      9.636      9.636    24.14  0.000
Type_1*Depth_1           3     13.308     15.339      5.113    12.81  0.000
Flow_1*Type_1*Depth_1    3      9.543      9.543      3.181     7.97  0.000
Error                   74     29.543     29.543      0.399
Total                   89    874.584
```

General Linear Model: Conductivity versus Flow, Stream Type, Depth

```
Factor        Type          Levels        Values
Flow          fixed         2             High, Low
Type          fixed         4             Chronic, Limed, Main, Neutral
Depth         fixed         2             Ground, Surface

Analysis of Variance for Conductivity, using Adjusted SS for Tests

Source                  DF     Seq SS     Adj SS     Adj MS        F      P
Flow_1                   1    5612.30    5024.58    5024.58    84.18  0.000
Type_1                   3     208.33     271.75      90.58     1.52  0.217
Depth_1                  1       9.86       5.04       5.04     0.08  0.772
Flow_1*Type_1            3     653.52     676.32     225.44     3.78  0.014
Flow_1*Depth_1           1      94.46     123.89     123.89     2.08  0.154
Type_1*Depth_1           3     334.06     341.69     113.90     1.91  0.136
Flow_1*Type_1*Depth_1    3      62.84      62.84      20.95     0.35  0.789
Error                   74    4417.02    4417.02      59.69
Total                   89   11392.40
```

APPENDIX 3. CHAPTER 3 – STATISTICS

General Linear Model: Number versus Shelf Group, Treatment, Period

```
Factor        Type         Levels         Values
Shelf Group   fixed        3              Top2, Middle6, Bottom2
Treatment     fixed        2              Control, Acid
Period        fixed        6              Pre-, during 1 - 4, Post-

Analysis of Variance for Number, using Adjusted SS for Tests

Source                   DF     Seq SS       Adj SS       Adj MS        F        P
Shelf Group              2      3020.55      3020.55      1510.28       399.37   0.000
Treatment                1      0.01         0.01         0.01          0.00     0.959
Period_1                 5      0.02         0.02         0.00          0.00     1.000
Shelf Gr*Treatmen        2      5801.42      5801.42      2900.71       767.05   0.000
Shelf Gr*Period_1        10     530.02       530.02       53.00         14.02    0.000
Treatmen*Period_1        5      0.05         0.05         0.01          0.00     1.000
Shelf*TM*Period_1        10     189.28       189.28       18.93         5.01     0.000
Error                    2484   9393.64      9393.64      3.78
Total                    2519   18935.00
```

General Linear Model: Opercular Rate versus Sentinel Type, Treatment, Period

```
Factor        Type         Levels         Values
Shelf Group   fixed        3              Top2, Middle6, Bottom2
Treatment     fixed        2              Control, Acid
Period        fixed        6              Pre-, during 1 - 4, Post-

Analysis of Variance for OR, using Adjusted SS for Tests

Source               DF     Seq SS      Adj SS      Adj MS      F         P
Sentinel             1      77018       77018       77018       396.14    0.000
TM                   1      475205      475205      475205      2444.20   0.000
Period               5      13154       13154       2631        13.53     0.000
Sentinel*TM          1      192750      192750      192750      991.40    0.000
Sentinel*Period      5      36058       36058       7212        37.09     0.000
TM*Period            5      77755       77755       15551       79.99     0.000
Sentinel*TM*Period   5      36750       36750       7350        37.80     0.000
Error                1656   321962      321962      194
Total                1679   1230653
```

One-Sample T

```
Test of mu = 0 vs mu > 0

Variable          N       Mean      StDev    SE Mean
Acid              7      2.571      2.440      0.922

Variable      95.0% Lower Bound         T        P
Acid                      0.780      2.79    0.016

Descriptive Statistics: Acid

Variable             N         Mean       Median       TrMean       StDev     SE Mean
Acid                 7        2.571        2.000        2.571       2.440       0.922
Variable       Minimum      Maximum           Q1           Q3
Acid             0.000        6.000        0.000        5.000

Descriptive Statistics: Control

Variable             N         Mean       Median       TrMean       StDev     SE Mean
Control              7      0.00000      0.00000      0.00000     0.00000     0.00000
Variable       Minimum      Maximum           Q1           Q3
Control        0.00000      0.00000      0.00000      0.00000
```

APPENDIX 4. CHAPTER 4 – PRELIMINARY EXPERIMENT (WINTER 2004)

Experimental animals

The preliminary experiment was carried out in December 2004, using 0+ Atlantic salmon collected from the Environment Agency hatchery at Cynrig, near Abergavenny in September of 2004. At the time of the experiment fish were approximately 7 – 8 months old and had previously been kept in a large age-specific aerated tank with a constant water supply of Afon Cynrig river water. Prior to the start of the experiment in Cardiff, fish were kept in a large tank, supplied with constant-flow de-chlorinated mains water, aerated at either end with air stones. Fish were fed every day, after dusk, to encourage nocturnal foraging. Once tested, fish were kept in a separate tank to avoid re-use. Following completion of the study, fish were humanely euthanased.

Test arena

The experiment was conducted within a transparent Perspex tank (approximate dimensions 120 cm L x 30 cm W x 60 cm D), in the constant-temperature aquarium to be used in the experiment proper. The test tank was supplied with flowing mains water (pH 7) into a low inlet at the upstream end of the tank, and drained freely at the opposite (downstream) end. The tank was divided into 20 zones of equal size (24 cm L x 15 cm W x 20 cm D [the tank was not filled to the top, hence the 20 cm D discrepancy]) with markers on the tank exterior, though these were for observational purposes only, and water flowed freely between all areas of the tank. Division of the tank created zones as follows: 5 from the upstream end to the downstream end (coded 1 to 5, left to right), from the front of the tank to the rear (coded front [F] and back [B]) and 2 vertical layers (coded upper [U] and lower [L]). A shelter (consisting of a 10 cm long hollow pipe sealed at one end with an internal diameter of 6 cm) was positioned in the centre of the tank over L3F/B, with the open end facing downstream towards L5F/B.

Experimental protocol

Experimental animals were transferred to the test tank at approximately 12:00 every evening, to allow acclimation over the next 5 hours. Observations were then made in near-darkness (using a red lamp to see the fish) between 17:00 and 19:00, by one of two observers. Once used, fish were transferred to a separate tank to avoid contamination with naïve fish. Following completion of the preliminary study, fish were humanely euthanased.

Each test comprised of three time periods: pre-treatment (0 – 30 minutes); treatment (30 – 90 minutes); post-treatment (90 – 120 minutes). Water conditions in these time periods in each treatment group are described in the table below (Table A2.1).

Table A2.1: Water chemistry in main test tank and shelter in before/during/after in each of four treatments (N = neutral water, A = acidified water).

	Pre-treatment		Treatment		Post-treatment	
	Tank	Shelter	Tank	Shelter	Tank	Shelter
1	N	N	N	N	N	N
2	N	N	N	A	N	N
3	N	N	A	N	N	N
4	N	N	A	A	N	N

During the pre-treatment and post-treatment periods, the test tank was supplied with flowing mains water which also passed through the shelter via a small open valve at the upstream end. In the treatment period, a header tank of acidified (pH 4) mains water (acidified to approximately pH 4 by mixing 12.5 ml 5M H_2SO_4 in 96 l water) was connected to either the shelter (treatment 2; the rest of the tank continued to be supplied with neutral mains water) or the main test tank (treatments 3 and 4), and allowed to flow through the shelter (treatment 4); in treatment 3, a small stopper was used at the upstream end of the shelter to prevent the acidified water from flowing through the shelter.

Fish position was recorded at the start of the experiment, and every subsequent movement into another section, or the shelter, noted against the current time. Opercular rate was recorded every 10 minutes throughout the experiment, by counting the number of gill openings over a 15 second period and multiplying by 4 to obtain a 1 minute count. Time spent in the shelter, if any, was also recorded and expressed as a proportion of the corresponding period. During the treatment period, pH was also recorded using a Hanna HI991000 pH/temperature meter every 10 minutes in three areas of the tank (inlet, shelter and outlet) to alternate with opercular rate observations.

Statistical analysis

GLMs were carried out to test the effects of treatment group and time period (i.e. pre-treatment, treatment and post-treatment) on activity minute^{-1} and opercular rate (using transformations to normalise data where necessary). Fish were not observed using the shelter in any treatment group, this variable was therefore excluded from the analysis.

Preliminary findings

The preliminary experiment showed that exposure to acidified water in the main body of the test tank significantly increased activity minute^{-1} (Figure A2.1), though there was no significant effect of time period, nor were any interaction effects observed.

Treatment also had a significant effect on opercular rate (Figure A2.2), which increased following exposure to acidified water, but as with activity there was no significant effect of time period, nor any significant interaction between factors.

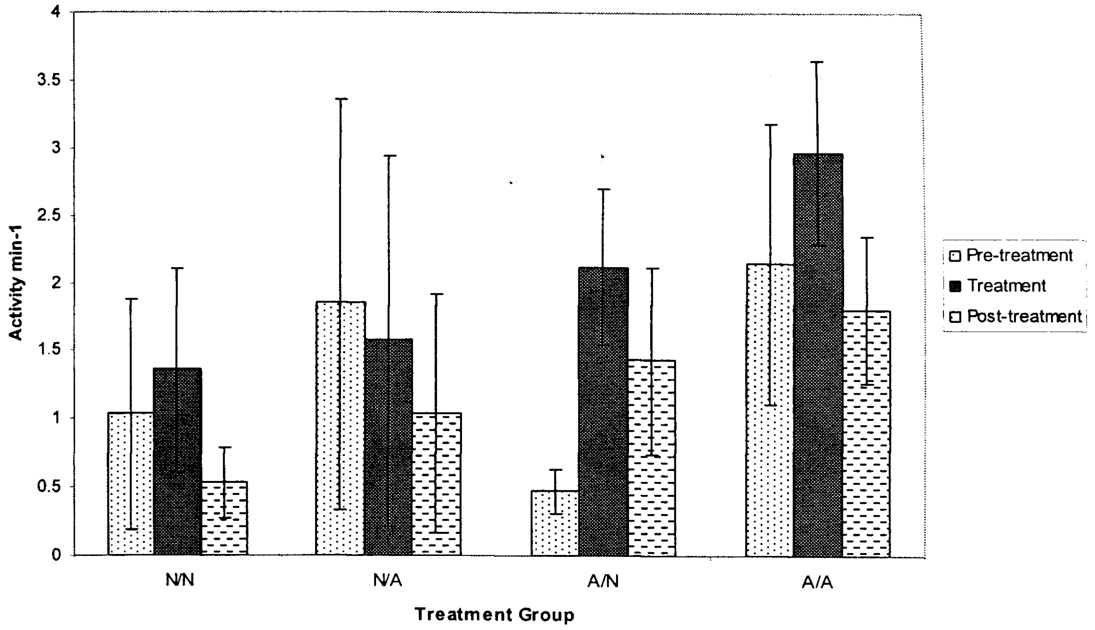


Figure A2.1: Mean activity min^{-1} ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups: Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter (GLM: $P_{treatment} = 0.048$, $P_{period} = 0.233$, $P_{interaction} = 0.927$).

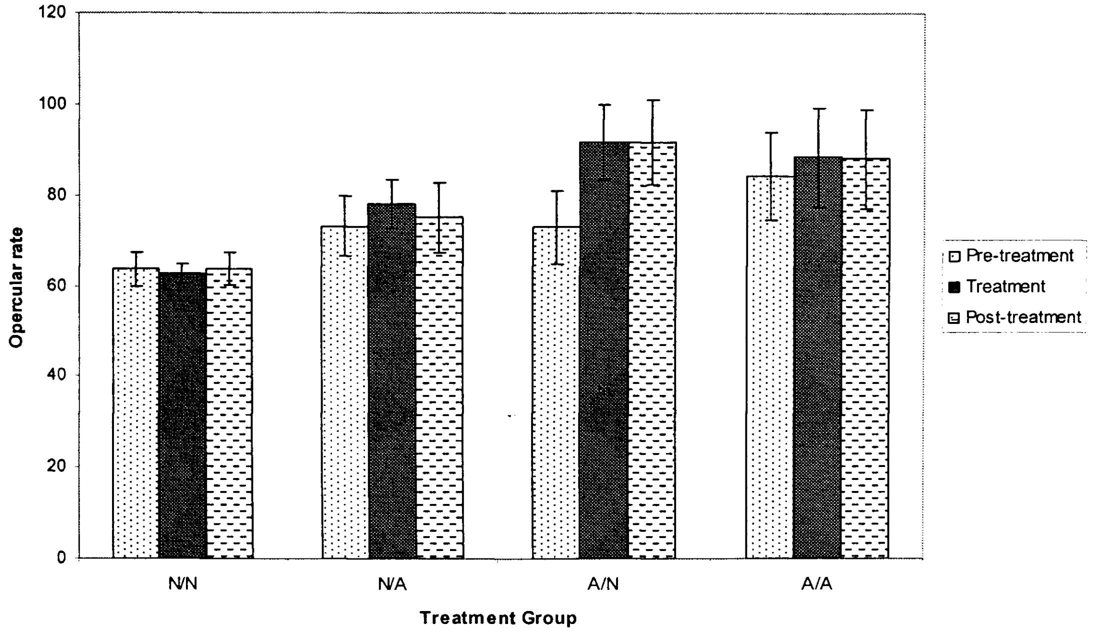


Figure A2.2: Mean opercular rate ± SE of fish during three test periods (pre-treatment, treatment and post-treatment) across four treatment groups: Neutral tank/Neutral shelter, Neutral tank/Acid shelter, Acid tank/Neutral shelter and Acid tank/Acid shelter (GLM: $P_{treatment} = 0.000$, $P_{period} = 0.384$, $P_{interaction} = 0.865$).

Results of the preliminary experiment suggested that Atlantic salmon do experience behavioural changes following exposure to acid exposure (such as the observed increase in activity), though no shelter use was observed. However, several shortcomings of the experimental set-up were realised which may account for the inconclusive results, for example:

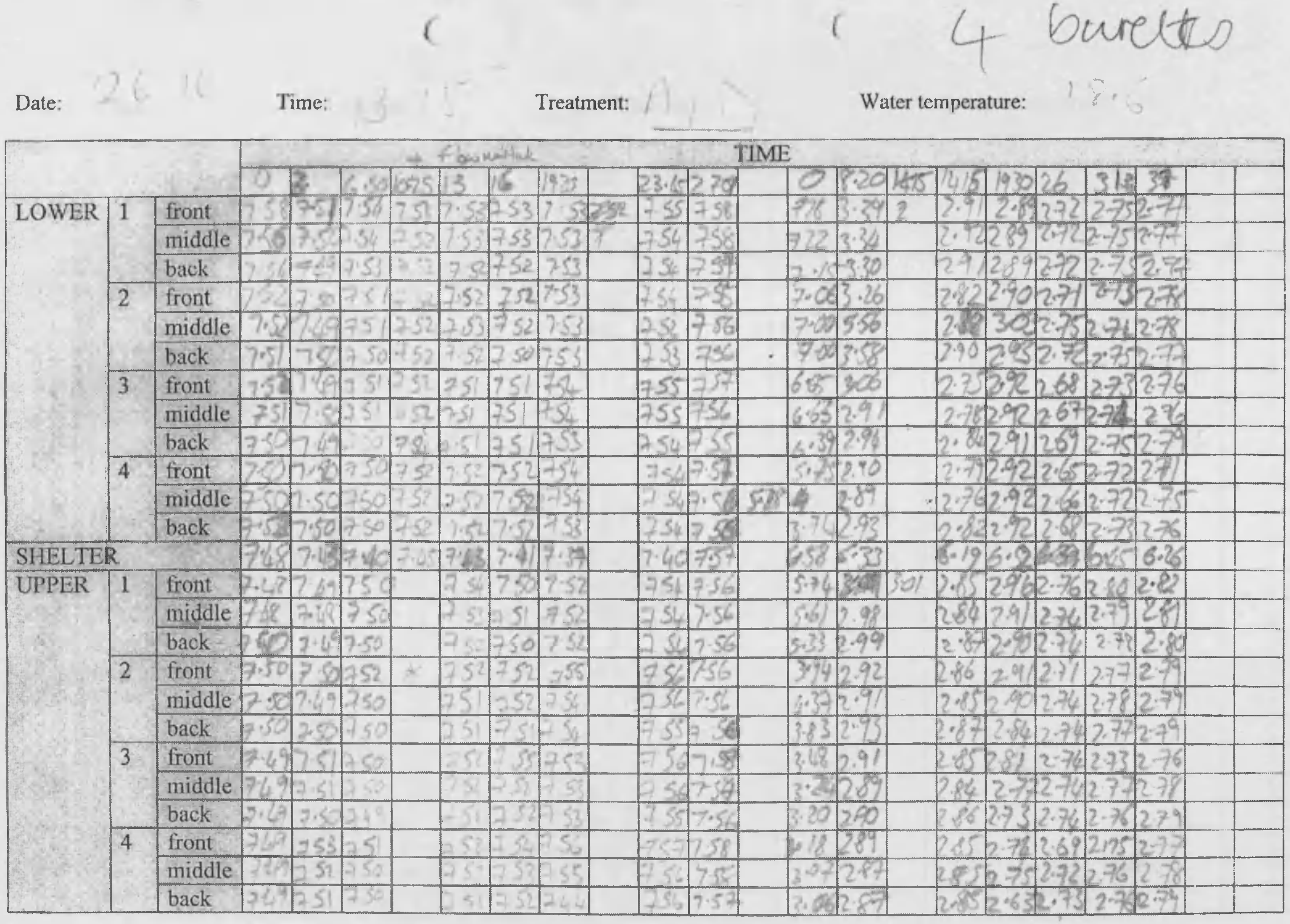
- fish were transferred to the test tank and the experiment was carried out only 5 hours later; in the 5 hours prior to the experiment the tank was still illuminated as part of the daytime light regime – the experiment was carried out immediately after 'dark', so it is perhaps likely that the sudden change in light regime increased stress levels of the fish even before acid was applied.
- no pilot trials were carried out in order to determine the sequence of acid additions necessary to maintain pH levels in the tank, pH was therefore monitored throughout the experiment in 3 locations within the tank using a pH probe, and the flow of acidified water from the header tank adjusted accordingly – this was disadvantageous for two reasons: (i) the movement of the pH probe within the tank would also have caused the fish additional stress; (ii) there may have been many more subtle variations in pH within the tank than those measured.
- the acidified water (pH 4) was pre-prepared in a header tank and added to the main body of water in the test tank (pH 7) where it was diluted rapidly, thus pH levels in the tank were too high to be considered an acid episode, or cause the fish any real stress.
- the tank was overly-large for the experiment – the shelter occupied a very small proportion of the tank (0.02 % of the total volume [cm^3 water]) so even with the observed increase in activity it was perhaps optimistic to assume that a single fish would encounter the shelter during the course of the experiment, particularly given the lack of any real change in water chemistry due to rapid dilution of the acid episode.

- division of the tank into only 20 zones meant that each zone was quite large (several times bigger than the size of a single fish, for example) and substantial movement could take place within a zone but would not be taken into account under the very either/or method of recording activity.
- manually recording movement was time consuming and fish often moved so quickly such that each some movements were impossible to record – however, development of a unique programme for the future experiment would render manual recordings obsolete and allow for a greater accuracy in the results.

The preliminary findings were thus essential in developing the experimental protocol to be used in a similar experiment in December of the following year; methods were refined as described in Chapter 4.

APPENDIX 5. CHAPTER 4 – PILOT WORK

Acid/Neutral: 26/10/05: 4 burettes



4 burettes

Date: 26 10 Time: 13 15 Treatment: [illegible] Water temperature: 18.6

→ flow halted

			TIME																		
			0	3	6.50	10.75	13	16	19.25		23.45	27.0		0	8.20	~~14.15~~	14.15	19.30	26	31	38
LOWER	1	front	7.58	7.51	7.56	7.51	7.53	7.53	7.53	7.52	7.55	7.58		7.76	3.39	2	2.91	2.89	2.72	2.75	2.74
		middle	7.56	7.50	7.54	7.52	7.53	7.53	7.53	7	7.54	7.58		7.22	3.34		2.92	2.89	2.72	2.75	2.74
		back	7.56	7.49	7.53	7.53	7.52	7.52	7.53		7.56	7.59		7.15	3.30		2.91	2.89	2.72	2.75	2.74
	2	front	7.52	7.50	7.51	7.52	7.52	7.52	7.53		7.56	7.58		7.06	3.26		2.82	2.90	2.71	2.73	2.78
		middle	7.52	7.49	7.51	7.52	7.53	7.52	7.53		7.52	7.56		7.00	5.56		2.88	3.02	2.75	2.76	2.78
		back	7.51	7.50	7.50	7.52	7.52	7.50	7.53		7.53	7.56		7.00	3.58		2.90	2.95	2.72	2.75	2.77
	3	front	7.52	7.49	7.51	7.52	7.51	7.51	7.54		7.55	7.57		6.85	3.06		2.75	2.92	2.68	2.73	2.76
		middle	7.51	7.50	7.51	7.52	7.51	7.51	7.54		7.55	7.56		6.65	2.91		2.78	2.92	2.67	2.74	2.76
		back	7.50	7.49	7.50	7.52	7.51	7.51	7.53		7.54	7.55		6.39	2.94		2.84	2.91	2.69	2.75	2.79
	4	front	7.50	7.50	7.50	7.52	7.52	7.52	7.54		7.54	7.57		5.75	2.90		2.79	2.92	2.65	2.72	2.71
		middle	7.50	7.50	7.50	7.52	7.52	7.52	7.54		7.54	7.58	5.88	[illegible]	2.89		2.76	2.92	2.66	2.72	2.75
		back	7.52	7.50	7.50	7.52	7.52	7.52	7.53		7.54	7.58		3.74	2.93		2.82	2.92	2.68	2.73	2.76
SHELTER			7.48	7.43	7.40	7.05	7.43	7.41	7.39		7.40	7.57		6.58	6.33		6.19	6.2	[illegible]	6.05	6.26
UPPER	1	front	7.47	7.49	7.50		7.54	7.50	7.52		7.54	7.56		5.74	[illegible]	5.01	2.85	2.96	2.76	2.80	2.82
		middle	7.48	7.49	7.50		7.53	7.51	7.52		7.54	7.56		5.61	2.98		2.84	2.91	2.76	2.79	2.81
		back	7.50	7.49	7.50		7.50	7.50	7.52		7.54	7.56		5.33	2.99		2.87	2.90	2.76	2.78	2.80
	2	front	7.50	7.50	7.52	*	7.52	7.52	7.55		7.56	7.56		3.94	2.92		2.86	2.91	2.71	2.77	2.79
		middle	7.50	7.49	7.50		7.51	7.52	7.54		7.56	7.56		4.37	2.91		2.85	2.90	2.74	2.78	2.79
		back	7.50	7.50	7.50		7.51	7.51	7.54		7.55	7.56		3.83	2.93		2.87	2.84	2.74	2.77	2.79
	3	front	7.49	7.51	7.50		7.52	7.55	7.53		7.56	7.58		3.48	2.91		2.85	2.81	2.76	2.73	2.76
		middle	7.49	7.51	7.50		7.52	7.53	7.53		7.56	7.59		3.24	2.89		2.84	2.77	2.74	2.77	2.78
		back	7.49	7.50	7.49		7.51	7.52	7.53		7.55	7.56		3.20	2.90		2.86	2.73	2.76	2.76	2.79
	4	front	7.49	7.53	7.51		7.52	7.54	7.56		7.57	7.58		3.18	2.89		2.85	2.76	2.69	2.75	2.77
		middle	7.49	7.51	7.50		7.51	7.53	7.55		7.56	7.55		3.07	2.87		2.85	2.75	2.72	2.76	2.78
		back	7.49	7.51	7.50		7.51	7.52	7.44		7.56	7.57		3.06	2.87		2.85	2.63	2.73	2.76	2.74

Date: Time: Treatment: Water temperature:

			TIME																				
			0	515	1030	15	1945	2310	29														
LOWER	1	front	2·92	296	3·08	3·21	3·35	3·60	459														
		middle	2·89	296	3·08	3·20	3·35	3·61	4·56														
		back	2·87	2·96	3·08	3·22	3·35	3·61	460														
	2	front	2·87	296	3·09	3·21	3·37	3·68	4·91														
		middle	2·84	3·02	3·09	3·21	3·36	4·02	4·67														
		back	2·86	2·96	3·09	3·23	3·39	3·69	4·85														
	3	front	2·85	2·92	3·10	3·23	3·41	3·76	4·96														
		middle	2·85	2·98	3·11	3·24	3·63	3·73	4·86														
		back	2·86	2·98	3·11	3·24	3·60	3·76	5·19														
	4	front	2·86	2·99	3·11	3·24	3·45	3·76	5·45														
		middle	2·86	2·99	3·11	3·25	3·45	3·80	5·15														
		back	2·86	3·00	3·11	3·26	3·44	3·80	5·12														
SHELTER			6·57	6·62	6·56	6·55	6·64	6·61	6·15														
UPPER	1	front	2·95	3·06	3·17	3·32	3·51	3·83	5·69														
		middle	2·93	3·04	3·15	3·31	3·51	3·85	5·63														
		back	2·94	3·04	3·16	3·31	3·57	4·08	5·86														
	2	front	2·95	3·04	3·19	3·30	3·52	3·90	5·54														
		middle	2·94	3·07	3·17	3·32	3·56	3·96	5·52														
		back	2·93	3·06	3·18	3·32	3·53	4·11	5·56														
	3	front	2·95	3·08	3·19	3·32	3·57	4·03	5·57														
		middle	2·93	3·07	3·18	3·32	3·56	4·02	5·57														
		back	2·93	3·05	3·18	3·32	3·55	4·13	5·63														
	4	front	2·94	3·07	3·22	3·24	3·60	4·12	5·62														
		middle	2·96	3·07	3·21	3·37	3·83	4·56	6·07														
		back	2·94	3·07	3·17	3·36	3·39	4·29	5·82														

Acid/Neutral: 27/10/05: 4 burettes

4 burettes

Date: 27/10 Time: 12 am tops adjusted Treatment: A/N Water temperature: 19.6°C

			TIME																				
			0	320	615		0	615	800	1750	2508	35		0	5	9	1215	16	20	23	25		
LOWER	1	front	736	736	760		715	703	596	605	3.33	5.15		669	696	707	715	724	730	733	735		
		middle	736	735	738		7.11	703	696	610	3.18	5.21		6.85	695	709	715	724	730	733	735		
		back	736	735	737		710	703	695	6.10	3.03	5.38		6.70	699	707	716	724	730	733	735		
	2	front	735	736	737		706	704	[illegible]	666	3.11	5.38		670	696	707	716	724	730	733	735		
		middle	734	736	735		715	711	653	671	3.09	6.06		6.99	703	708	716	727	730	733	735		
		back	734	734	733		711	711	671	612	2.98	~~945~~	545	689	704	709	717	726	730	733	735		
	3	front	736	735	736		706	706	620	[illegible]	3.04	5.93		682	702	709	717	726	730	733	735		
		middle	736	735	736		695	701	588	614	2.98	5.92		6.81	700	708	717	725	730	733	735		
		back	736	737	737		697	700	501	601	2.63	5.85		6.79	700	708	716	725	730	733	736		
	4	front	736	736	738		692	700	563	604	[illegible]	605		6.78	699	708	716	724	730	733	734		
		middle	736	736	736		697	697	498	603	2.75	6.14		[illegible]	699	708	717	726	730	733	734		
		back	736	736	733		697	698	496	602	2.71	6.10		7.01	700	708	717	725	730	733	734		
SHELTER			732	731	731		713	715	646	679	6.47	6.55		695	7.18	720	725	728	730	7.31	733		
UPPER	1	front	734	734	737		708	710	597	627	312	648		690	710	715	721	727	730	733	735		
		middle	734	734	737		706	708	595	624	3.3[illegible]	648		690	708	714	720	727	731	733	735		
		back	734	734	736		7.05	707	600	623	3.20	652		687	706	713	720	727	730	733	736		
	2	front	734	734	739		700	704	593	626	317	648		687	703	714	721	727	733	733	737		
		middle	734	735	738		699	702	580	622	316	649		687	703	712	721	727	732	733	737		
		back	734	735	739		699	703	575	619	322	610		686	702	712	720	[illegible]	732	733	737		
	3	front	734	738	739		700	703	568	622	330	651		688	703	713	721	727	732	735	738		
		middle	734	737	738		699	703	573	626	327	653		682	703	712	721	727	732	736	737		
		back	734	732	738		699	702	556	596	329	653		689	707	712	721	727	732	736	738		
	4	front	734	738	740		701	702	583	616	340	656		689	704	713	721	727	732	734	736		
		middle	734	737	739		699	700	583	574	362	653		690	704	714	720	728	733	734	738		
		back	734	737	738		699	700	575	588	342	650			703	715	721	728	733	734	738		

~ 10

4?2?

shelter tank empty

Date: 2/11/05 Time: 1126 Treatment: A/N Water temperature: 19.6°C

			TIME													
			0	2 15	4 15	6 20		0	3·55	9 40	15 25	21 40	25 11	30 18	35 31	
LOWER	1	front	723	731	740	736		7·26	3·89	3·67	603	555	443	589	626	
		middle	722	731	738	735		7·21	3·63	3·66	599	539	496	593	627	
		back	722	736	737	737		7·15	3·63	3·62	595	526	499	593	627	
	2	front	726	731	736	736		7·06	3·65	3·73	595	505	522	591	625	
		middle	725	730	735	736		7·08	3·24	6·17	604	528	629	595	635	
		back	725	731	735	736		6·76	6·96	4·05	648	420	535	596	634	
	3	front	725	731	735	736		6·74	3·77	3·92	557	416	543	607	630	
		middle	726	732	735	737		6·64	3·03	3·96	592	401	545	608	647	
		back	726	731	734	736		6·72	3·07	3·61	592	347	508	604	641	
	4	front	725	731	735	736		5·73	2·90	3·68	595	465	508	581	629	
		middle	726	732	734	7·36		554	3·09	3·77	596	489	315	594	633	
		back	726	732	735	736		4·33	3·12	3·77	592	346	434	595	627	
SHELTER			728	731	733	735		6·78	6·44	697	689	662	677	670	656	
UPPER	1	front	728	732	735	734		5·45	3·39	5·80	628	398	590	638	644	
		middle	728	732	734	736		5·95	3·41	5·79	620	381	591	632	643	
		back	728	732	736	737		5·99	3·46	5·77	615	402	588	631	642	
	2	front	728	732	735	737		5·53	3·41	578	612	406	593	630	638	
		middle	727	732	736	738		5·08	3·32	570	590	413	594	622	635	
		back	728	732	735	737		5·03	3·31	580	576	389	596	623	634	
	3	front	728	732	735	736		409	3·24	580	589	404	597	625	632	
		middle	729	732	736	738		3·85	3·28	582	559	492	606	623	636	
		back	729	732	736	739		3·91	3·28	581	521	471	589	598	598	
	4	front	728	732	735	738		3·20	3·26	5·64	560	426	591	615	626	
		middle	729	732	735	738		3·54	3·25	555	538	427	595	434	632	
		back	730	732	735	737		3·39	3·27	584	382	412	596	550	632	

Acid/ Neutral: 2/11/05: 2 burettes

Date: Time: Treatment: Water temperature:

			TIME													
			0	3	515	740	1040	1300	1505	1730	1920	2135	2460	2624		
LOWER	1	front	653	671	690	704	713	720	725	728	733	735	736	740		
		middle	650	672	690	704	713	720	725	728	733	731	736	741		
		back	648	674	691	704	716	721	725	728	732	731	736	741		
	2	front	647	674	692	704	715	720	725	728	734	736	736	741		
		middle	647	675	693	705	716	721	726	728	733	735	737	739		
		back	647	678	694	707	717	721	725	728	732	735	737	740		
	3	front	649	678	693	707	716	722	725	728	732	736	734	735		
		middle	649	676	696	708	717	722	727	728	734	735	738	740		
		back	650	677	693	706	716	721	729	728	734	735	737	739		
	4	front	648	678	693	706	714	722	727	728	733	736	738	739		
		middle	648	692	694	706	717	723	727	728	733	736	738	740		
		back	650	677	694	706	717	722	726	728	733	736	738	739		
SHELTER			700	7.02	711	719	722	725	726	730	733	734	736	737		
UPPER	1	front	672	692	705	713	718	725	727	729	733	734	734	736		
		middle	669	690	702	712	718	724	729	729	733	735	736	738		
		back	669	687	701	712	719	725	728	729	733	735	738	738		
	2	front	669	687	701	712	718	723	728	729.	733	736	737	739		
		middle	669	686	700	711	719	724	728	731	732	736	737	739		
		back	669	686	700	710	719	725	728	730	734	735	737	739		
	3	front	669	686	700	712	718	724	727	730	733	737	737	739		
		middle	668	686	701	712	719	725	728	731	734	737	739	740		
		back	669	687	700	712	719	724	728	731	733	736	737	739		
	4	front	670	6.87	700	712	719	725	728	731	733	736	738	739		
		middle	671	6.87	700	712	719	725	728	732	735	736	739	739		
		back	671	6.87	700	713	719	725	728	731	736	736	738	740		

2 burettes

Date: 2/11 Time: 1.40pm Treatment: A/N Water temperature: 18.3°C – [illegible]

			TIME													
			0	235	428	710		0	415	825	1145	1620	1921	2240	2652	3002
LOWER	1	front	7.36	739	739	743		708	649	578	640	499	591	622	614	599
		middle	739	739	739	742		703	647	603	640	521	594	624	613	600
		back	739	738	738	741		700	621	608	638	521	595	624	613	600
	2	front	7.38	740	740	740		693	634	615	637	518	598	624	612	601
		middle	7.37	739	739	741		695	660	617	637	520	606	625	611	599
		back	7.38	739	738	740		654	459	622	640	559	616	630	615	599
	3	front	738	740	739	740		647	510	613	478	550	606	634	619	602
		middle	738	740	740	742		583	565	614	390	542	605	627	617	603
		back	738	739	739	741		604	401	583	333	510	606	625	616	600
	4	front	737	740	7.40	741		606	495	576	349	487	604	627	482	564
		middle	739	740	7.40	742		473.	514	596	300	506	602	596	538	571
		back	739	739	7.40	743		508	505	589	410	512	604	592	564	5.49
SHELTER			735	736	7.35	737		595	684	6.87	6.63	663	675	681	677	671
UPPER	1	front	737	739	739	740		655	6.20	637	492	586	644	624	610	612
		middle	737	739	740	741		656	616	634	400	585	628	622	609	602
		back	737	739	740	742		652	614	633	430	585	626	623	610	601
	2	front	738	739	741	743		650	611	635	400	585	623	623	610	601
		middle	740	739	741	742		650	613	635	401	587	620	619	610	599
		back	739	738	741	741		641	612	632	372	584	622	619	608	600
	3	front	739	739	741	742		646	615	628	407	579	622	598	518	598
		middle	740	740	742	743		629	614	631	433	581	621	583	537	598
		back	739	739	741	742		632	615	625	397	582	620	587	562	596
	4	front	739	739	742	742		624	615	623	421	582	619	596	563	594
		middle	740	739	742	744		626	614	627	409	586	618	591	581	593
		back	739	739	742	743		617	589	628	437	541	620	596	554	577

Acid/Neutral: 2/11/05: 2 burettes

7·03
7·21 = 25 H2O turned on [illegible]

Date: Time: 1·40am Treatment: Water temperature:

			TIME													
			3324	3246		0	343	640	922	1231	1445	1701	1931	2135	2352	2553
LOWER	1	front	589	538		531	599	636	661	685	697	705	717	721	728	730
		middle	591	546		538	613	636	662	686	697	707	715	721	727	730
		back	593	558		527	617	638	663	686	698	707	715	721	726	730
	2	front	593	564		517	611	637	664	687	698	708	716	721	727	730
		middle	597	560		5·65	607	638	665	686	698	706	716	721	727	730
		back	610	570		537	609	648	668	689	701	708	716	721	727	729
	3	front	586	581		4·89	616	650	666	690	701	707	716	721	726	730
		middle	583	480		5·22	609	647	666	689	701	708	717	721	728	730
		back	562	418		4·28	604	644	666	687	700	707	716	721	726	730
	4	front	544	392		4·55	602	641	665	688	699	708	716	721	726	730
		middle	557	396		4·19	607	641	665	687	699	709	717	721	728	730
		back	547	411		4·23	607	640	666	686	698	708	716	721	726	730
SHELTER			677	6·82		658	6·72	693	701	909	714	719	721	725	727	729
UPPER	1	front	587	548		5·83	627	661	684	702	706	715	719	725	728	729
		middle	586	551		5·79	628	658	681	697	704	715	720	725	728	730
		back	589	554		5·77	630	659	680	696	705	713	719	724	728	731
	2	front	575	555		5·75	630	657	680	695	705	716	719	724	728	731
		middle	569	508		578	629	658	679	695	706	713	719	724	728	732
		back	564	476		592	628	656	681	695	706	712	719	724	728	732
	3	front	559	475		589	629	656	680	696	705	713	719	724	728	732
		middle	552	500		583	629	656	680	696	706	716	720	724	729	731
		back	538	433		583	628	655	679	694	706	714	719	724	729	731
	4	front	4·26	422		581	630	656	680	695	705	714	719	725	727	733
		middle	4·23	388		579	629	657	680	695	706	713	719	724	729	733
		back	4·62	393		582	630	657	681	695	706	714	721	724	730	732

at 3mm e 0(3)

Date: 2/11 Time: 3 pm Treatment: PN Water temperature: 10.7

slowed down

			TIME													
			0	319	555	810		0	302	7·10	1212	1610	2028	2413	29·04	3321
LOWER	1	front	7·47	7.38	739	740		685	644	588	419	356	379	385	426	571
		middle	745	738	739	740		684	645	593	428	356	371	377	413	573
		back	743	739	738	740		683	647	595	453	358	366	382	410	575
	2	front	742	739	739	740		683	616	596	460	365	369	378	431	578
		middle	742	738	739	740		684	645	606	442	369	482	380	524	578
		back	740	738	737	741		667	602	590	513	365	345	402	473	573
	3	front	741	737	738	740		685	647	562	426	371	387	385	483	579
		middle	741	738	739	740		681	637	554	385	370	392	386	489	581
		back	740	738	737	741		671	617	521	373	307	336	332	405	511
	4	front	741	738	739	740		560	447	358	343	336	367	325	406	552
		middle	740	739	738	740		591	520	409	307	320	309	317	412	558
		back	739	738	738	740		618	498	427	337	324	324	344	401	359
SHELTER			737	734	737	736		700	683	6·79	6·67	666	652	677	635	682
UPPER	1	front	734	737	738	740		662	623	4·37	3·80	363	358	395	543	604
		middle	737	732	738	739		660	622	505	383	369	387	391	559	602
		back	737	737	738	739		657	624	515	367	362	368	386	553	602
	2	front	730	739	738	740		655	620	503	374	363	377	390	554	601
		middle	728	739	738	739		652	614	446	375	369	369	391	531	598
		back	737	737	737	739		651	614	435	372	353	346	373	528	601
	3	front	737	737	741	739		651	595	425	354	366	384	366	536	596
		middle	739	739	740	740		650	597	438	369	363	385	408	539	597
		back	739	739	739	740		646	600	425	354	349	353	372	494	576
	4	front	739	739	738	739		616	577	386	345	349	380	367	511	578
		middle	740	739	740	740		615	569	335	390	361	396	395	543	555
		back	739	739	740	741		629	578	360	359	348	355	367	478	579

Date: 2/11 Time: 320pm cont Treatment: A/N Water temperature:

			TIME													
			0	151	450	801	1063	1315								
LOWER	1	front	617	631	662	685	702	712								
		middle	616	632	662	685	701	712								
		back	615	633	662	686	700	713								
	2	front	616	634	662	686	702	713								
		middle	616	638	662	687	700	711								
		back	617	640	663	688	700	713								
	3	front	620	637	668	688	701	714								
		middle	618	638	667	688	701	715								
		back	617	637	666	687	700	714								
	4	front	602	636	664	686	700	713								
		middle	605	635	663	687	700	714								
		back	602	635	665	686	699	714								
SHELTER			639	723	726	719	7·20	725								
UPPER	1	front	644	657	684	706	7·08	726								
		middle	637	657	683	702	7·08	723								
		back	632	659	683	700	7·08	720								
	2	front	629	658	684	699	7·08	719								
		middle	627	657	684	698	7·08	718								
		back	628	657	684	697	7 08	719								
	3	front	625	657	683	698	9.08	7·19								
		middle	627	657	682	700	709	7·18								
		back	625	656	682	698	708	719								
	4	front	625	656	681	698	708	7·19								
		middle	626	657	681	698	708	7·19								
		back	625	657	681	698	708	7·22								

Acid/Neutral: 3/11/05: 2 burettes

H₂O flow - middle at, middle of tank.

2 burettes cooler on

Date: 3/11　Time: 9.45　Treatment: A/N　Water temperature: 17·8°C

before turned back on!

			TIME												
			0	228	529	832		0	450	914	1411	2205	3046	3722		
LOWER	1	front	739	741	762	744		7·13	613	532	513	368	3·33			
		middle	739	741	741	744		708	612	525	535	363	3·32	↑ stopped acid flow to let tank cool down.		
		back	739	741	741	744		706	610	524	551	360	3·31			
	2	front	738	741	741	744		680	608	503	507	357	3·31			
		middle	739	741	740	744		675	601	502	474	344	3·32			
		back	739	741	740	744		684	604	507	554	354	3·31			
	3	front	739	741	741	744		637	588	425	478	339	3·30			
		middle	739	741	741	744		584	588	361	425	341	3·26			
		back	739	741	741	744		625	537	404	419	346	326			
	4	front	739	741	741	744		622	520	391	437	344	326			
		middle	739	741	741	744		628	546	394	417	344	324			
		back	739	741	741	744		567	499	411	426	341	326			
SHELTER			739	729	734	7·40		715	644	654	654	395	6·36			
UPPER	1	front	739	740	740	744		652	565	406	558	345	3·32			
		middle	739	741	741	745		644	561	500	565	345	3·36			
		back	739	741	741	744		640	560	508	569	345	3·49			
	2	front	739	740	741	746		636	554	369	552	342	3·42			
		middle	739	741	741	746		633	552	419	548	341	3·27			
		back	739	740	74	746		631	549	435	541	340	3·42			
	3	front	739	741	741	745		627	539	428	532	340	3·26			
		middle	739	741	740	746		623	542	402	518	340	3·26			
		back	739	740	742	746		619	537	400	518	339	3·26			
	4	front	739	740	742	745		618	539	409	477	338	3·26			
		middle	739	741	742	746		618	540	410	513	339	3·26			
		back	739	741	743	746		483	537	409	414	337	3·26			

Acid/Neutral: 3/11/05: 2 burettes

Date: 3/11 Time: 210pm Treatment: A/N Water temperature: 18 C.

			TIME												
			0	235	616	843		0	347	558	1006	1428	1803	2142	2519	2903
LOWER	1	front	743	743	741	743		724	625	583	359	346	346	344	341	341
		middle	741	742	742	742		718	626	582	358	346	346	345	341	341
		back	742	742	742	742		714	626	584	353	346	347	346	342	341
	2	front	741	742	742	744		711	623	569	357	345	346	343	340	341
		middle	741	742	741	744		704	622	569	356	346	345	344	341	340
		back	741	741	742	743		702	624	568	356	353	346	348	341	341
	3	front	741	742	741	741		700	569	520	353	346	347	347	335	338
		middle	741	742	741	743		700	381	546	351	342	346	335	336	337
		back	742	741	742	743		695	552	403	348	330	344	337	335	333
	4	front	741	741	741	742		680	534	376	347	335	342	338	336	333
		middle	741	742	742	743		653	547	396	347	340	345	341	334	333
		back	741	741	742	742		491	573	358	346	339	342	333	335	336
SHELTER			739	739	739	739		708	570	636	580	656	642	6·44	649	643
UPPER	1	front	742	740	741	742		655	604	402	350	352	346	345	342	339
		middle	741	740	740	742		649	604	406	348	349	346	344	340	337
		back	741	740	740	743		643	600	403	348	347	346	343	340	337
	2	front	741	740	741	743		640	598	397	349	346	344	341	339	337
		middle	741	740	741	742		643	600	401	347	346	343	341	339	336
		back	741	741	742	742		640	594	401	347	346	343	342	339	336
	3	front	740	741	742	742		638	586	384	347	345	344	341	338	333
		middle	741	741	742	743		639	592	392	347	344	343	339	335	335
		back	741	741	742	742		638	586	346	344	345	341	334	332	334
	4	front	740	740	741	742		628	554	352	344	343	341	336	337	334
		middle	741	741	742	742		628	569	371	346	343	341	339	338	335
		back	741	741	742	742		607	570	328	318	342	339	339	337	332

turned down acid

Date: 3/11 Time: 210at Treatment: A/N Water temperature: 17·8°C

			TIME													
			3257	3656		0	304	539	835	1130	1508	1811	2100	2340	2624	
LOWER	1	front	334	332		330	336	344	351	365	386	408	444	503	549	
		middle	334	332		330	337	343	351	363	382	408	444	508	550	
		back	335	332		3·30	338	343	352	366	383	409	444	505	550	
	2	front	335	332		330	337	344	353	362	384	409	447	510	551	
		middle	335	332		3·30	336	344	351	362	383	439	448	508	550	
		back	336	337		3·31	336	344	353	367	388	425	448	508	552	
	3	front	337	332		330	338	344	353	365	385	410	488	522	554	
		middle	334	327		329	336	344	353	365	383	409	466	516	554	
		back	332	326		3 28	336	343	352	363	384	408	463	513	554	
	4	front	330	323		3·29	337	344	353	364	387	409	459	516	554	
		middle	330	328		330	338	345	355	365	387	412	466	518	557	
		back	326	329		3 29	337	345	354	366	389	413	467	519	558	
SHELTER			666	651		6·43	645	646	653	659	616	658	6·36	6·67	670	
UPPER	1	front	336	336		339	342	353	363	376	398	4·32	493	595	579	
		middle	334	332		337	342	351	362	375	400	428	488	568	577	
		back	333	331		336	342	352	362	375	400	428	488	565	576	
	2	front	332	330		335	342	351	366	378	390	429	489	560	576	
		middle	333	331		334	341	350	360	380	395	426	488	554	575	
		back	334	331		335	342	350	359	375	398	427	493	550	575	
	3	front	332	330		337	342	350	360	376	396	443	495	549	576	
		middle	331	330		334	341	349	359	382	396	433	500	548	577	
		back	332	330		336	341	349	361	378	394	437	501	550	578	
	4	front	331	330		335	341	350	362	378	404	439	498	551	578	
		middle	329	330		334	341	351	376	392	405	440	499	550	579	
		back	327	326		335	344	351	364	380	419	442	502	555	580	

Acid/Neutral: 4/11/06: 1 burette

drip/sec (30m 1 burette pH 7.28

No [illegible] sector

Date: 4/11 Time: 9.40am Treatment: A/N Water temperature: 16.9

			TIME													
			0	403	721	1153	1530	1831	2135	2504	2815	3126	3505			
LOWER	1	front	715	669	650	635	620	604	587	572	546	497	408			
		middle	706	669	657	655	619	605	589	570	543	493	403			
		back	702	660	657	635	618	605	588	567	541	476	401			
	2	front	695	669	652	634	619	608	590	564	544	467	402			
		middle	667	669	653	634	619	607	590	565	542	475	406			
		back	678	669	645	633	623	608	587	565	542	496	400			
	3	front	677	668	645	632	619	604	502	563	480	491	400			
		middle	677	687	565	631	617	605	542	561	518	417	387			
		back	676	661	608	626	615	602	561	553	510	385	391			
	4	front	677	652	629	626	608	513	525	496	484	412	394			
		middle	676	657	602	629	610	555	556	534	507	465	393			
		back	676	660	621	601	637	574	562	540	509	443	[illegible]			
SHELTER			711	705	689	681	677	665	666	658	658	649	650			
UPPER	1	front	682	668	644	626	620	603	585	565	526	430	384			
		middle	679	664	641	625	617	600	583	561	517	437	385			
		back	677	662	639	625	616	600	583	558	513	432	384			
	2	front	676	660	639	626	614	599	581	556	513	427	383			
		middle	674	660	640	625	613	598	582	558	513	426	380			
		back	679	659	639	624	614	596	578	541	508	409	384			
	3	front	672	657	636	625	613	593	569	533	498	402	376			
		middle	672	656	637	623	610	594	573	547	500	415	380			
		back	692	656	638	622	578	593	579	550	485	394	378			
	4	front	669	653	634	616	584	591	566	486	444	406	366			
		middle	671	654	636	597	596	592	572	525	484	410	377			
		back	671	652	627	609	596	523	570	537	488	408	375			

Date: 4[illegible] Time: (2:30) or 14[illegible] Treatment: A/N Water temperature: 16·6 °C L 50

Lower H2O [illegible] slower

			TIME													
			0	~~410~~ 431	314	619	922	1302	1615	1908	2206	2439				
LOWER	1	front	373	389	405	434	486	532	555	572	590	601				
		middle	372	389	404	447	488	533	555	572	590	600				
		back	376	390	407	449	4·94	537	555	573	590	600				
	2	front	376	389	405	441	4·93	537	556	575	590	590				
		middle	371	388	406	438	492	536	560	574	591	600				
		back	376	392	416	456	520	538	559	576	590	600				
	3	front	376	395	406	440	496	541	559	580	591	601				
		middle	372	384	400	441	491	537	559	579	590	601				
		back	370	386	404	437	4·89	538	557	576	589	600				
	4	front	370	386	405	432	4·86	535	554	575	589	600				
		middle	371	388	406	439	4·91	536	555	575	590	600				
		back	371	387	406	443	4·92	537	555	574	590	600				
SHELTER			635	6·38	642	644	6·57	658	667	674	663	675				
UPPER	1	front	380	400	427	478	530	558	579	617	618	613				
		middle	379	399	427	479	529	557	576	603	613	611				
		back	381	403	436	482	530	558	597	602	606	611				
	2	front	3·79	400	423	484	528	556	594	596	604	609				
		middle	380	400	423	473	527	552	573	594	603	609				
		back	380	397	431	469	527	554	578	593	601	608				
	3	front	380	398	431	476	526	554	575	592	601	608				
		middle	379	398	427	471	526	551	575	590	600	608				
		back	379	398	426	468	525	550	573	590	598	607				
	4	front	378	398	424	468	524	546	570	589	597	607				
		middle	379	398	425	469	526	547	571	588	597	607				
		back	379	398	425	467	524	548	571	588	597	607				

HP

Date: 4.11.05 Time: 4.pm Treatment: A/N Water temperature:

Acid/Neutral: 4/11/05: Hand pippetting

			TIME											
								200x4		300µl×4	300µl	300 × 4				
			0	10µM	0.20	2.34	5.11		7.17	9.34	11.45	14.20	16.10	17.50	19.34	21.21
LOWER	1	front	7.47	in 4cm	7.38	7.04	6.78		6.61	5.58	5.30	5.27	4.25	4.26	4.36	4.57
		middle	7.46		7.35	7.03	6.76		6.56	5.63	5.37	5.35	4.26	4.25	4.36	4.57
		back	7.46		7.30	6.94	6.74		6.52	5.70	5.45	4.50	4.25	4.26	〃	4.58
	2	front	7.46		7.24	6.89	6.60		6.44	5.73	5.43	4.55	4.25	4.25	4.39	4.58
		middle	7.45		7.15	6.85	6.68		6.43	5.78	5.4	4.52	4.26	4.24	4.56	4.58
		back	7.46		7.09	6.83	6.67		6.42	5.78	5.43	4.23	4.25	4.37	4.58	4.63
	3	front	7.45		7.07	6.80	6.63		6.40	5.81	5.41	4.16	4.25	4.35	4.41	4.63
		middle	7.46		7.05	6.79	6.62		6.38	5.77	5.36	4.27	4.23	4.29	4.40	4.62
		back	7.45		7.04	6.78	6.60		6.39	5.76	5.39	4.11	4.23	4.28	4.41	4.56
	4	front	7.45		7.05	6.76	6.58		6.39	5.76	5.41	4.14	4.23	4.27	4.39	4.55
		middle	7.45		7.03	6.76	6.58		6.36	5.79	5.39	4.20	4.23	4.28	4.36	6 ↓
		back	7.46		7.03	6.77	6.59		6.35	5.71	5.35	4.21	4.22	4.27	4.36	↓
SHELTER			7.45		7.29	7.06+	6.89+		6.71	6.35	6.5+	5.87	5.6+	5.9+	6.08	6.04
UPPER	1	front	7.45		7.10	6.96	6.76		6.50	6.0↓	5.81	4.42	4.30	4.78	4.67	4.8
		middle	7.44		7.08	6.90	6.72		6.44	6.02	5.74	4.31	4.28	4.31	4.58	4.76
		back	7.45		7.07	6.88	6.66		6.42	5.78	5.72	4.28	4.26	4.34	4.57	4.72
	2	front	7.46		7.06	6.85	6.65		6.39	5.96	5.72	4.26	4.26	4.35	4.56	4.71
		middle	7.44		7.05	6.83	6.64		3.38	5.92	5.72	4.25	4.24	4.36	4.56	4.70
		back	7.45		7.05	6.82	6.62		.37	5.92	5.63	4.25	4.24	4.38	4.57	4.73
	3	front	7.45		7.04	6.81	6.62		.35	5.92	5.64	4.26	4.24	4.4	4.56	4.75
		middle	7.45		7.04	6.80	6.62		.35	5.92	5.62	4.23	4.24	4.36	4.52	4.74
		back	7.45		7.04	6.81	6.61		.34	5.91	5.61	4.21	4.23	4.31	4.47	4.69
	4	front	7.44		7.04	6.79	.60		.34	5.91		4.22	4.24	4.30	4.47	4.67
		middle	7.46		7.04	6.80	.60		.35	5.91		4.22			4.48	4.68
		back	7.45		7.04	6.80	.60		.34	5.91	↓	4.23	↓	↓	4.48	4.69

Date: Time: 4pm cont Treatment: Water temperature:

			TIME	100x2												
			23.15	25.12												
LOWER	1	front	4.76	4.94												
		middle	4.76	4.85												
		back	4.77	4.32												
	2	front	4.77	4.04												
		middle	4.77	4.23												
		back	4.81	4.31												
	3	front	4.80	4.22												
		middle	4.82	4.54												
		back	4.85	4.44												
	4	front	4.82	4.25												
		middle	4.81	4.55												
		back	4.80	4.50												
SHELTER			6.10	6.05												
UPPER	1	front	5.0-	4.74												
		middle	4.97	4.65												
		back	4.93	4.53												
	2	front	4.93	4.50												
		middle	4.94	4.57												
		back	4.94	4.50												
	3	front	4.95	4.50												
		middle	4.93	4.50												
		back	4.93	4.50												
	4	front	4.91	4.52												
		middle	4.90	4.51												
		back	4.87	4.50												

Date: 6 11 05 Time: 2pm Treatment: A/N Water temperature: ~~19.5~~ 17.7

			TIME													
					↓300×4				180×4			70×4		170×2		
			0	↓	0	↓ 2.06	↓ 4.25	↓ 6.28	↓ 8.52	10.25	12:04	↓ 14.06	15.58	↓ 18.06	19.40	21.15
LOWER	1	front	7.16		7.01	6.65	6.23	5.61	4.80	4.32	4.75	4.50	4.82	4.86	4.40	4.41
		middle	7.17		6.97	6.53	6.17	5.63	4.53	4.32	4.78	4.60	4.83	4.45	4.35	4.41
		back	7.16		6.97	6.55	6.14	5.65	4.10	4.34	4.81	4.84	4.84	4.30	4.35	4.41
	2	front	7.18		6.94	6.50	6.10	5.25	4.44	4.36	4.84	4.68	4.83	4.40	4.40	4.41
		middle	7.19		6.91	6.49	6.08	5.20	4.27	4.37	4.85	4.69	4.84	4.55	4.4	4.42
		back	7.19		6.90	6.48	6.07	5.02	4.37	4.40	4.91	4.80	4.83	4.55	"	4.43
	3	front	7.19		6.90	6.45	6.04	4.60	4.27	4.60	4.81	4.90	4.85	4.50	4.39	4.49
		middle	7.19		6.88	6.44	6.04	4.81	4.13	4.35	4.76	4.63	4.79	4.24	4.40	4.42
		back	7.19		6.86	6.43	6.04	4.86	4.16	4.38	4.76	4.55	4.75	4.30	4.39	4.41
	4	front	7.19		6.82	6.39	6.04	4.89	4.17	4.34	4.74	4.52	4.77	4.33	"	4.43
		middle	7.19		6.83	6.39	6.05	4.78	4.23	4.31	4.65	4.53	4.77	4.36	"	4.41
		back	7.19		6.82	6.40	6.05	5.00	4.25	4.33	4.73	4.53	4.77	4.44	"	4.40
SHELTER			7.20		7.0+	7.0+	6.4+	6+	6+	6+	6+	6+	6.1+	6+	5.4	5.79
UPPER	1	front	7.21		6.94	6.54	6.23	5.38	4.44	4.6	5.28	4.91	5.0	4.57	4.5	4.63
		middle	7.21		6.93	6.50	6.13	5.26	4.34	4.6	5.10	4.89	5.0	4.48	4.47	4.47
		back	7.22		6.90	6.47	6.10	5.18	4.30	4.65	5.09	4.85	4.9	4.41	4.42	4.48
	2	front	7.21		6.88	6.44	6.07	5.15	4.29	4.67	5.10	4.84	4.88	4.40	4.41	4.31
		middle	7.22		6.87	6.45	6.05	5.13	4.28	4.61	5.09	4.80	4.87	4.35	4.43	4.46
		back	7.25		6.87	6.43	6.05	5.11	4.30	4.73	5.10	4.80	4.85	4.35	4.41	4.47
	3	front	7.23		6.85	6.42	"	5.10	4.33	4.76	5.12	4.81	4.85	4.35	4.41	4.52
		middle	7.22		6.85	6.43	"	5.10	4.29	4.61	5.11	4.76	4.84	4.38	4.40	4.50
		back	7.23		6.84	6.42	6.04	5.08	4.27	4.63	5.08	4.72	4.83	4.38	4.41	4.41
	4	front	7.22		6.84	6.42	"	5.07	4.26	4.57	5.05	4.72	4.83	4.39	4.4	4.48
		middle	7.22		6.84	6.42	"	5.07	4.25	4.55	5.01	4.72	4.83	4.39	4.4	4.45
		back	7.23	↑	6.83	6.42	"	5.07	4.26	4.57	5.01	4.72	4.82	4.39	4.4	4.47

Acid/Neutral: 6/11/05: Hand pippetting

Date: Time: Treatment: Water temperature:

			|70×2	, 80×4	| 70×2			TIME		|70×2		39.41			
			22.55	25.00	21.08	29.14	30.43	32.22	33.44	35.08	36.51	38.12		O	4.20	26
LOWER	1	front	4.56	5.21	4.64	4.48	4.42	.44	.53	4.71	4.38	4.50		5.24	6.1	7.0
		middle	4.55	4.92	4.49	4.19	4.42	..43	.54	4.73	4.47	4.17		5.34	6.1	
		back	4.60	5.10	4.65	4.51	4.42	.43	.54	4.74	4.53	4.57		5.35	6.10	
	2	front	4.65	5.08	4.68	4.56	4.42	.43	.54	4.74	4.32	4.57		5.44	"	
		middle	4.67	5.14	4.82	4.62	4.42	.43	.54	4.78	4.40	4.52		.46	"	
		back	5.00	5.15	4.93	4.53	4.42	.43	.53	.78	4.53	4.69		.53		
	3	front	4.87	5.27	4.78	4.42	4.42	.45	.54	77	4.54	4.68		.50		
		middle	4.68	4.94	4.75	4.45	4.41	.44	.52	83	4.47	4.53		.50		
		back	4.65	4.93	4.67	4.37	4.42	.43	.53	.75	4.40	4.52		.52		
	4	front	4.63	4.92	4.64	4.38	4.41	..43	.53	.72	4.37	4.58		5.55		
		middle	4.62	4.99	4.62	4.37	4.41	.43	.52	.72	4.35	4.52		.55		
		back	4.64	4.98	4.62	4.34	4.42	.43	.51	.71	.40	4.52		.54		
SHELTER			6+	6+	6+	5.8	5.8	6+	6+	6+	6.05	6+		6+	6+	
UPPER	1	front	5.15	5.50	5.3	4.55	4.57	4.73	.71	5.0	4.75	4.7		5.58		
		middle	5.14	5.35	5.04	4.45	4.49	.53	.67	4.91	4.57	.65		.61		
		back	5.24	5.4	5.04	4.43	4.44	.50	.67	4.85	4.54	.70		.68		
	2	front	5.23	5.4	5.05	4.44	4.43	.50	.67	4.91	4.54	.69		.69		
		middle	5.19	5.38	4.92	4.42	4.43	.47	.68	4.88	.52	.65		.69		
		back	5.15	5.34	4.94	4.43	4.43	.45	.67	4.85	.51	.72		.68		
	3	front	5.22	5.44	4.93	4.42	4.43	.52	.70	4.93	.53	.74		.69		
		middle	5.15	5.35	4.91	4.42	4.42	.50	.68	4.91	.53	.4.55		.67		
		back	5.18	5.35	4.89	4.41	4.42	.50	.63	4.80	.50	4.67		.68		
	4	front	5.10	5.36	4.88	4.41	4.42	.50	.64	4.95	.41	4.67		.70		
		middle	5.08	5.36	4.85	4.41	4.42	.50	.61	4.83	.48	4.67		.70		
		back	5.10	5.34	4.85	4.41	4.42	.50	.61	4.83	.47	4.67		.70		

APPENDIX 6. CHAPTER 4 – STATISTICS

Two-way ANOVA: % rank for shelter use versus Treatment, Period

```
Analysis of Variance for % rank
Source       DF      SS       MS       F        P
Treatmen      3   12093     4031    5.81    0.001
BDA           2    8246     4123    5.94    0.004
Interaction   6    7893     1316    1.90    0.089
Error        96   66594      694
Total       107   94827
```

Sheirer-Ray-Hare

```
Total SS    Total DF    Total MS    Factor       SS       SS/MS     Chi Sq      P
94827       107         886.234     Treatment    12093    13.6454   0.996570    0.003430
                                    Period       8246     9.3045    0.990460    0.009540
                                    Interaction  7893     8.9062    0.821079    0.178921
```

General Linear Model: Activity versus Treatment, Period

```
Factor         Type          Levels         Values
Treatment      fixed         4              N/N, N/A, A/N, A/A
Period         fixed         3              Before, During, After

Analysis of Variance for Activity, using Adjusted SS for Tests

Source         DF      Seq SS         Adj SS         Adj MS         F       P
Treatment      3       9.2575         7.8195         2.6065         6.48    0.001
Period         2       1.7838         1.8426         0.9213         2.29    0.108
TM*Period      6       4.0648         4.0648         0.6775         1.69    0.186
Error          77      30.9573        30.9573        0.4020
Total          88      46.0634
```

General Linear Model: Opercular Rate versus Treatment, Period

```
Factor         Type          Levels         Values
Treatment      fixed         4              N/N, N/A, A/N, A/A
Period         fixed         3              Before, During, After

Analysis of Variance for Opercula, using Adjusted SS for Tests

Source         DF      Seq SS         Adj SS         Adj MS         F       P
Treatment      3       11925.8        10550.3        3516.8         16.54   0.000
Period         2       7431.5         7277.8         3638.9         17.12   0.000
TM*Period      6       3069.2         3069.2         511.5          2.41    0.034
Error          84      17856.8        17856.8        212.6
Total          95      40283.3
```

APPENDIX 7. CHAPTER 5 – STATISTICS

Two-way ANOVA: % rank for shelter use versus Treatment, Period

```
Analysis of Variance for % rank
Source         DF        SS         MS         F        P
Treatment       3     12434       4145      2.90    0.038
Period          2      3894       1947      1.36    0.260
Interaction     6      1800        300      0.21    0.973
Error         120    171248       1427
Total         131    189376
```

Sheirer-Ray-Hare

```
Total SS    Total DF    Total MS    Factor        SS       SS/MS      Chi Sq      P
189376      131         1445.62     Treatment     12434    8.60116    0.964908    0.035092
                                    Period        3894     2.69366    0.739936    0.260064
                                    Interaction   1800     1.24514    0.025404    0.974596
```

General Linear Model: Activity versus Treatment, Period

```
Factor         Type          Levels          Values
Treatment      fixed         4               H1, H2, W1, W2
Period         fixed         3               Before, During, After

Analysis of Variance for Activity, using Adjusted SS for Tests

Source         DF       Seq SS          Adj SS          Adj MS          F       P
Treatment      3        7.6484          6.7266          2.2422          7.49    0.000
Period         2        4.4465          4.3564          2.1782          7.27    0.001
TM*Period      6        1.6125          1.6125          0.2688          0.90    0.500
Error          97       29.0507         29.0507         0.2995
Total          108      42.7582
```

General Linear Model: Opercular Rate versus Treatment, Period

```
Factor         Type          Levels          Values
Treatment      fixed         4               H1, H2, W1, W2
Period         fixed         3               Before, During, After

Analysis of Variance for OR, using Adjusted SS for Tests

Source         DF       Seq SS          Adj SS          Adj MS          F       P
Treatment      3        21523.8         18946.6         6315.5          51.44   0.000
Period         2        11910.4         12182.2         6091.1          49.61   0.000
TM*Period      6        8318.0          8318.0          1386.3          11.29   0.000
Error          67       8225.7          8225.7          122.8
Total          78       49977.8
```