THE EFFECT OF ACIDIFICATION ON THE SURVIVAL OF AMERICAN EEL

by

Craig Reynolds

Submitted in partial fulfillment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
March 2011

DALHOUSIE UNIVERSITY

DEPARTMENT OF BIOLOGY

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "THE EFFECT OF ACIDIFICATION ON THE SURVIVAL OF AMERICAN EEL" by Craig Reynolds in partial fulfillment of the requirements for the degree of Master of Science.

Dated: March 31, 2011

Supervisor: _____

Readers: _____

_____

DALHOUSIE UNIVERSITY

DATE: March 31, 2011

AUTHOR: Craig Reynolds

TITLE: THE EFFECT OF ACIDIFICATION ON THE SURVIVAL OF AMERICAN EEL

DEPARTMENT OR SCHOOL: Department of Biology

DEGREE: MSc CONVOCATION: October YEAR: 2011

_____

Signature of Author

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The geographic range of the panmictic American eel (*Anguilla rostrata*) has contracted in recent years because of the pronounced decline in recruitment of glass eels and elvers to the Laurentian Basin. In consequence, the American eel was assessed in 2006 as a species of special concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). The evident sensitivity of American eel status to elver recruitment highlights the importance of understanding both the mechanisms contributing to the delivery of glass eels from the Sargasso Sea to continental waters and mortality following their recruitment to coastal and inland waterways. The potential for variability in environmental quality at localized geographic scales to affect American eel productivity and hence the status of the species and the fisheries it supports is not fully understood. The Atlantic coast of Nova Scotia is an ideal location to examine the relationship between water quality and American eel productivity. Within Nova Scotia there is wide natural variation in freshwater pH, which has been further increased in recent years by the effects of acid precipitation. This variation occurs over a small geographic range of several hundred kilometres that overlaps an area of high elver influx. As low environmental pH is known to adversely affect aquatic ecosystems, it has been identified as a possible threat to elver survival.

In this study, the effect of low pH on elver survival was examined in both laboratory and field based trials using wild glass eel/elvers that were captured upon entry to fresh water. Trials examined the mortality rate of elvers at pH levels within the range of 4.0 - 7.0 over a 10 day period. The relationship between elver development and mortality at low pH was also examined through pigmentation analysis. Laboratory and

field based studies resulted in zero mortality among elvers in natural and artificial acidic environments with pH levels as low as 4.0., thus indicating that the American eel is fully acid tolerant upon initial migration into fresh water. Sub-lethal effects of acidification were explored by examining the hematological parameters of river resident yellow-phase American eels exposed to varying levels of acidity in the laboratory. The level of acidification proved not to be a factor in determining both hematocrit and blood plasma osmolarity levels, as there were no significant differences in these variables between eels exposed to acidic conditions and those exposed to control neutral pH conditions. These results suggest that through the use of a highly effective mechanism for regulating blood ion concentration, the eel is able to tolerate low pH conditions.

List of Abbreviations and Symbols Used

AIC	Akaike Information Criteria
ANC	Acid Neutralizing Capacity
ANOVA	Analysis of Variance
ASF	Atlantic Salmon Federation
ATPase	Adenosine Triphosphate Catalyzing Enzyme
Ca^{2+}	Calcium ion
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
Cl^-	Chloride ion
CO_2	Carbon dioxide
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
DFO	Fisheries and Oceans Canada
DOC	Dissolved Organic Carbon
H^+	Hydrogen ion
H_2SO_4	Sulphuric acid
HCO_3^-	Bicarbonate ion
K^+	Potassium ion
MRCs	Mitochondrial Rich Cells
MS-222	Tricaine Methanesulfonate
N	Number
Na^+	Sodium ion
PIT	Passive Integrated Transponder
SRPR	Species at Risk Public Registry

Acknowledgements

I would like to thank Dr. Rod Bradford for introducing me to fish biology and fisheries and providing me with the initial work opportunity that brought me to Nova Scotia. That initial vote of confidence laid the foundation for everything that followed. The ability to conduct this research and pursue this degree has proven to be an invaluable experience. Your guidance and mentorship throughout this process has played an integral role in shaping my current career path.

Thank you to Dr. Paul Bentzen for the opportunity to study at Dalhousie and welcoming me as a graduate student in your lab. You always had an open door and made the time for your students. I appreciate your support, advice and guidance.

Adam Cook, first and foremost thank you for your friendship. I consider myself very lucky to have had you in the lab with me working on our degrees. You were always there whenever I had a question or was having difficulty and I appreciate that. You really went out of your way to help me with my work and I've really learned a lot from you. I've completed this degree and gained a good friend.

To my parents thank you for all your love and support throughout my education. I realize that I wouldn't be where I am today if I didn't have such amazing parents. You taught me the value of education and that hard work really does pay off in the end.

Stephanie, I love you, thank you for being there with me supporting me this whole time. This was the hardest thing I've ever done and I couldn't have done it without you. You gave me the encouragement I needed when times were tough. You believed in me and I knew that no matter what happened you loved me. Thank you for wearing your pink rain boots to go elver fishing at 3am and for the countless hours you put in directly helping me with this project. It really showed me early on just how much you care.

Chapter 1: Introduction

1.1 The American Eel in Canada

Biodiversity is important from an ecological, economic and cultural perspective (Pimentel *et al.* 1997, Posey 1999, Chapin *et al.* 2000). Any loss in biodiversity weakens the natural environment, resulting in ecosystems that are potentially less stable, increasingly fragile and more vulnerable to extreme events (Naeem and Li 1997, Purvis and Hector 2000). As humans, we rely on a biologically diverse natural environment to provide us with the necessities of life as well as the basis of our economy (Pimentel *et al.* 1997, Chapin *et al.* 2000). Our cultural diversity is closely linked to the Earth's biodiversity (Posey 1999). Biodiversity can be viewed as an essential intrinsic value worth protecting.

Conservation biology is a multidisciplinary science that has developed to address the loss of biological diversity (Soulé 1985). The field of conservation biology focuses on evaluating and identifying threats to biological diversity and developing practical approaches to prevent the extinction of species (Soulé 1985, Wilson 1992). It is the applied science of maintaining the earth's biologicial diversity (Hunter and Gibbs 2007). Ultimately, conservation requires an interdisciplinary approach combining both scientific and economic principles in order to develop a sustainable management plan to protect and conserve that which is threatened (Chapin *et al.* 2000, Hunter and Gibbs 2007).

The biodiversity of North American fish species has declined in recent years and an increasing number of fish species are becoming at risk of extinction as a result of anthropogenic factors (Jelks *et al.* 2008). Currently within Canada alone, the number of freshwater and marine fish species that have been assessed by the Committee on the

Status of Endangered Wildlife in Canada (COSEWIC) as endangered, threatened or special concern is more than 80 (SRPR 2010).

In 2006, COSEWIC assessed the American eel (*Anguilla rostrata*) as a species of special concern, as there has been a pronounced decline in recruitment of glass eels and elvers to the Laurentian Basin in recent years (Castonguay *et al.* 1994, Cassleman 1997, Haro *et al.* 2000, DFO 2010). Historically, the American eel has one of the largest ranges of any fish species in the western hemisphere encompassing fresh waters, estuaries and marine waters of the western North Atlantic (Scott and Crossman 1973, Tesch 2003). In Canada, this area includes all accessible fresh water, estuarine and coastal areas connected to the Atlantic Ocean, as far north as the mid-Labrador coast and as far inland as Niagara Falls in the Great Lakes (Scott and Crossman 1973). Due to this historical widespread distribution the American eel is considered an important component of Canadian biodiversity.

1.2 Life History

The American eel species is comprised of a single breeding population that spawns in only one place in the world, the Sargasso Sea, a 3-million square-kilometre area of the North Atlantic Northeast of the West Indies (Schmidt 1922, Tesch 2003). After hatching, young eels develop into leaf-shaped leptocephali, and are distributed across the species range with the aid of ocean currents (Schmidt 1922, Kleckner and McCleave 1982, Tesch 2003). As they cross the continental shelf, leptocephali metamorphose into unpigmented glass eels that are fully capable of directed swimming (Antunes and Tesch 1997, Tesch 2003, Wuenschel and Able 2008,). Glass eels detrain from the Gulf Stream and move towards continental waters through the use of ocean

currents and active swimming (Kleckner and McCleave 1985, McCleave 1993, Arai *et al.* 2000, Powles and Warlen 2002, Wuenschel and Able 2008). Overall, the recruitment of individuals to a particular geographic area is a stochastic process likely influenced by the ontogenetic timing of metamorphosis, which in itself is inherently plastic (McCleave 1993, Arai *et al.* 2000, Powles and Warlen 2002).

American eels have traditionally been viewed as a catadromous species, migrating as juveniles from sea water to fresh water growth habitat and returning to sea water to spawn. However, recent studies have shown that catadromy is not obligate for the American eel but rather a facultative life-history option as American eels have been shown to complete their entire life-cycle within the marine environment. (Jessop *et al.* 2002, Daverat *et al.* 2006, Lamson *et al.* 2006, Jessop *et al.* 2008). It has been proposed that newly arriving glass eels make migratory choices based on internal energetic status (Edeline *et al.* 2005, Edeline *et al.* 2006, McCleave and Edeline 2009) and thyroid hormonal activity (Edeline *et al.* 2004). Increased energetic status and thyroid gland activity has been linked to the active colonization of river habitats and freshwater-seeking behaviour (Edeline *et al.* 2004, Edeline *et al.* 2005, Edeline *et al.* 2006).

As glass eels approach the coast they begin to develop pigment and become elvers, a small version of the adult eel ranging between 50 to 90 mm. The timing of elver arrival to estuarine waters varies across the species range, generally occurring progressively later as distance from the Sargasso increases (Jessop 1998a, Powles and Warlen 2002, Sullivan *et al.* 2006). In Atlantic coastal Nova Scotia, the migration of elvers into fresh water occurs over a nine week period between late April and late June (approximately 14 months after hatching) (Jessop 1998a). Over this period there are

typically several arrival waves of varying magnitude, as elver abundance throughout the run tends to follow a sinusoidal wave pattern linked to the spring-neap tidal cycle (Kleckner and McCleave 1982, Martin 1995, Jessop 1998a, Jessop 2003, Sullivan *et al.* 2006).

Upon attaining body lengths of greater than 30cm eels are termed yellow eels on the basis of their ventral pigmentation. The yellow eel is the primary growth phase of the species. Eels are carnivores feeding on a wide variety of fishes and invertebrates. The sexual maturation of eels occurs anywhere between five to twenty years of age and is largely determined by environment (Jessop 1987, Oliveira 1999, Krueger and Oliveira 1999). Smaller rivers with high population densities and low productivity tend to produce males whereas larger lakes and rivers with increasing resource opportunities are conductive to females (Krueger and Oliveira 1999).

Each winter, sexually maturing eels from across the species range migrate to the Sargasso Sea spawning grounds. Eels exhibit remarkably high swimming efficiency and low energy costs, and complete this journey without feeding, as the gut begins to degenerate before the onset of migration (Pankhurst and Sorensen 1984, van Ginneken and van den Thillart 2000). The onset of spawning migration from different areas over the species range varies to allow for synchronous arrival at the spawning grounds (Jessop 1987, McGrath *et al.* 2003, Verrault *et al.* 2003). This synchronous arrival of all potential spawners creates a genetic "mixing pot" and the entire species is considered to form a single population (Avise 1986, Wirth and Bernatchez 2003). As mating and larval dispersal are random, there are no genetically distinguishable watersheds or regional "stocks" as there are for anadromous species such as Atlantic salmon (*Salmo salar)*. As a

result, the American eel can be viewed as a single stock (Avise 1986, Wirth and Bernatchez 2003, COSEWIC 2006). The American eel is considered a semelparous species, as individuals die shortly after spawning (Helfman *et al.* 1987).

1.3 Status

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assessed the American eel as a species of special concern, a species that may become threatened or endangered because of a combination of biological characteristics and identified threats. As data on the entire Canadian component of the species is not available, the status of American eel is described using region-specific indicators of abundance. The Lake Ontario – Upper St. Lawrence component of the population has seen its numbers reduced by 99% since the 1970's (Castonguay *et al.* 1994, Casselman *et al.* 1997, DFO 2010). As this component of the population is known to produce large-bodied eels that are almost exclusively female (COSEWIC 2006), concern has been expressed that the population fecundity of the species may be negatively influenced as a result of this decline. Within the Maritime Provinces, indices of eel abundance have fluctuated inter-annually without trend since 1996 on the Atlantic Coast of Nova Scotia and show a generally increasing trend from 1997 to 2008 in the southern Gulf of St. Lawrence (DFO 2010). This observed pattern of reduced abundance in peripheral areas (Lake Ontario – Upper St. Lawrence) and relative stability in areas closer to the breeding grounds (Maritimes) suggests an overall contraction of the area of distribution that may be consistent with an overall decline in species abundance (COSEWIC 2006). This evident sensitivity highlights the importance of understanding both the mechanisms contributing to the delivery of young from the Sargasso Sea to continental waters and

mortality following their recruitment to coastal and inland water. The potential for local-scale variability in environmental quality to affect American eel productivity, and hence the status of the species and the fisheries they support, is not fully understood. Despite the uncertainty that exists, all recent assessments of the status of American eel in Canada agree that there is a cause for concern and that further study and research is warranted (Haro *et al.* 2000, COSEWIC 2006, DFO 2010).

The Canadian government has adopted a precautionary approach to American eel management, with an immediate goal of reducing overall human-induced mortality by 50% (COSEWIC 2006, DFO 2010). Thus, understanding the factors that may affect mortality rates of American eel is crucial for the proper management, protection and conservation of the species.

The American eel has a complex life history and is dependent on a wide range of aquatic habitats, subjecting it to a wide range of possible threats in essentially all aquatic habitats in eastern North America. Threats affecting any life stage, in any geographic area of the range, and in any array of habitats, have the potential to affect the abundance of all life stages of the species throughout the range.

Although the leading causes of eel mortality have not yet been defined, potential key threats have been identified, most of which are anthropogenic. Fishing, dams (restricting migration/access to habitat, mortality during migration) and chemical contaminants (bioaccumulation, river acidification) were all listed in recent status reports as possible threats to the sustainability of the American eel population (COSEWIC 2006, DFO 2010).

1.4 Conservation Issues and Causes for Concern within the Maritimes Region

1.4.1 Fishing

As high levels of fishery-induced mortality can directly result in population decline and possible extinction (Baum *et al.* 2003, Myers and Worm 2005), a balance must exist between the economic success of a fishery and the overall health and sustainabilty of the stock. Conservation of the resource is the primary objective, as sustainability must be ensured to maintain an economically viable resource. The American eel is an economically important species to Canadians, and large (yellow and silver) eel fisheries have historically existed throughout the species' range (COSEWIC 2006). The only fishery in Canada for elvers occurs during April-July on the Atlantic and Fundy coasts of Nova Scotia and New Brunswick (Jessop 1998b). Begun in 1989 in response to the Asian aquaculture industry's high demand for *Anguilla* elvers, the elver fishery expolits elvers recruiting to rivers. Elver fisheries only exist in rivers where there is no large eel fishing therefore the elver fishery is the sole source of fishing mortality in these rivers. Total catch varies annually, with 2,862 kg reported in 1996 (Jessop 1998b) and approximately 2,000 kg reported in 2007 (DFO, 2010). Currently the price per kg averages around $1000 resulting in a multi-million dollar industry.

Over-exploitation of elvers in the fishery has the potential to reduce the number of future spawners, negatively affecting the reproductive capacity of the species. A study conducted by Jessop (2000) on the recruitment of elvers to the East River, Chester, Nova Scotia found that 31-52% of arriving elvers were removed by the fishery. It has been suggested that elver fisheries can tolerate a high exploitation rate because the natural mortality is intrinsically high particularly in many Nova Scotia rivers where fishing

occurs (Jessop 2000). The East River is acidic and at the time of the study pH ranged from 4.7-5.0. It was hypothesized that acidification may be a factor in the observed high natural mortality rate (Jessop 2000).

1.4.2 The Potential Threat of Acidification

Of all Canadian provinces, Nova Scotia has the highest percentage of fish habitat that has been damaged by acidification. Early studies by Watt et al. (1979, 1983) and Kerekes et al. (1982) demonstrated that surface waters in the province, particularly those that drain the Southern Uplands (Figure 1), were acidified to a level which would result in deleterious effects to aquatic ecosystems.

According to the Atlantic Salmon Federation, acid precipitation has caused the extirpation of the salmon populations in 14 rivers in Nova Scotia's Southern Uplands region, and populations in another 50 rivers are threatened (ASF 2010). Watt (1987) estimated that 33% of Atlantic salmon productivity had been lost in this region. Acidification of Nova Scotia's Southern Uplands region is also thought to have contributed to the decline of the Atlantic whitefish (*Coregonus huntsmani)* (DFO 2006), an endangered species endemic to Nova Scotia, whose native habitat has been reduced to only three lakes within a single watershed (Bradford *et al.* 2004).

The American eel is a species of economic importance in the Maritimes region and is a traditional food source and culturally important to the Mi'kmaq people (COSEWIC 2006). Due to the panmictic nature of the species, regional isolated threats unique to a specific area of the species range, such as acidification, have the potential to not only affect local eel abundance but can also affect the entire species by reducing overall reproductive capacity (COSEWIC 2006).

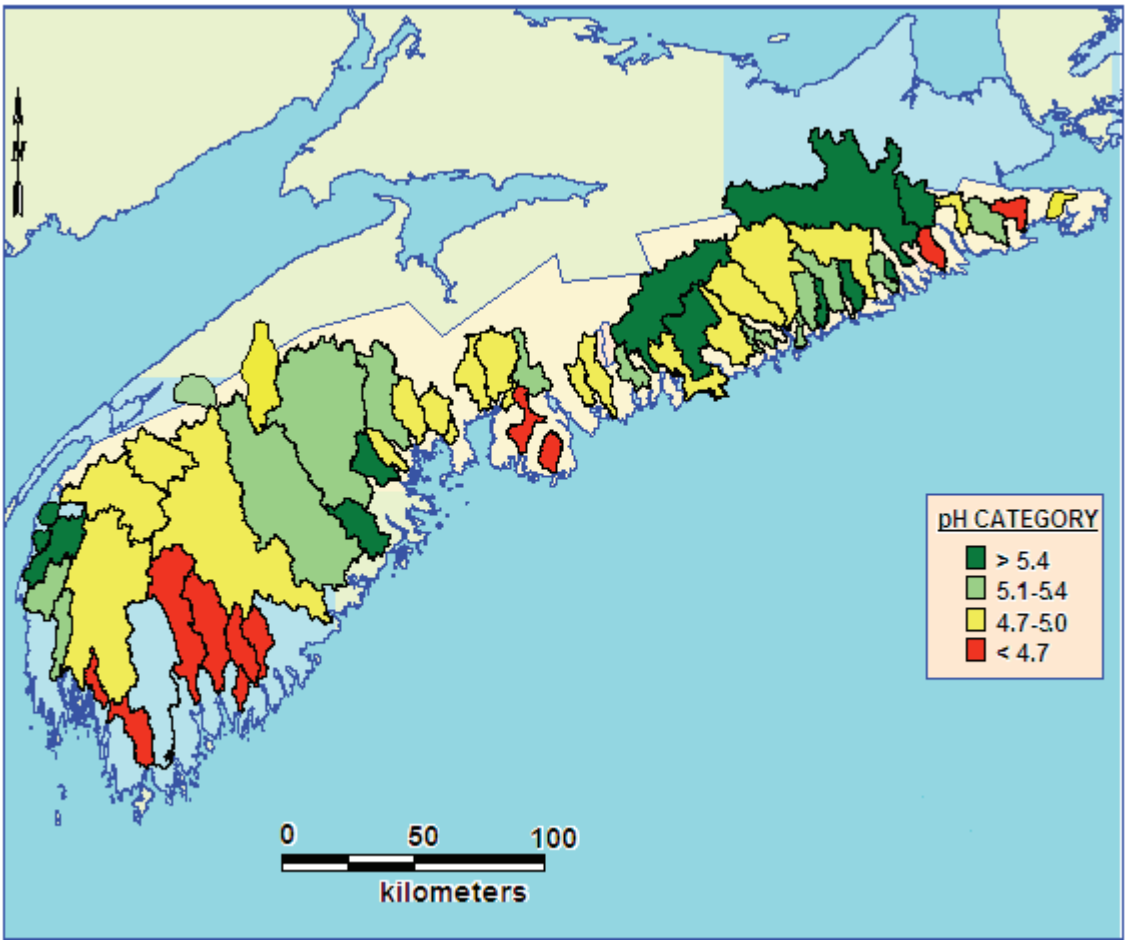

Figure 1: Mean annual pH of 48 rivers of the Southern Upland region of Nova Scotia classified into four categories (Watt 1987)

1.5 Thesis Statement

Freshwater eels are generally considered to tolerate and survive low pH environments (pH 4.1- 5.5) (Graham 1993, Lacroix 1987); however, these observations are for older eels, rather than elvers. A laboratory study of European eel elvers (*Anguilla anguilla)* reported a high rate of elver mortality at pH values of 4.6–5.1; however, dissolved labile aluminum concentrations exceeded 0.18 mg/L and mortality was attributed to aluminum toxicity rather than acid toxicity (Fjellheim *et al.* 1985).

Acidification of watersheds results in increased concentrations of aqueous aluminum in surface waters (Hooper and Shoemaker 1985). Aluminum toxicity is generally considered to be the major cause of fish mortality in low pH environments as inorganic labile aluminum binds to fish gills inhibiting the organism's ability to respire (Baker and Schofield 1982). However, in waters with elevated organic acid concentrations, such as those found in southwestern Nova Scotia, there is little toxic effect as aluminum is complexed with organic acids, thus rendering it unavailable to fish gills (Lacroix and Kan 1986, Lacroix and Townsend 1987).

In the absence of toxicity caused by aluminum, the main pH associated factor directly affecting the density and production of fishes is hydrogen ion activity (H^+) (Lacroix 1989). In the Southern Uplands of Nova Scotia, increased mortalities of salmon eggs, fry, and parr occur at low pH levels (4.2-5.0), and the lethal effects have been attributed to high hydrogen ion concentrations (H^+) at low environmental calcium concentrations (Lacroix 1985, Lacroix and Townsend 1987, Lacroix *et al.* 1990, Farmer 2000).

Currently, there is no data on the acid tolerance of American eel elvers that can be applied to assess the impact of low pH hydrogen ion activity on elver mortality, and whether survival is likely to vary among river drainages of varying acidity.

This thesis addresses these questions through the following studies:

1) A controlled laboratory experiment challenging elvers and young river resident eel to a range of water pH levels representative of those found among Nova Scotia rivers. 2) A field study examining elver survival within Nova Scotia rivers. 3) An examination of the relationship between elver pigmentation (an indicator of development stage) and acid tolerance. 4) The determination of possible sub-lethal effects of acidification by examining hematological parameters associated with acid stress in river resident eels.

As acidification varies widely among Maritime rivers, should low pH be found to increase mortality, the relative effect of elver fisheries on eel mortality could vary among rivers based on their pH level. This information could be used by managers to both minimize the effect of the fishery on overall mortality and increase conservation efforts. Elver fisheries would be most justified in low pH rivers with high natural mortality rates. The effect of the fishery on overall mortality would be lower as a high percentage of elvers removed by the fishery would likely die later on as a result of low pH. Likewise conservation efforts could include collecting elvers from areas of low pH and stocking them in environments with higher productivity, increasing the chance of survival.

Within a fishing season and over the course of an elver run, there is temporal variability in both the development and condition of the elvers. As the run progresses elvers decrease in both length and weight, whereas pigmentation increases (Haro and Krueger 1988, Jessop 1998a, Sullivan *et al.* 2006). The increased pigmentation and the

smaller length and weight of later arriving older elvers are likely a result of metamorphosis occurring earlier, resulting in a reduced migration speed and later estuarial arrival (Wuenschel and Able 2008). Pigmentation can therefore be used as a proxy for development as higher pigmented elvers are generally older upon estuarial arrival (Powles and Warlen 2002).

By examining the acid tolerance of elvers arriving at different times during the period of upstream migration, it would be possible to assess differential mortality over the course of an elver run. Among fish, the juvenile stages of development are shown to be the most susceptible to the effects of low pH (Rask 1983, McCormick and Leino 1999). If there is differential mortality based on pigmentation and development then this information could also be used to reduce the relative impact of fishing mortality by limiting fishing to the period when acid-related mortality is highest.

Thus, the aim of this study is not only to acquire information on the acid tolerance of elvers in order to develop an objective basis to assess potential eel production in Maritime rivers but also to determine any among-river or temporal variability on the impact of fishing mortality relative to potential production. By attempting to define and quantify the effect of acidification on mortality this study has the potential to contribute directly to the conservation and management strategy of the American eel.

Chapter 2: The Effect of Acidification on the Status of American Eel within Maritime Rivers

2.1 The Acidification of Nova Scotia's Southern Upland Rivers

Nova Scotia is an ideal location to examine the tolerance of American eel to environmental acidification because of the wide variation in freshwater pH among rivers and the absence of aluminum toxicity (Lacroix and Kan 1986, Watt 1987) (Figure 1). Acidification varies throughout the province as watersheds differ in their acid neutralizing capacity (ANC) (Howell and Brooksbank 1987, Clair *et al.* 2007). The quantity of acid-neutralizing carbonates present in watershed bedrock and soils determines the buffering capacity of the watershed and the extent to which acid deposition acidifies surface waters (Shilts 1981, Clair *et al.* 2007). In southwestern Nova Scotia, watershed bedrock is either igneous (granite) or metamorphic (quartzite and slate) in origin and soils are generally thin (Shilts 1981). These watersheds are deficient in carbonates and hence are vulnerable to acidification (Gorham 1957, Hayes and Anthony 1958, Shilts 1981). It is in these regions where pH can reach levels as low as 4.0 (Kerekes *et al.* 1982).

Acidification of Nova Scotia surface waters occurs as a result of both anthropogenic and natural factors (Kerekes *et al.* 1982, Gorham *et al.* 1986, Kerekes *et al.* 1986, Ginn *et al.* 2007, Ginn *et al.* 2007b). Weather and atmospheric circulation patterns carry industrial pollutants such as sulfur and nitrogen dioxide from highly industrialized areas (Eastern United States, Southern Ontario) towards Atlantic Canada (Shaw 1979). These pollutants react with atmospheric moisture to produce sulfuric and nitric acids which are deposited as rain or other types of precipitation. Despite a

significant reduction in emissions over the past 20 years the surface water pH of Nova Scotia lakes and rivers remains low (Watt *et al.* 2000, Clair *et al.* 2002, Jefferies *et al.* 2003).

In addition, many watersheds in southwestern Nova Scotia contain high levels of dissolved organic carbon (DOC) as a result of the large amounts of organic matter (peat mosses, bogs) that are present in the drainage basin. This leads to coloured surface waters high in natural organic (fluvic and humic) acids (Gorham 1957, Hayes and Anthony 1958, Kerekes *et al.* 1982, Kerekes *et al.* 1984, Gorham *et al.* 1998). These organic acids contribute significantly to the high acidity of surface waters of Nova Scotia (Oliver 1983, Gorham *et al.* 1986, Kerekes *et al.* 1986, Gorham *et al.* 1998, Ginn *et al.* 2007, Ginn *et al.* 2007b). Water that drains from high DOC basins have pH values approximately one unit lower than those of well-drained DOC-free catchments (Kerekes *et al.* 1986).

In order to properly assess the mechanisms and extent to which hydrogen ion activity (low pH) may specifically affect American eel, it is important to first understand the physiological pathway by which low pH results in the mortality of freshwater teleosts. The discovery of the first acidified aquatic ecosystems due to anthropogenic factors (Cogbill and Likens 1974, Galloway *et al.* 1976, Summers and Whelpdale 1976, Gorham 1976) and the resulting fish mortalities (Beamish and Harvey 1972, Beamish 1974, Leivestad and Muniz 1976, Beamish 1976, Magnuson *et al.* 1984) stimulated effort into environmental and ecological studies concerning the effects of low pH on aquatic ecosystems (Farmer *et al.* 1980, Fromm 1980, Spry *et al.* 1981, Brown 1982, Leivestad 1982). The observed fish mortalities and significant losses of Atlantic salmon in rivers of southwest Nova Scotia have largely been attributed to a failure of the salmon's ability to

ionoregulate as a direct result of increased hydrogen ion activity (low pH) (Lacroix 1985, Lacroix and Townsend 1987, Lacroix *et al.* 1990, Farmer 2000).

2.2 Freshwater Ion Regulation in Teleost Fish and the Physiological Stress Caused by Acidified Water

In fresh water, fish are hypertonic to their environment, and are constantly faced with an osmotic influx of water and diffusive loss of ions across the gills. In order to maintain ionic balance and body fluid homeostasis, fish must actively recover ions from the water to replace those that are passively lost. This is accomplished by both a decrease in the relative permeability of the gill to ion loss and the development of elaborate mechanisms of active ion uptake at the fish gill. Mitochondria-rich cells (MRCs) located on the epithelium of the gill filament are the primary sites of ion uptake in fish (Perry 1997, Marshall and Grosell 2006). These cells actively uptake Na^+ and Cl^- ions in exchange for internal H^+ and HCO_3^- in order to maintain electroneutrality (Krogh 1937, Maetz and Garcia Romeu 1964, Kerstetter *et al.* 1970) (Figure 2). Na^+ and Cl^- uptake occur independently of one another through two morphologically similar types of mitochondria-rich cells that specialize in either Na^+ or Cl^- uptake (Goss *et al.* 2001, Galvez *et al.* 2002).

In acidified water, high concentrations of H^+ ions (pH 4-6) reduce both the active uptake of Na^+ and Cl^- into cells and also increase the passive efflux of ions out of cells (McWilliams 1980, McDonald and Wood 1981, McWilliams 1982, Wood and McDonald 1982, McDonald 1983, Wood 1989). This negatively affects the ability of the fish to maintain and regulate their internal ion concentration.

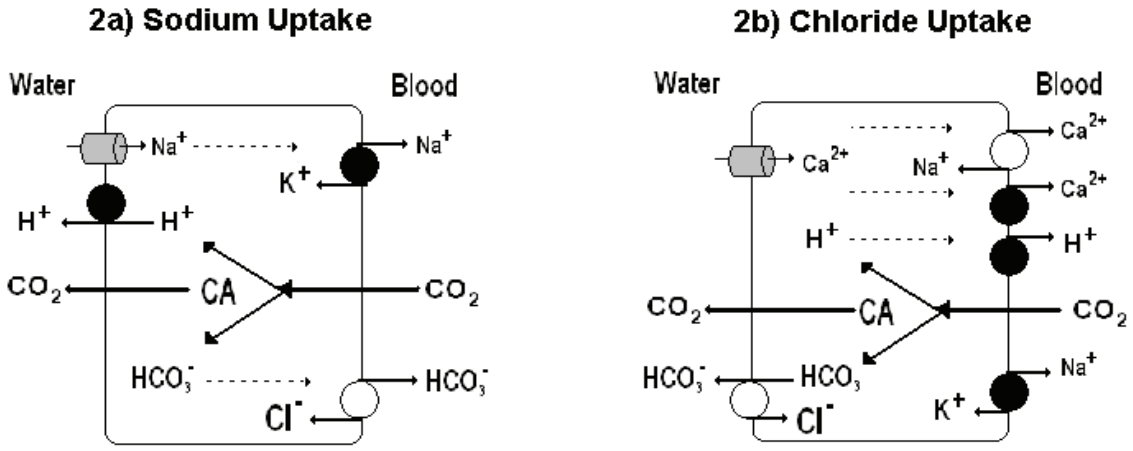

Figure 2: Schematic diagram showing the proposed mechanism of ion uptake across the gills of freshwater teleosts. Active transport mechanisms are represented by filled circles, passive exchangers driven by ionic gradients by unfilled circles and ion channels by shaded cylinders. (Adapted from Perry *et al.* 2003)

2.2.1 Acidification and Na^+ Uptake

The most widely accepted model of Na^+ uptake in freshwater fish is that of an apical vascular-type H^+-ATPase (proton pump) electrically linked with an epithelial Na^+ channel (Lin and Randall 1995, Marshall 2002, Evans *et al.* 2005, Hwang and Lee 2007, Evans 2008) (Figure 2a). Functional evidence was first observed by Avella and Bornancin (1989) and Lin and Randall (1991), who demonstrated apical vascular-type H^+-ATPase activity in the gill epithelium of rainbow trout *(Oncorhynchus mykiss)*. It is proposed that MR cells located on the gill epithelium of freshwater fish actively extrude H^+ thereby creating a negative electrochemical potential gradient (in the order of -100 mV) (Marshall and Grosell 2006). External sodium is thus driven down this gradient entering the cell through an epithelial sodium channel in the apical membrane. This membrane potential is strong enough to drive the uptake of sodium under extremely low environmental concentrations (Na^+ <1 mM) (Marshall and Grosell 2006). Na^+ then exits into the blood on the basolateral side of the cell via a Na^+/K^+ ATP-ase pump (Perry *et al.* 2003, Evans *et al.* 2005) and/or an Na^+/HCO_3^- co-transporter (Parks *et al.* 2007).This model of Na^+ uptake is supported by evidence for the existence of a vascular-type H^+-ATPase and epithelial sodium channels in fish gills (Lin *et al.* 1994, Sullivan *et al.* 1995, Wilson *et al.* 2000, Perry *et al.* 2003, Lin *et al.* 2006, Horng *et al.* 2007). Additionally, results of pharmacological studies show that bafilomycin (selective inhibitor of vascular H^+-ATPase) and phenamil (specific Na^+ epithelial channel inhibitor) significantly reduced Na^+ uptake in both tilapia and carp (Fenwick *et al.* 1999), zebrafish (Boisen *et al.* 2003, Esaki *et al.* 2007) and rainbow trout (Lin and Randall 1993, Bury and Wood 1999, Grosell and Wood 2002, Reid *et al.* 2003, Parks *et al.* 2007).

Given this proposed model of ion uptake, under acidic conditions, the increase of H^+ ions inhibits Na^+ uptake through either direct competition with Na^+ for access to the epithelial transport channels or by an indirect effect caused by an increased resistance against the vascular-type H^+-ATPase to pump H^+ out of the cell, resulting in the loss of the outward proton gradient that is used to drive inward sodium flux (Wood 1989; Lin and Randall 1991, 1995; Wood 2001; Marshall and Grosell 2006).

2.2.2 Acidification and Cl^- Uptake

The mechanism behind base extrusion and Cl^- uptake in the fish gill is less well understood. There is evidence for the presence of an apical Cl^-/HCO_3^- exchanger (Sullivan *et al.* 1996, Wilson *et al.* 2000); however the mechanism of function is still under debate (Evans *et al.* 2005, Tresguerres *et al.* 2006, Hwang and Lee 2007). Due to a strong electrochemical resistance (-60 mV) against anion uptake the ability for this to be an electroneutral exchange of the two is not likely (Marshall and Grosell 2006). It has been proposed that Cl^- uptake is fueled by actively pumping H^+ from the cell to the blood via a proton-ATPase located on the basolateral side of the cell, resulting in HCO_3^- accumulation inside the cell as a result of cellular CO_2 hydration via carbonic anhydrase (Marshall and Grosell 2006, Tresguerres *et al.* 2006) (Figure 2b). This increase of intracellular HCO_3^- would then drive and allow for the apical exchange of HCO_3^-/Cl^- (Marshall and Grosell 2006). Additionally, it has been suggested than H^+ extrusion may effectively titrate and remove HCO_3^- at the gill surface (Marshall 2002). This removal of external HCO_3^- would aid HCO_3^- extrusion and favor Cl^- uptake. Cl^- would then exit the cell into the blood via CFTR anion channels on the basolateral membrane (Marshall 2002, Marshall and Grosell 2006, Tresguerres *et al.* 2006).

The mechanism by which the increase in environmental H^+ ions affects Cl^- uptake has yet to be satisfactorily explained. It has been proposed that a depletion of intercellular HCO_3^- occurs as a result of HCO_3^- being used to buffer against the increased internal acidosis via the bicarbonate buffer system (Wood 2001, Marshall and Grosell 2006). This would result in a lower amount of HCO_3^- available to act as a co-transporter in exchange for external Cl^-, thus inhibiting Cl^- uptake. Therefore under conditions of low environmental pH both Na^+ and Cl^- uptake are significantly reduced and or inhibited as the fish is unable to replenish its ions from environmental sources.

2.2.3 Passive Ion Efflux

Equally important to ion uptake in freshwater fish is the ability to limit diffusive loss by reducing the rate of ion depletion. Much of the gill is comprised of pavement cells which form a continuous epithelial sheet of the lamellae and interlamellar regions and are joined to each other and to mitochondria-rich cells by tight intercellular junctions (Wood *et al.* 2002, Evans 2005 *et al.*, Marshall and Grosell 2006). With fresh water on the outside, these cells act as a passive barrier to internal ion loss due to their high electrical resistances and low permeability (Wood and Part 1997, Wood *et al.* 2002).

While inhibiting active uptake of needed ions, acidic waters also affect the ability of a fish to regulate its internal ion control by disrupting paracellular tight junctions and stimulating diffusive efflux (Gonzalez and Dunson 1989, Freda *et al.* 1991). It is well documented that tight junctions are major barriers to the movement of water and ions through the paracellular pathway and are a dominant feature regulating the permeability of many epithelial tissues (Wood *et al.* 2002, Marshall and Grosell 2006). Low ion permeability of the tight junction is dependent on calcium binding to the membrane

bound junctional proteins (Hunn 1985, Madara 1988). By forming cross-links with ligands on the surface and in the intercellular cement, calcium acts to increase membrane stability and reduce the permeability of the paracellular pathways. At low pH, H^+ ions disrupt the paracellular tight junctions between cells by leaching and displacing calcium from the junctional proteins (McDonald 1983, McDonald *et al.* 1989). This greatly increases the permeability of these junctions leading to a profound increase in outward ion diffusion (ion efflux) from the fish.

2.2.4 Lethal Effects

The main etiology behind the cause of death from acidic water and low environmental pH is the ionic dilution of blood plasma that results from the aforementioned disturbance in ionoregulation, leading to hematological disturbances and ultimately circulatory failure (Milligan and Wood 1982, Lacroix 1985, Wood 1989, Lacroix *et al.* 1990, Farmer 2000). Under normal conditions plasma ionic concentrations and body cell ionic concentrations are in equilibrium. Under acute acid stress, ions are lost more rapidly from the blood plasma than from blood and muscle cells, resulting in the osmotic flux of water from the plasma into the cells. As a consequence, red blood cells swell and plasma volume is reduced. This results in increased hematocrit and a rise in blood viscosity. Increased resistance and elevated arterial pressure results in the heart being unable to circulate the thicker blood at a rate sufficient to supply oxygen, leading to death from circulatory collapse. When blood plasma sodium or chloride levels fall more than 30% below normal, death can occur within hours (Milligan and Wood 1982, Wood 1989).

Most of the aforementioned research and conclusions are based on studies using salmonids as model species. Currently, there is no data on the acid tolerance of American eel elvers that can be applied to assess the impact of low pH on elver mortality. As acidification has been identified as a possible threat to the status of the species (Jessop 2000, COSEWIC 2006), the subject warrants an in depth study.

2.3 Methods

2.3.1 Tolerance of Elvers to Artificially Acidified Water

American eel elvers (N~2,000, length 5-7cm) were dip netted from the mouth of the Mushamush River (Halifax County, Nova Scotia) (Figure 3) during the evening tide on May 23rd 2007. Elvers were transported in holding tanks to the Aquatron Laboratory facilities at Dalhousie University. Aeration was supplied with an aquarium pump set with air stones to maintain oxygen levels during transport. Immediately upon arrival 1500 elvers were distributed evenly among 15 experimental cylindrical tanks (100 per tank) (66cm x 41cm, width x depth). The remaining elvers (n=500) were placed in a holding tank and not used during the experiment. Aquatron water was continuously supplied via a flow-through system of five header tanks (56 cm x 30 cm x 36 cm). Each header tank was connected to three experimental tanks that varied in their location within the room. Therefore the setup was able to examine five separate experimental conditions in triplicate (three tanks each).Water was supplied from the header tanks to the experimental tanks at a rate of 1L/min. Each experimental tank was configured to maintain a constant volume of 25 L.

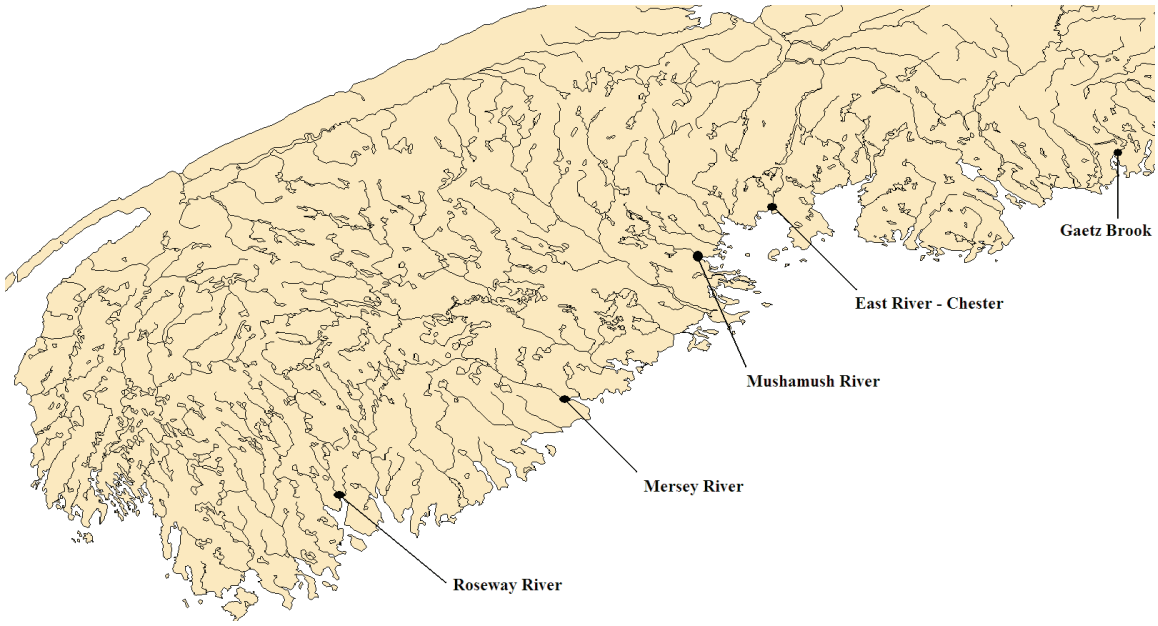

Figure 3: Map of southwest Nova Scotia displaying the location of the elver collection sites for the laboratory trials (East River, Mushamush, Gaetz Brook), the location of the rivers used in the field trials (East, Mushamush, Roseway) and the source of adult yellow eels for the hematological trial (Mersey).

The water of each individual header tank can be set to a desired pH value (Figure 4). For the purpose of these experiments the values chosen were neutral 7.0-7.2, 5.5, 5.0, 4.5 and 4.0. This range of pH values corresponds to the range of ambient pH levels experienced by eels within Nova Scotia rivers (Watt 1987). The acidity of the water in the header tanks was lowered by pumping a 0.01M solution of sulfuric acid H_2SO_4 into the header tanks. pH was continuously measured by probes placed in each header tank.

The probe was connected to an electrochemical controller that was set to the desired treatment level. When the pH of the header tank was above the set treatment level, the controller signaled the dosing pump to move acid from the concentrated acid tank to the header tank. Once the desired treatment pH level was reached in the header tank the controller sent another signal to the dosing pump to stop pumping acid. The water in the header tank was constantly mixed and aerated by a diffusing ring. Water of the desired pH then flowed from the header tank into the three connected experimental tanks.

The initial trial was conducted between May 25th 2007 and June 7th 2007. pH was gradually lowered to the treatment levels over the first 72-hours. This 72-hour duration was chosen as it mimics the natural time-course of pH experienced by individuals moving between habitats (estuary to fresh water) or during freshets and/or spring snowmelt. Once the desired treatment levels were reached, mortality was recorded daily for each tank over a 10 day period and expressed as cumulative percent mortality from day zero (i.e. day 1 =10 mortalities = 10%, day 2 =10 mortalities = 20%). pH readings were taken daily from each experimental tank throughout the trial by an independent pH

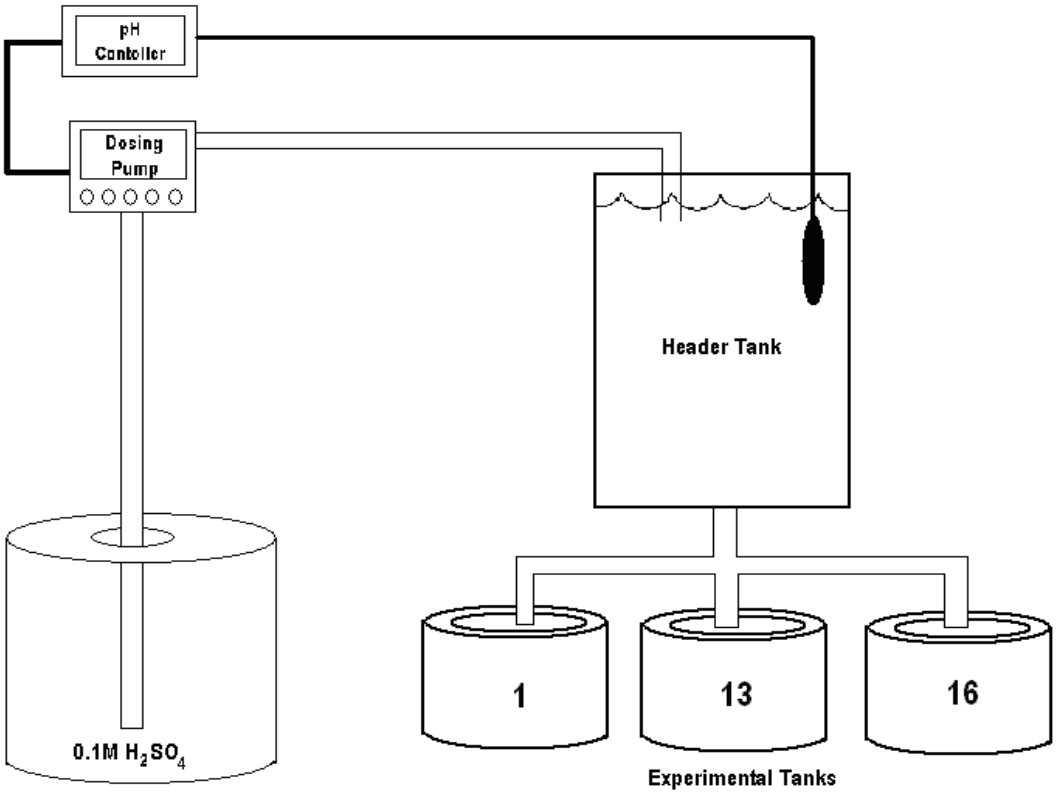

Figure 4: Schematic diagram representing the method by with pH is controlled in laboratory trials.

probe to confirm the set treatment levels. An Analysis of Variance (ANOVA) with repeated measures was conducted to test for significant differences in the mortality curves between the fixed pH treatments by examining both the treatment effect and the interaction between treatment and time.

This protocol was repeated for a sample of 1,500 elvers collected from the East River- Chester (Halifax County, Nova Scotia) (Figure 3) on June 10th 2007 and examined between June 12th 2007 and June 25th 2007. A third trial was conducted using a sample of 120 juvenile eels (8-14 cm) collected from Gaetz Brook (Halifax County, Nova Scotia) (Figure 3) on July 12th 2007. Thirty eels were used per treatment level (neutral, 5.0, 4.5, 4.0) and examined between July 17th 2007 and July 26th 2007.

2.3.2 Elver Survival in Response to Naturally Occurring pH

Three rivers were chosen based on their pH at the time of study: Roseway River, Shelburne County, Nova Scotia (pH 4.2), East River-Chester, Halifax County, Nova Scotia (pH 4.7) and Mushamush River, Lunenburg County, Nova Scotia (pH 7.0) (Figure 3). In order to monitor and assess mortality within the river, elvers were placed in fixed pens and held over a 14 day time period. Elver pens were constructed out of plastic aquaria (30.5 cm x 17.8 cm x 20.3 cm). Circular holes (7.5 cm diameter) were drilled into the sides of the pen to allow for water flow and the pens were lined with screening to prevent elver escape. Each pen was affixed to a plywood base (150cm x 60cm) and anchored to the riverbed using natural rocks. The sites within each river were located within 5km from the estuary. All elvers used in the trials were collected from the estuary of the East River-Chester on May 18th, 2008. One hundred elvers were placed in each of

three pens within each river on May 19th, 2008. Elvers were counted and mortalities recorded every 2-3 days over the 14 trial period. pH of the river was monitored using a Metrohm pH meter. An ANOVA with repeated measures was used to test for significant differences in mortality between rivers by examining both the river (pH) effect and the interaction between river pH and time.

2.3.3 The Effect of Elver Pigmentation (Development) on pH Tolerance

Prior to the laboratory trials (Section 2.3.1) a sample of 50 elvers were randomly selected to assess pigmentation. Pigmentation was assessed using digital image analysis, with images taken by an Epson Perfection 1670 scanner. The elvers were euthanized in an immersion bath with an overdose of MS-222. Each individual elver was then laid out on the scanning deck laterally in the same orientation. Digital images were produced using a resolution of 1200 dpi. Using the digital image analysis program Imagetool (UTHSCSA), an area was selected for each elver consisting of a straight line from the gill arch to the posterior end along the lateral line and back to the gill arch along the dorsal line (Neave *et al.* 2006). The histogram function in Imagetool creates a mean grey value profile for the area selected. Each pixel is assigned a number value on a scale of 0 to 256 based on its intensity (light = 256 dark =0). The program is able to generate a histogram profile based on the intensities and assigns an overall mean value for the selected area. This value is a direct representation of the pigmentation of the elver (Figure 5). In order to ease graphical interpretation mean values were multiplied by -1 so that a value of -256 would indicate the complete absence of any pigmentation. This process was repeated for elvers from the second and third trials (Section 2.3.1) using the same image parameters

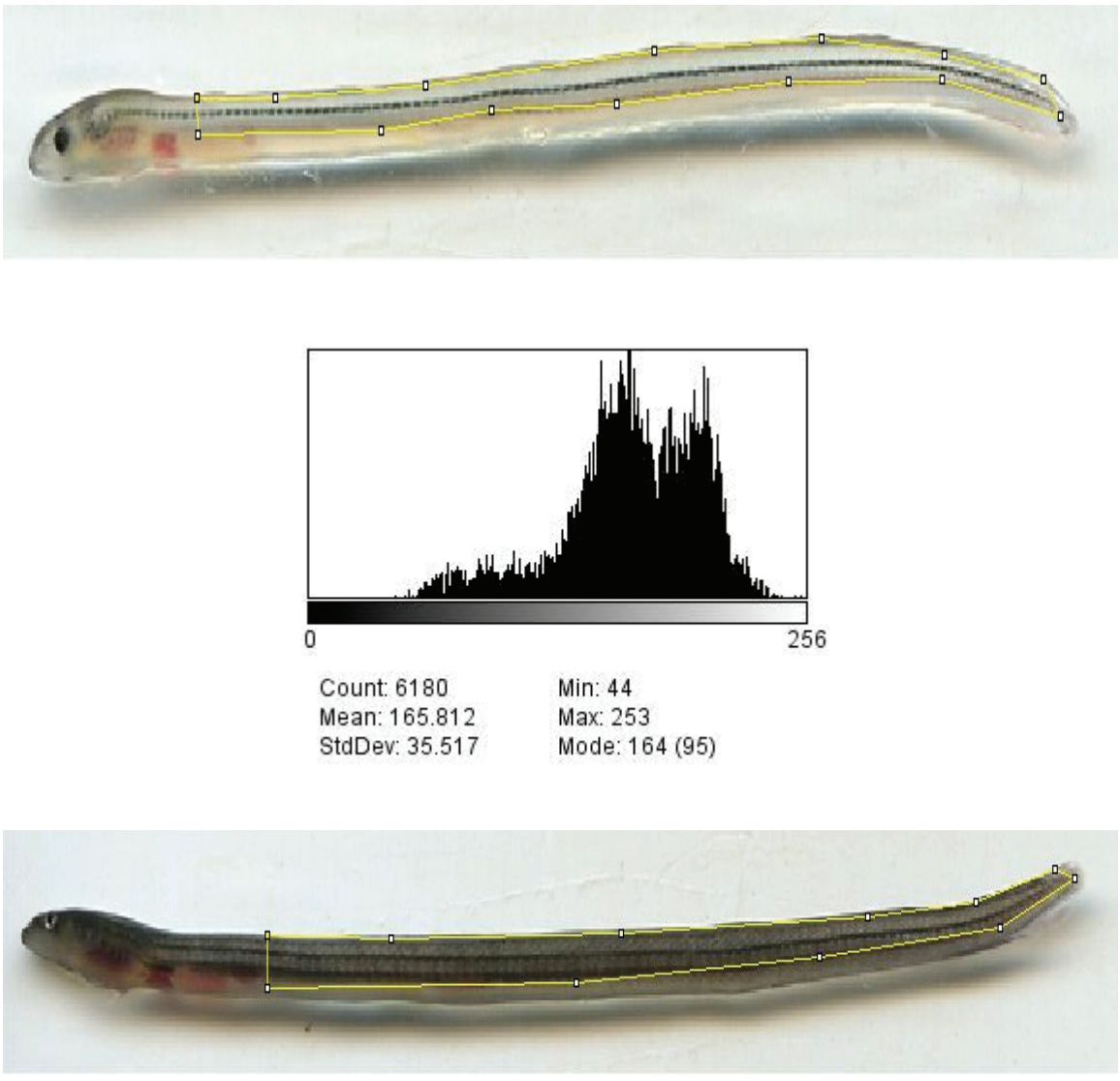

Figure 5: Area selected for digital image analysis and resulting mean pigmentation value for an individual elver from Trial 1 (upper) and Trial 3 (lower).

and protocol. An ANOVA was conducted to test for significant differences in pigmentation between the three different experimental trials (runs). A repeated measures ANOVA was used on the data collected from section 2.3.1 to test for significant differences in the mortality curves between fixed elver runs at a given pH.

2.3.4 The Physiological Effect of Acidified Water on Eels

Yellow eels (N~100) were collected from the Mersey River, Queens County, Nova Scotia (Figure 3) during November 2007. They were transported to the Dalhousie Aquatron and kept in a holding tank at neutral pH. Thirty adult eels were implanted with Passive Integrated Transponder (PIT) tags allowing them to be individually monitored throughout the course of this study. In April of 2008 the 30 tagged eels were moved to the pH laboratory in the Aquatron and distributed evenly among six tanks. Three treatment levels were examined: neutral pH (7.0-7.2), 5.5 and 4.5. Two tanks were used per treatment resulting in 10 eels per treatment. In order to establish a baseline for the experiment, hematocrit and blood plasma osmolarity readings were taken prior to lowering the pH. Each eel was sedated in an immersion bath of MS-222 and approx. 0.5 mL of whole blood was removed using a lithium heparinized syringe. A sample of the blood was drawn into a microhematocrit capillary tube (Fisher Scientific) sealed with critoseal (Oxford Labware) and spun using a microhematocrit centrifuge (Clay Adams Readacrit). Percent hematocrit readings were obtained by dividing the length of the red blood cell portion by the length of the entire sample. In order to determine blood plasma osmolarity the remainder of the whole blood sample was centrifuged at 130 rpm for 5 minutes (Hettich Instruments) and the blood plasma was drawn. An osmomoter was used to determine the osmolarity of a 50 μL sample of plasma.

After a 24 hour recovery period the water was lowered to the desired pH over the next 72 hours. Hematocrit and plasma osmolarity was measured on each eel 2, 5, 10 and 20 days post-exposure. Post-exposure values were subtracted from pre-trial values to give an index of change, and these values were used for statistical analysis.

The effects of pH on eel hematocrit and osmolarity levels were analyzed separately using linear mixed effects models in the statistical package R (R Development Core Team 2009). Linear mixed effects analysis uses the observed data to create a linear model that examines the fixed effect of pH in determining the dependent variable while accounting for the random effects associated with sampling individual eels over time. In total, three models were examined for each variable (hematocrit and osmolarity):

Model 1. Without intercept and without slope varying by treatment (pH has no effect on the variable)

$$Y_{ipt} = \alpha + a_i + (\beta + b_i)t + \varepsilon_{ipt}$$

i = individual eel, p = pH treatment, t = time, α = y-intercept, a_i = random effect on intercept by eel, β = slope, b_i = random effect on slope by eel, ε = error term

Model 2. With intercept varying by treatment, without slope varying by treatment (pH is significant in determining the variable, however there is no significant interaction between pH and time effecting the variable)

$$Y_{ipt} = \alpha + \Delta\alpha_{pH} + a_i + (\beta + b_i)t + \varepsilon_{ipt}$$

α = y-intercept at neutral pH, $\Delta\alpha_{pH}$ = change in intercept by pH treatment

Model 3. Intercept and slope varying by treatment pH (There is a significant interaction between pH and time on the variable, pH and time are both significant in determining the variable)

$$Y_{ipt} = \alpha + \Delta\alpha_{pH} + a_i + (\beta + \Delta\beta_{pH} + b_i)t + \varepsilon_{ipt}$$

β = slope at neutral pH, $\Delta\beta_{pH}$ = change in slope by pH treatment

Akaike's information criterion (AIC) was used to identify the model that best fit the data. An ANOVA was conducted between the models and a chi square test was used to determine any significant difference between the models in explaining the data.

2.4 Results

2.4.1 Tolerance of Elvers to Artificially Acidified Water

pH levels were maintained at the desired set treatment level throughout each laboratory trial. There was zero elver mortality across all the pH treatments tested during the 10 day period in each of the experimental trials (Table 1), therefore pH did not affect mortality under experimental conditions. There were no differences in the mortality curves between pH treatments as there was no variation in the dependent variable (mortality).

2.4.2 Elver Survival in Response to Naturally Occurring pH

During the time period of the experiment trial (May 19th, 2008 – June 2nd 2008) the pH of the rivers was as follows: Roseway 4.2, East River-Chester 4.7, Mushamush 7.0. There was zero elver mortality across all the rivers tested during the field trial (Table 2), therefore pH did not affect mortality under naturally occurring environmental

Table 1: Percent survival of elvers (N = 1500 per trial, 100 per tank) exposed to artificially acidified water.

Trial 1: May 25th 2007 - June 7th 2007															
pH	4.0			4.5			5.0			5.5			Neutral		
Tank	4	5	10	2	6	9	3	8	15	7	11	12	1	13	16
% Survival 10 days post exposure	100%			100%			100%			100%			100%		
Trial 2: June 12th 2007 - June 25th 2007															
pH	4.0			4.5			5.0			5.5			Neutral		
Tank	4	5	10	2	6	9	3	8	15	7	11	12	1	13	16
% Survival 10 days post exposure	100%			100%			100%			100%			100%		
Trial 3: July 17th 2007 - July 26th 2007															
pH	4.0			4.5			5.0			Neutral					
Tank	4	5	10	2	6	9	3	8	15	1	13	16			
% Survival 10 days post exposure	100%			100%			100%			100%					

Table 2: Percent survival of elvers (N=900, 300 per river, 100 per box) observed in Nova Scotia rivers of varying pH.

Field Trial: May 19th 2008 – June 2nd 2008									
River	Roseway (pH 4.2)			East – Chester (pH 4.7)			Mushamush (pH 7.0)		
Box	1	2	3	1	2	3	1	2	3
% Survival 10 days post exposure	100%			100%			100%		

conditions over the duration of the experiment. There were no differences in the mortality curves between rivers as there was no variation in the dependent variable (mortality).

2.4.3 The Effect of Elver Pigmentation (Development) on pH Tolerance

Pigmentation was significantly different among elver runs and increased during the season (Figures 6 and 7). As there was zero mortality within each of the three trials tested in Section 2.1, there was no difference in the mortality curves of separate elver runs at a specific pH. Pigmentation and development was not a factor in determining pH tolerance as the eels exhibited 100% survival at all stages of development.

2.4.4 The Physiological Effect of Acidified Water on Eels

Hematocrit and osmolarity values decreased during treatments (Figures 8 and 9), however they remained within the range of normal eel heamtocrit levels (Kreutzmann and Nasev 1979, Gill and Epple 1993, Acierno *et al.* 1997, van Ginneken *et al.* 2005, Caruso *et al.* 2010). Based on the AIC results of the tested statistical models (Table 3), the second model would appear to best explain both the hematocrit and osmolarity data. However, even though the AIC was slightly lower for these models than the others, there was no statistically significant difference between any of the models tested (Table 3). Therefore, in both cases the null hypothesis (Model 1) cannot be rejected (Figure 10, Figure 11). The more complex models that incorporate a pH effect did not significantly add to the explanation of the data. pH had no significant effect in determining the predicted hematocrit and/or osmolarity value at a given time post-exposure.

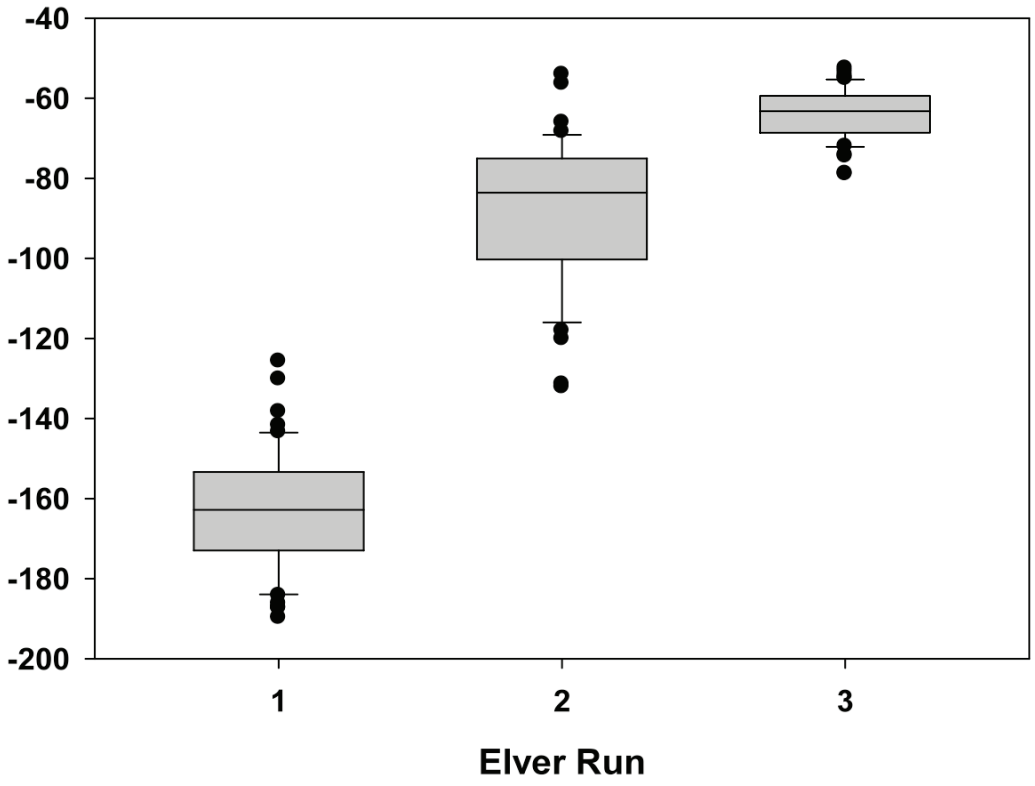

Figure 6: Box and whisker plot representing pigmentation values for elvers of varying runs. An index of zero represents full pigmentation. The line within the box represents the median with the box representing data within the 25th to 75th percentile. Whiskers represent the 10th and 90th percentile. Outliers are represented by circles.

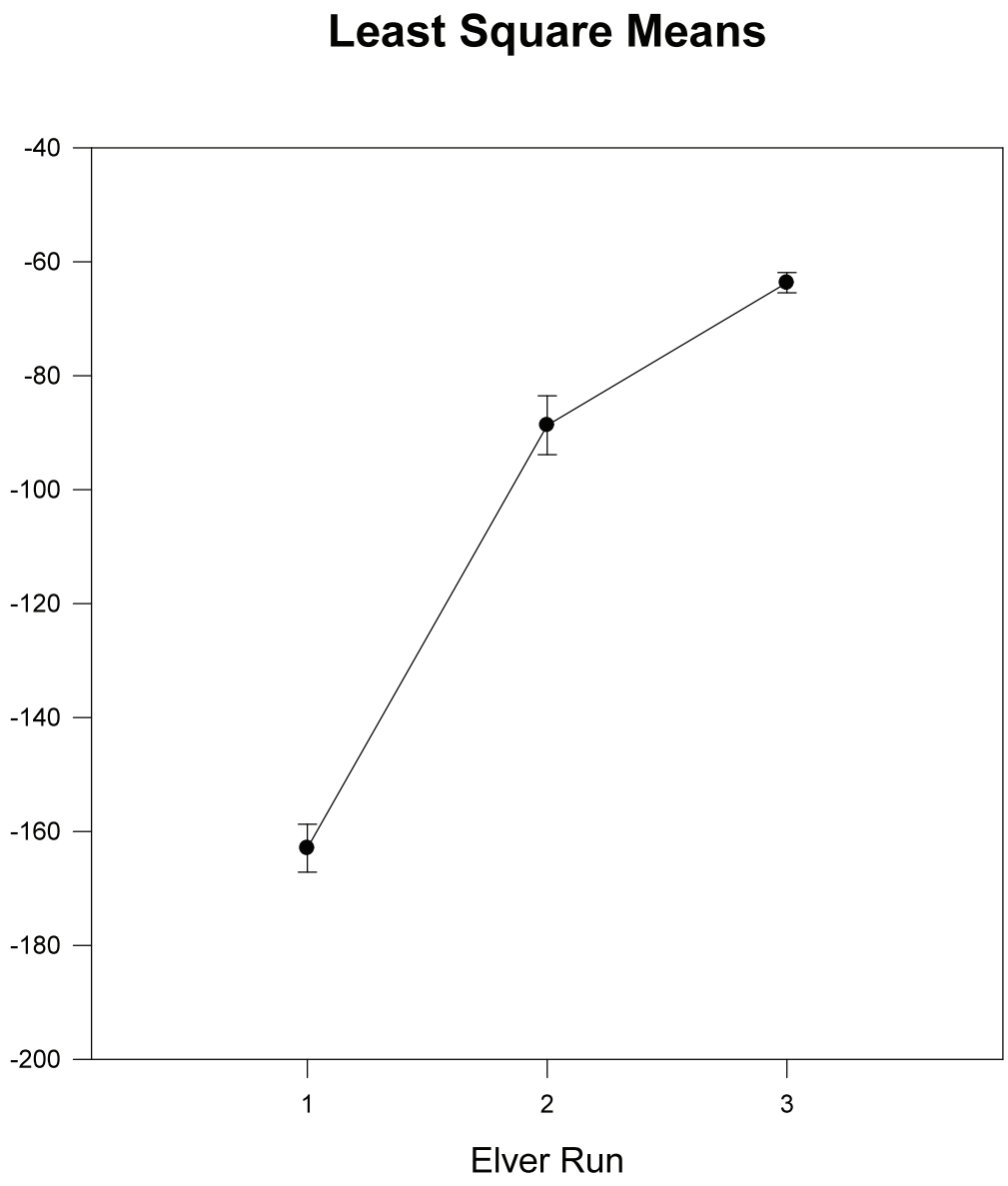

Figure 7: Results of the single factor ANOVA examining the pigmentation index of elver runs. (F-ratio 700.041; df 2,145; $p<0.001$).

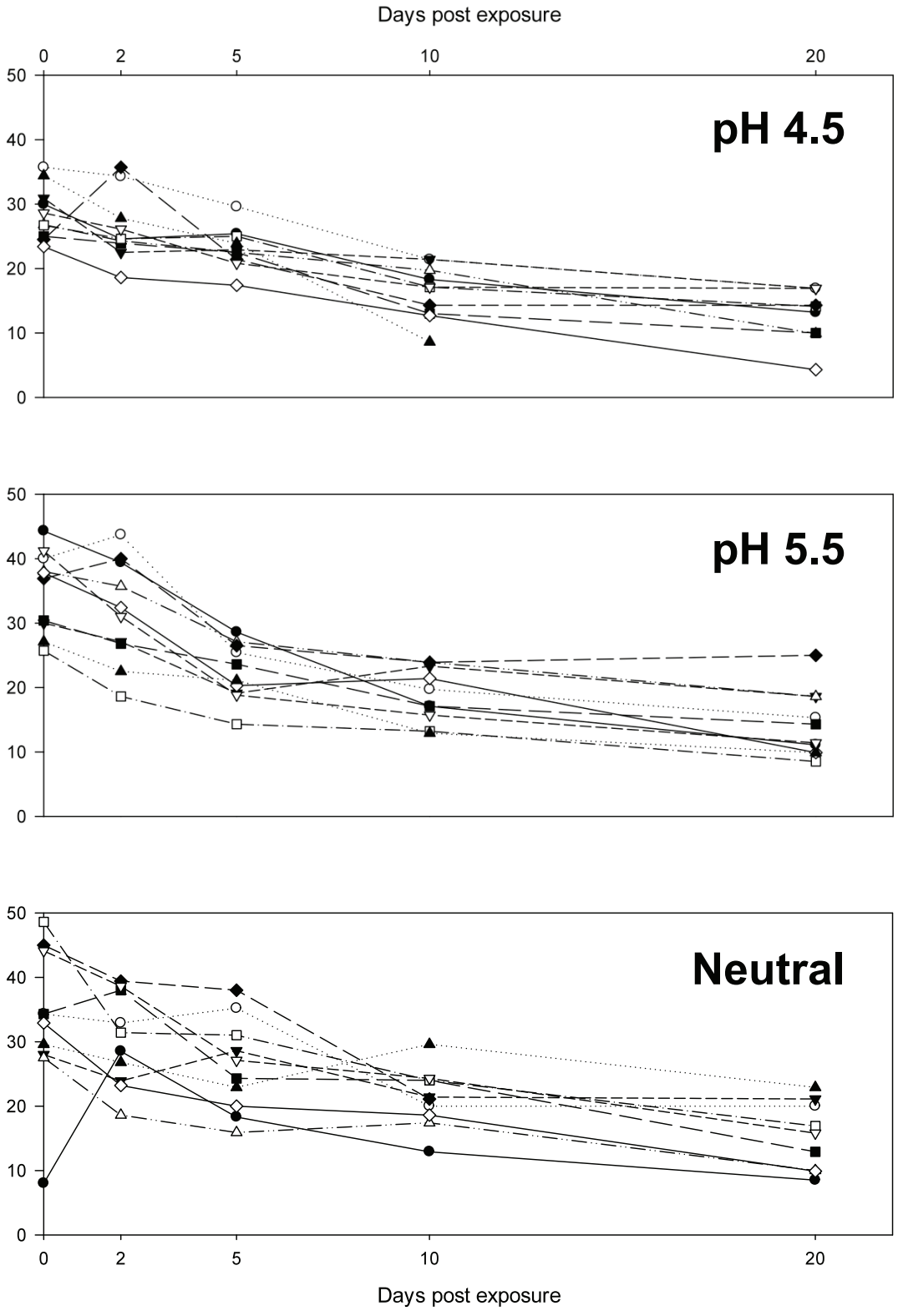

Figure 8: Hematocrit values of American eels after exposure to varying levels of acidity. Each line represents an individual eel within a specific pH treatment.

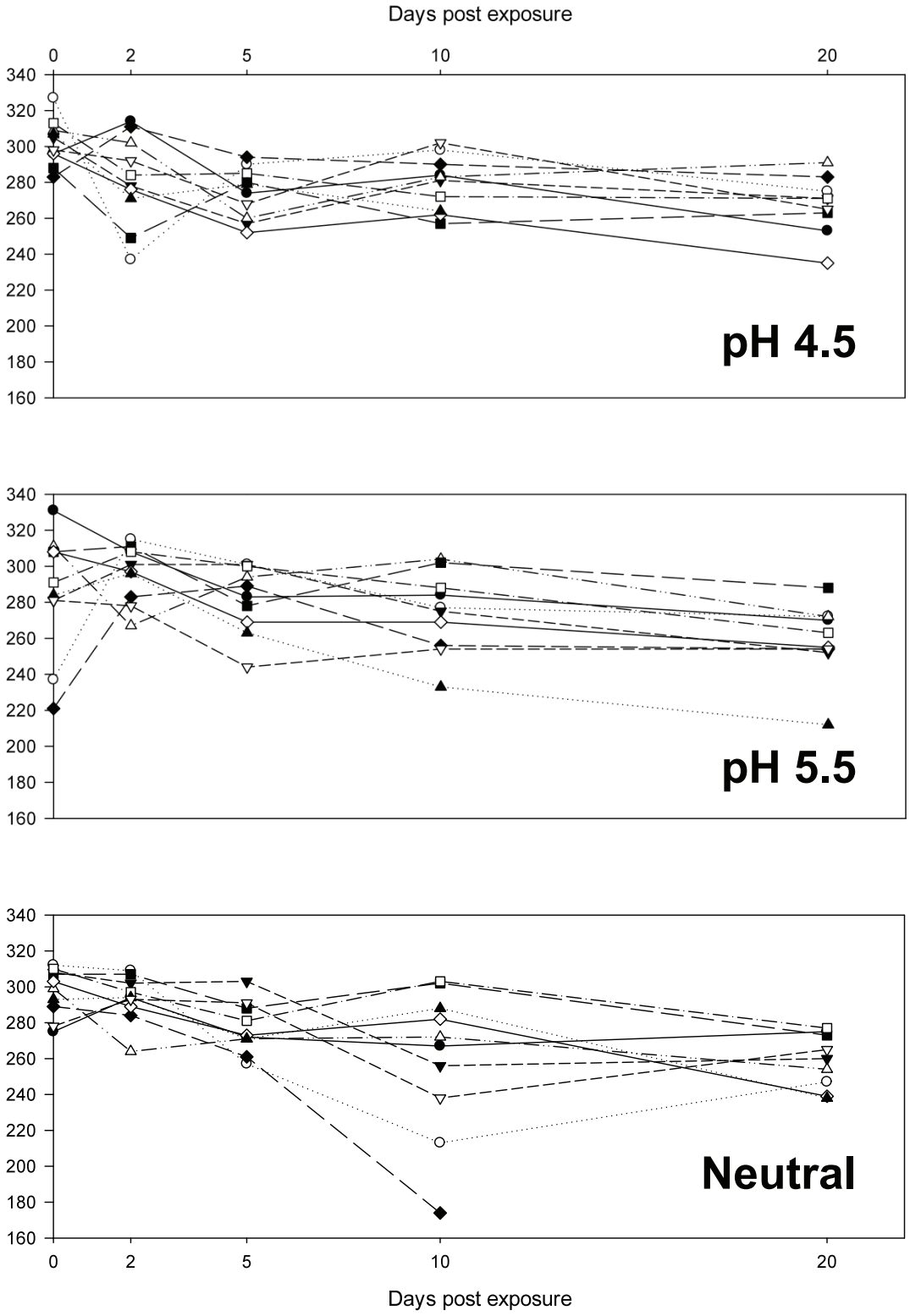

Figure 9: Osmolarity values of American eels after exposure to varying levels of acidity. Each line represents an individual eel within a specific pH treatment.

Table 3: Summary statistics for mixed effects linear model analysis on the physiological effect of acidified water on eels

Akaike information criterion (AIC)		
	Hematocrit	Osmolarity
Model 1	753.6722	1103.297
Model 2	748.2005	1090.388
Model 3	755.8605	1091.638
ANOVA and chi-square test for significance between the models		
	Hematocrit	Osmolarity
Model 1 vs. Model 2	$p = 0.3144$	$p = 0.1347$
Model 1 vs. Model 3	$p = 0.6011$	$p = 0.2394$

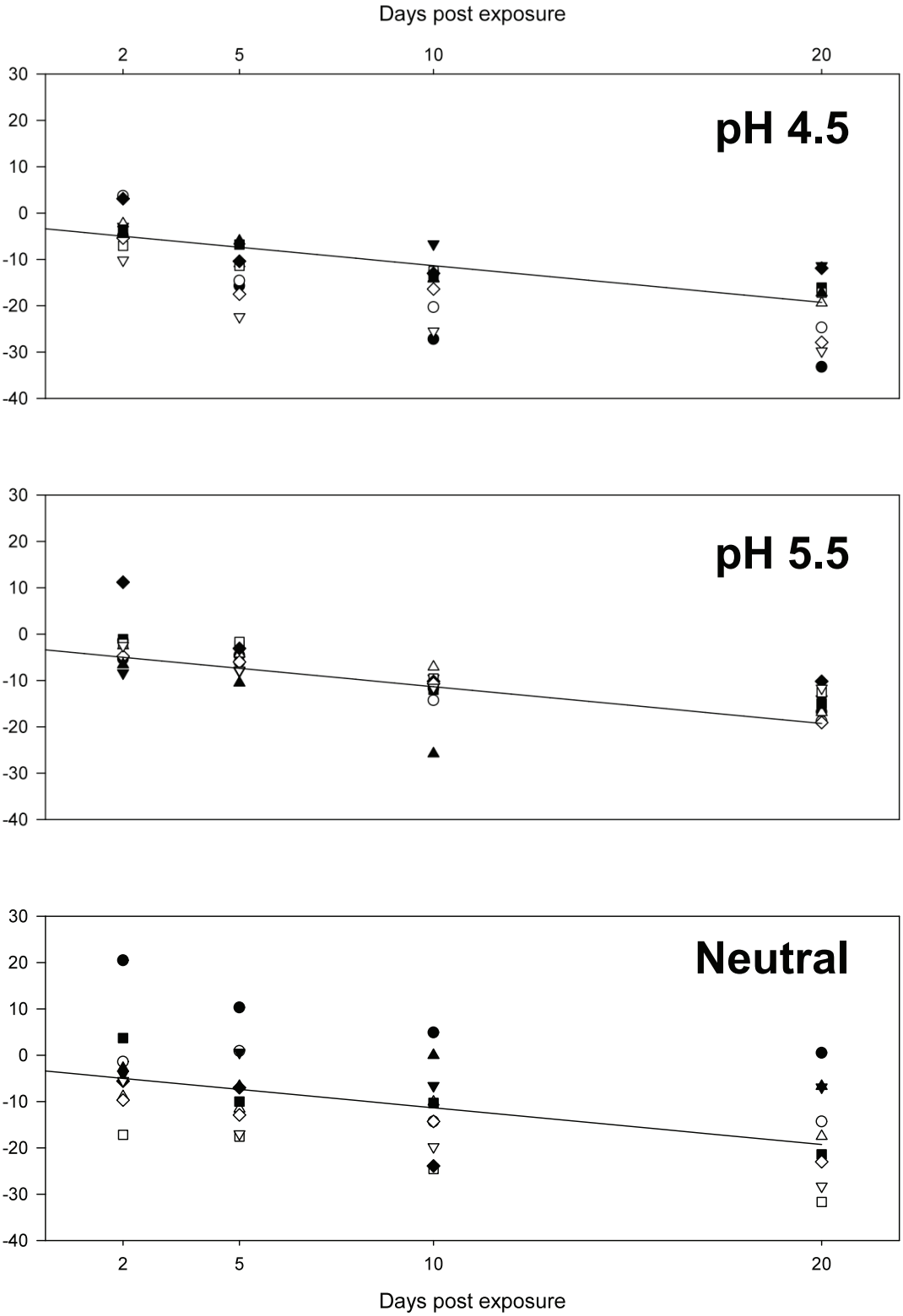

Figure 10: Deviation from pre-exposure hematocrit levels for individual eels (represented by symbols) following exposure to artificially acidified water. The linear equation represents the predicated hematocrit at a given time as expressed by Model 1 of the mixed effects analysis.

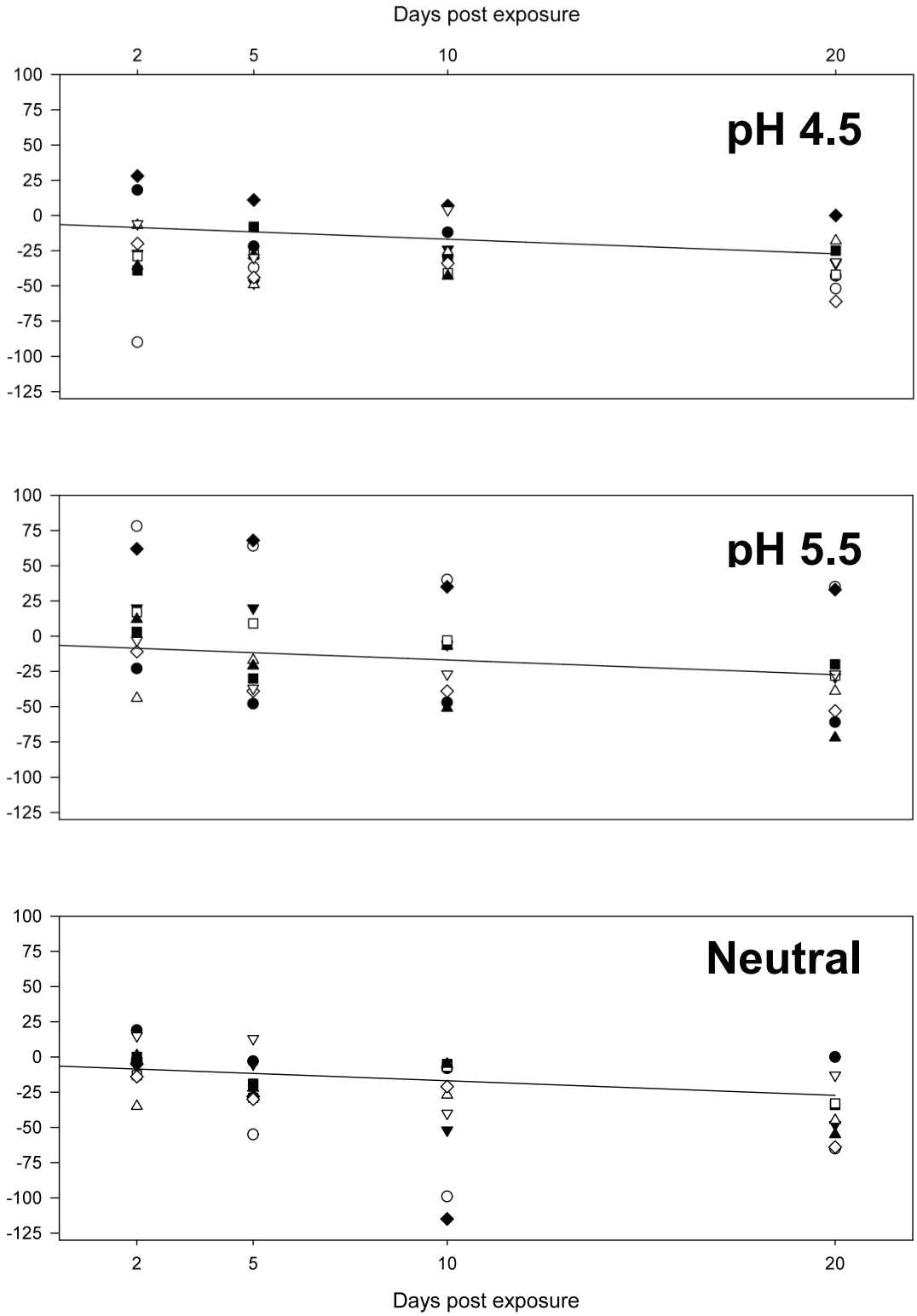

Figure 11: Deviation from pre-exposure osmolarity levels for individual eels (represented by symbols) following exposure to artificially acidified water. The linear equation represents the predicated osmolarity at a given time as expressed by Model 1 of the mixed effects analysis.

2.5 Discussion - Adaptation to Low pH: The American Eel, an Acid Tolerant Species

My results show that American eel elvers at all stages of development are capable of surviving in acidic water with a pH as low as 4.0 for periods of at least ten days. Moreover, statistical analyses of blood hematocrit and plasma osmolarity revealed no evidence of adverse physiological effects of exposure to low pH. Although observed hematocrit and osmolarity levels decreased and fluctuated under control and experimental conditions, observed values remained within the range of normal eel heamtocrit levels. (Johansson-Sjobeck *et al.* 1975, Gill and Epple 1993, Acierno *et al.* 1997, van Ginneken *et al.* 2005, Caruso *et al.* 2010). Conversely, studies examining the effect of acidification on Atlantic salmon reported significant differences between experimental and control groups with heamatocrit values 50% above normal in fish exposed to low pH (Lacroix 1985, Lacroix and Townsend 1987).

Given that juvenile stages of development are the most susceptible to the effects of low pH (Rask 1983, McCormick and Leino 1999) and that the examination of river-resident eels showed no significant difference in their hematological variables between acidic and control conditions, it can be concluded that the American eel is an acid tolerant species. This suggests that the American eel can maintain its ion balance under acidic conditions. The question remains as to the mechanism by which the eel is able to adapt and withstand the physiological stress caused by low pH water. Further study into the influx and efflux patterns of ions under acidic conditions would help elucidate the mechanism of ion transport in eels and determine the magnitude of effect pH may have on ion regulation. However, using the current knowledge of eel physiology and the

mechanisms of acid adaptation in other teleost species, a strong hypothesis can be formulated as to the rationale behind the eel's acid tolerance.

Fish that inhabit acidic waters need to develop a specific strategy in order to survive and maintain ion regulation. Acid tolerance rises from both an ability to limit the increase in membrane permeability caused by low pH and an ability of the ion transport mechanism to resist or to recover from low pH inhibition. As death is likely to occur when approximately 30% of body Na^+ is lost (Milligan and Wood 1982), the magnitude of ion loss is the major determinant of survival time during exposure to water of low pH (Wood 1989). The variation in acid tolerance among species can be described by the pH threshold at which large ion losses occurs. Thus, the ability to restrict the effect of low pH on membrane permeability is crucial to survival. Previous studies of North American fishes have found a strong inverse correlation between the magnitude of stimulation of Na^+ efflux at a given pH and overall tolerance of low pH (Gonzalez and Dunson 1987, 1989; Freda and McDonald 1988).

In particular, the banded sunfish (*Enneacanthus obesus*) exhibits a superior tolerance to low pH. It is capable of surviving direct transfer to pH 3.5 and reproducing populations have been found in pH 3.7 (Graham and Hastings 1984). The banded sunfish uses an ionoregulatory strategy focused on low branchial permeability and resistance of the efflux stimulation caused by low pH. This resistance has been attributed to a very high branchial affinity for Ca^{2+}. A high branchial affinity of tight junctions for Ca^{2+} would act to maintain their integrity in the face of elevated H^+ levels in the surrounding water. Gonzalez and Dunson (1989) estimated that at pH 3.25 a Ca^{2+} concentration of only 19 μmol/L was sufficient to reduce efflux by 50% relative to measurements in Ca^{2+}

free water. Estimates of the necessary calcium concentrations to reduce efflux by 50% in rainbow trout are 20-35 times higher at even higher pH levels (Freda and MacDonald 1988). As a result of their low branchial permeability, at neutral pH sunfish lose Na^+ at a rate less than 50 nmol g^{-1} h^{-1}, approximately one tenth the loss rate of rainbow trout and common shiners (*Notropis cornutus*) (Gonzalez and Dunson 1987, Freda and MacDonald 1988). Thus, the sunfish is less reliant on ion uptake compared to other species. As influx is completely inhibited at pH 4.0 (Gonzalez and Dunson 1989), the ionoregulatory pattern displayed by sunfish of low intrinsic permeability reflects the importance of preventing efflux stimulation at low pH. These characteristics suggest a strategy of adapting to low pH by preventing ion loss.

2.5.1 Physiological Gill Structure: A Morphological Basis for Acid Tolerance

A study by McDonald *et al.* (1991) examining the gill morphology of common shiner, rainbow trout, smallmouth bass *(Micropterus dolomieu)*, yellow perch (*Perca flavescens)* and banded sunfish found a morphological basis for pH tolerance as species with lower gill MRC densities proved to be more acid tolerant. In addition, ion transport activity is also related to MRC density as decreasing density accompanied decreasing transport activity (Perry and Gross 1992). Histological evidence has shown that eels possess an extremely low MRC fractional area on the filament epithelium (11,328 $\mu m^2/mm^2$) when compared to other species (tilapia (*Oreochromis mossambicus)* 85,194 $\mu m^2/mm^2$, rainbow trout 146,333 $\mu m^2/mm^2$) (Perry and Gross 1992, Goss and Perry 1994, Perry 1997).

The basic principle of maintaining ionic homeostasis is that ion uptake must be equal to that of ion loss. With this in mind, it could be theorized that the gill density of

MRCs reflects the intrinsic ion permeability of the epithelium. Species that show low rates of ion efflux due to their low epithelial permeability would thus have a lower MRC density and low rate of uptake. Conversely, species that have a high epithelial permeability would need additional proliferation of MRCs to compensate their need for an increased ion uptake capability. Therefore the low gill MRC density that exists in eels is indicative of a low membrane permeability and superior ability to maintain membrane integrity when subjected to low pH conditions.

In the freshwater environment, ions are constantly being lost to the environment via the paracellular pathway between gill epithelial cells (McDonald *et al.* 1989). It is well established that paracellular ion permeability is determined by the tight junction, with the depth of the junction and the permeability of the channel being roughly correlated (Freda *et al.* 1991, Marshall and Grosell 2006). Three types of paracellular junctions exist on the gill epithelium: pavement cell-pavement cell, MRC-pavement cell and MRC-MRC. As the pavement cell-pavement cell junction is the most numerous, it is likely the main paracellular leak pathway. As stated previously, under conditions of low pH the tight junctions between cells are disrupted and ion efflux is stimulated. McDonald *et al.* (1991) reported that pavement cell-pavement cell paracellular tight junction depth is 1.6 times greater in yellow perch than in rainbow trout. Given that perch are able to maintain ion control at a lower pH than trout, this indicates that an increasing depth of tight junctions between adjacent gill-pavement cells corresponds to increasing acid tolerance. Freda *et al.* (1991) also found that in rainbow trout the length of tight junctions between pavement cells and MR cells decreased by 25% after 1 hour of exposure to pH 4.0; however, there were no significant differences in pavement cell -pavement cell

junctions. This decrease in the length of MRC-pavement cell tight junctions would likely increase the permeability of the branchial epithelium to ions via this specific paracellular pathway. Therefore it could be proposed that a greater density of MRCs results in an increasing number of pavement cell-MRC junctions and a higher susceptibility to ion loss via this pathway under low pH conditions. Although the depth of paracellular tight junctions in eels in unknown, the low density of MRCs on the gill would provide an advantage in maintaining membrane integrity and resisting the increased ion efflux associated with low pH. Thus, the physiological structure of the eel gill would be beneficial in increasing the eel's ability to tolerate low pH conditions.

2.5.2 Ion Uptake and the Role of Diet

If an individual can maintain membrane stability and control ion efflux, survival is then dependent upon continued active ion uptake. Tolerance to low pH is enhanced when there is an increased availability of sodium in the freshwater environment, often caused by aerial deposition (Shuter and Ihssen 1991).

Freda and McDonald (1988) observed a correlation between pH tolerance and Na^+ transport kinetics. The most acid tolerant species proved to have the highest transport affinity for Na^+ uptake. Due to competition between H^+ and Na^+ for the uptake transport sites, species with high affinity mechanisms would be best adapted to continue ion uptake under low pH.

In the waters of the Rio Negro, a tributary of the Amazon River, fish encounter some of the most dilute, naturally acidic waters on earth. The pH of the Rio Negro is around 5.5 with Na^+, Cl^- and Ca^{2+} levels generally less than 50 μmol/L, however, forest streams routinely exhibit pHs as much as 2 pH units lower and ion levels only 20% of

those in the main river (Gonzalez *et al.* 2006). Many fish species enter these forest streams to reproduce, and many naturally migrate between circumneutral and low-pH environments. Studies have shown that neon (*Paracheirodon innesi)* and cardinal (*Paracheirodon axelrodi)* tetras are able to survive prolonged exposure to pH 3.5 as they experience only a small transitory stimulation of ion loss, and ion uptake is uninhibited (Gonzalez and Preest 1999, Gonzalez and Wilson 2001). In neon tetras, uptake continues at pH 3.25, the lowest pH at which active uptake has been observed (Gonzalez and Preest 1999). However, at pH 3.25 a fourfold greater stimulation of Na^+ loss occurs and fish die within 6-8 hours, illustrating how the ability to inhibit ion loss is the primary limiting factor in mortality. Kinetic analysis of ion uptake mechanisms in these species showed a high transport capacity with neon tetras having the highest Na^+ affinity ever recorded (Gonzalez and Preest 1999). Ion regulation and survival at low pH in these species is characterized by their high-affinity, high capacity ion transport systems. These differences in gill ion permeability at low pH, as well as differences in Na^+ transport kinetics provide the physiological basis for interspecific differences in tolerance to low pH.

The transport kinetics of ion uptake in the American eel has yet to be examined. However, the closely related European eel (*Anguilla anguilla*) has a body Na^+ turnover rate of only 1.1% per 24 hours under control conditions (neutral pH, [Na^+] 50μM, [Cl^-] 10μM, [Ca^{2+}] 10μM, [K^+] 10μM, [Mg^{2+}] <1μM) compared to 19% for rainbow trout and higher for other salmonids (Grosell *et al.* 2000). This means that the internal Na^+ pool is depleted more rapidly in rainbow trout than in European eel. As mortality is likely to occur when the plasma Na^+ concentration is reduced by 30% (Milligan and Wood 1982),

assuming a total inhibition of sodium uptake as a result of low pH, mortality would occur within 38 hours for the rainbow trout compared to 655 hours (27.3 days) for the European eel.

Perhaps even more significant is that the American eel lacks any appreciable Cl^- uptake (Hyde and Perry 1987, 1989). Cl^- uptake is less than 1% of the values reported for trout under resting conditions (Goss *et al.* 1995). From this, one can conclude that the eel is much less reliant on the active uptake of ions from the environment compared to other species due to their high resistance to ion loss.

The American eel is an opportunistic scavenger feeding on a wide variety of fishes and invertebrates. As such, food sources are often abundant. The diet of the American eel may play a key role in the eel's apparent acid tolerance. Dietary salt can play a crucial role in maintaining internal ion levels as it is able to replace and compensate for the branchial ion loss that occurs as a result of low pH exposure (Smith *et al.* 1989, D'Cruz and Wood 1998). Laboratory studies have found an increased food intake of acid exposed fish (Dockray *et al.* 1996, D'Cruz *et al.* 1998), and it is theorized that this increased appetite is stimulated by the internal loss of Na^+ that occurs in a low pH environment (Salman and Eddy 1987). When fish are fed adequately in conditions of low pH, ionoregulatory disturbances are much reduced or do not occur at all. (Kwain *et al.* 1984, Sadler and Lynam 1986, Wilson *et al.* 1994, Dockray *et al.* 1996, D'Cruz *et al.* 1998). High salt diets have also been shown to increase chloride cell number and Na^+/K^+ ATPase, resulting in enhanced ionic uptake (Salman and Eddy 1987). Thus, dietary salt has the potential benefits of both replacing branchial ion loss and stimulating branchial uptake. It is therefore plausible that species with high dietary salt intake have less need

for strong active ion transport capabilities. Although one can not pinpoint the exact salt content of the American eel's dietary intake in the wild, one can speculate that an opportunistic carnivorous scavenger such as the American eel may be able to compensate the deleterious effects of low pH on ion uptake through the increased intake of dietary salt.

2.5.3 Facultative Catadromy as an Adaptation to Environmental Challenges

It is unlikely that there is a single unifying mechanism common to freshwater fishes for dealing with the challenges of life in low pH environments. A close examination of freshwater fishes from diverse habitats around the world would likely yield a range of novel and diverse osmoregulatory strategies specifically adapted for each habitat (Patrick and Wood 1999, Hirata *et al.* 2003, Katoh *et al.* 2003).

Eels of the genus *Anguilla* are thought to be derived from a marine ancestor that completed its migration loop within the ocean (Tsukamoto *et al.* 2002). It has been theorized that the ancestral Anguillid eel was a tropical marine species with a migration loop extending close to coastal waters. This likely resulted in the ancestral eel developing an adaptive behaviour of regularly migrating into fresh water as a result of a possible cline in food abundance and/or to avoid both inter and intra species competition, resulting in increased productivity and a reproductive advantage (Gross *et al.* 1988, Edeline 2007).

Recent studies have shown that catadromy is not obligate for eels of the Genus *Anguilla* (American – *A. rostrata*, European – *A. anguilla* and Japanese – *A. japonica*) as some individuals may only migrate into fresh water for short periods and/or complete their entire their life-cycle in the marine environment (Tsukamoto *et al.* 1998, Daverat *et al.* 2006, Lamson *et al.* 2006, Jessop *et al.* 2008). For this reason, catadromy is viewed as

a facultative life history option. These different life cycle options are likely to be differentially vulnerable to exploitation, habitat degradation, and climate change (Secor 1999, 2007, Edeline 2007). Where environmental conditions fluctulate unpredictably, natural selection favours risk-spreading behaviours (Orr, 2007). Catadromy in American eel may be an adaptive strategy to make the population more resilient to selective agents (Cairns *et al.* 2009).

As dispersal of leptocephali and migration of elvers into fresh water is random and occurs over a large geographical area, eels encounter a wide variety of environments and an individual eel must be able to tolerate the specific conditions of the environment it encounters in order to survive. The freshwater migrating eel would need to be resilient and adaptable to a wide variety of environmental conditions. By minimizing the passive efflux of ions at the gill and through the dietary uptake of ions, the eel would be more adept to maintain ion balance and able to tolerate a wide range of pH's and salinities.

Chapter 3: Conclusion

The aim of this research was to acquire information on the acid tolerance of American eel in order to develop an objective basis to assess potential eel production in Maritime rivers and among-river variability in elver fishing mortality relative to potential production. My results show that the American eel is physiologically able to withstand and survive in water with a pH as low as 4.0.

As ion imbalance is a main cause of fish mortality in low pH water these results suggest that the eel is able to maintain ion regulation under conditions of low pH. Although the exact mechanism of ion regulation in the American eel is unknown, evidence suggests that the eel's inherent tolerance to low pH is primarily a result of its ability to restrict ion loss. As a result of this apparent tolerance, the pH of a particular watershed should not be used as a proxy for determining levels of eel mortality. Low pH should not be viewed as contributing to the direct mortality of elvers entering a river. As low pH is not a direct cause of elver mortality it would not dampen the effect of elver fishing mortality in low pH rivers as each elver recruited to the fishery can be viewed as a potential spawner regardless of the pH of the watershed being fished.

It should be noted that this study only examined the physiological impact low pH has on American eel survival. Conditions of low pH may reduce the general aquatic productivity of a river resulting in the possibility of acid impacts on eel populations via trophic effects and the alteration of food supply. If low pH reduces general aquatic productivity, then there may be less for eels to eat, and their growth and possibly numbers may be reduced. However, it is also possible that low pH may be advantageous for eels. As low pH is known to negatively affect other fish species (salmonids), it could

reduce the amount of inter-species competition resulting in a higher level of invertebrate food abundance for eels.

Migrations between freshwater and estuarine/marine environments have been documented for numerous Atlantic Canadian eel stocks, including the East River – Chester (Jessop *et al.* 2002, 2006, 2008). It has been suggested that these inter-habitat migration patterns are influenced by environmental conditions, productivity and inter and intra specific competition (Daverat *et al.* 2006, Edeline 2007, McCleave and Edeline 2009). As a result, the American eel may be able to mitigate any decline in aquatic productivity and food abundance caused by low pH by migrating to the estuarine environment.

While both the marine and freshwater contingents are important to the conservation of the species, most of the anthropogenic challenges faced by the American eel occur primarily in freshwater habitat (COSEWIC 2006). Further losses of freshwater eels may have serious demographic effects as freshwater eels typically have higher fecundity and sexually mature at a larger size than marine resident eels (McCleave and Edeline 2009). Low pH has been shown to affect fecundity in other species (Heibo and Vollestad 2002, Vuorinen *et al.* 2004) and a decrease in reproductive output as a result of low pH could lower the potential productivity of the watershed.

From this research, I conclude that low environmental pH should not be viewed as a factor which directly threatens the continued survival of the American eel. Due to the facultative nature of catadromy in eels and given the fact that eels are physiologically tolerant of acidified water, acidity should not be viewed as a primary factor related to mortality, thus having little to no impact on overall abundance. Studies such as this are

important in order to increase our knowledge and understanding of the causes of mortality and to identify and focus on significant sources of possible population decline. Although current data does not allow for a determination of a decline in eel abundance in the Maritime region, the significant decline in the St. Lawrence region may be an early warning sign of an overall species decline. The goal of the current American eel management plan is to reduce eel mortality from all sources by 50%. Without knowing the causes or sources of the decline and concern it is almost impossible to create a management plan that can effectively conserve and protect a species.

The American eel is an important part of Canadian biodiversity benefiting Canadians both on an economic and cultural basis. It is an economically important species for those who fish it and is inextricably linked to Mi`kmaq culture. As the causes of the possible decline for American eel remain to be fully elucidated, continued research must be conducted in order to ensure the health of the species.

REFERENCES

Acierno R, Maffia M, Rollo M, Storelli C, (1997) Buffer capacity in the blood of the hemoglobinless Antarctic fish *Chionodraco hamatus*. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology,* 118(4):989-992.

Antunes C, Tesch FW, (1997) A critical consideration of the metamorphosis zone when identifying daily rings in otoliths of European eel, *Anguilla anguilla* (L). *Ecology of Freshwater Fish, 6*(2):102-107.

Arai T, Otake T, Tsukamoto K, (2000) Timing of metamorphosis and larval segregation of the Atlantic eels *Anguilla rostrata* and *A-anguilla*, as revealed by otolith microstructure and microchemistry. *Marine Biology,* 137(1):39-45.

ASF (2010) Issues – Acid rain. *Atlantic Salmon Federation* http://www.asf.ca/issues.php?id=1

Avella M, Bornancin M, (1989) A new analysis of ammonia and sodium-transport through the gills of the fresh-water rainbow-trout (*Salmo gairdneri*). *Journal of Experimental Biology,* 142:155-175.

Avise JC, Helfman GS, Saunders NC, Hales LS, (1986) Mitochondrial DNA differentiation in north Atlantic eels: population genetic consequences of an unusual life history pattern. *Proceedings of the National Academy of Sciences of the United States of America,* 83(12):4350-4354.

Baker JP, Schofield CL, (1982) Aluminum toxicity to fish in acidic waters. *Water Air and Soil Pollution,* 18(1-3):289-309.

Baum JK, Myers RA, Kehler DG, Worm B, Harley SJ, Doherty PA, (2003) Collapse and conservation of shark populations in the northwest Atlantic. *Science,* 299(5605):389-392.

Beamish RJ, (1974) Loss of fish populations from unexploited remote lakes in Ontario, Canada as a consequence of atmospheric fallout of acid. *Water Research,* 8(1):85-95.

Beamish RJ, (1976) Acidification of lakes in Canada by acid precipitation and resulting effects on fishes. *Water Air and Soil Pollution,* 6(2-4):501-514.

Beamish RJ, Harvey HH, (1972) Acidification of la cloche mountain lakes, Ontario, and resulting fish mortalities. *Journal of the Fisheries Research Board of Canada,* 29(8):1131-1143.

Boisen AMZ, Amstrup J, Novak I, Grosell M, (2003) Sodium and chloride transport in zebrafish soft water and hard water acclimated (*Danio rerio*). *Biochimica Et Biophysica Acta-Biomembranes,* 1618(2):207-218.

Bradford RG, Longard DA, Longue P, (2004) Status, trend, and recovery considerations in support of an allowable harm assessment for Atlantic whitefish (*Coregonus huntsmani)*. *Canadian Science Advisory Secretariat Research Document,* 2004/109:38p.

Brown DJA, (1982) The effect of pH and calcium on fish and fisheries. *Water Air and Soil Pollution,* 18(1-3):343-351.

Bury NR, Wood CM, (1999) Mechanism of branchial apical silver uptake by rainbow trout is via the proton-coupled Na^+ channel. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology,* 277(5):1385-1391.

Cairns DK, Secor DA, Morrison WE, Hallett JA, (2009) Salinity-linked growth in anguillid eels and the paradox of temperate-zone catadromy. *Journal of Fish Biology,* 47:2094-2114.

Caruso G, Maricchiolo G, Micale V, Genovese L, Caruso R, Denaro MG, (2010) Physiological responses to starvation in the European eel (*Anguilla anguilla)*: effects on haematological, biochemical, non-specific immune parameters and skin structures. *Fish Physiology and Biochemistry.* 36:71-83.

Cassleman JM, Marcogliese LA, Hodson PV, (1997) Recruitment index for the upper St. Lawrence River and Lake Ontario eel stock: A re-examination of eel passage at the R.H. Saunders hydroelectric generating station at Cornwall, Ontario. In R. E. Johnson (Ed.), *The American eel in eastern Canada: stock status and management strategies.* (pp. 157-164). Canadian Technical Report of Fisheries and Aquatic Sciences 2196.

Castonguay M, Hodson PV, Couillard CM, Eckersley MJ, Dutil JD, Verreault G. (1994) Why is recruitment of the American eel, *Anguilla rostrata*, declining in the St. Lawrence River and gulf. *Canadian Journal of Fisheries and Aquatic Sciences,* 51(2):479-488.

Chapin FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Díaz S, (2000) Consequences of changing biodiversity. *Nature*, 405:234-242.

Clair TA, Dennis IF, Scruton DA, Gilliss M, (2007) Freshwater acidification research in Atlantic Canada: A review of results and predictions for the future. *Environmental Reviews,* 15:153-167

Clair TA, Ehrman JM, Ouellet AJ, Brun G, Lockerbie D, Ro CU, (2002) Changes in freshwater acidification trends in Canada's Atlantic provinces: 1983-1997. *Water Air and Soil Pollution,* 135(1-4):335-354.

Cogbill CV, Likens GE, (1974) Acid precipitation in northeastern United States. *Water Resources Research,* 10(6):1133-1137.

COSEWIC (2006) *COSEWIC assessment and status report on the American eel (Anguilla rostrata) in Canada*. Ottawa: Committee on the Status of Endangered Wildlife in Canada.

Daverat F, Limburg KE, Thibault I, Shiao JC, Dodson JJ, Caron FO, Tzeng WN, Iisuka Y, Wickstrom H, (2006) Phenotypic plasticity of habitat use by three temperate eel species, *Anguilla anguilla*, *A. japonica* and *A. rostrata*. *Marine Ecology-Progress Series,* 308:231-241.

D'Cruz LM, Wood CM, (1998) The influence of dietary salt and energy on the response to low pH in juvenile rainbow trout. *Physiological Zoology,* 71(6):642-657.

DFO (2006) Recovery Strategy for the Atlantic Whitefish (*Coregonus huntsmani*) in Canada. *Species at Risk Act* Recovery Strategy Series. Fisheries and Oceans Canada, Ottawa, xiii + 42 pp.

DFO (2010) Status of American Eel and progress on achieving management goals. *DFO Canada Science Advisory Secretariat Science Advisory Report,* 2010/062.

Dockray JJ, Reid SD, Wood CM, (1996) Effects of elevated summer temperatures and reduced pH on metabolism and growth of juvenile rainbow trout (*Oncorhynchus mykiss)* on unlimited ration. *Canadian Journal of Fisheries and Aquatic Sciences,* 53(12):2752-2763.

Edeline E, (2007) Adaptive phenotypic plasticity of eel diadromy. *Marine Ecology Progress Series,* 341:229-232.

Edeline E, Dufour S, Briand C, Fatin D, Elie P, (2004) Thyroid status is related to migratory behaviour in *Anguilla anguilla* glass eels. *Marine Ecology Progress Series,* 282:261-270.

Edeline E, Dufour S, Elie P, (2005) Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. *Marine Ecology Progress Series,* 304:191-199.

Edeline E, Lambert P, Rigaud C, Elie P, (2006) Effects of body condition and water temperature on *Anguilla anguilla* glass eels migratory behaviour. *Journal of Experimental Marine Biology and Ecology,* 331:217-225.

Esaki M, Hoshijima K, Kobayashi S, Fukuda H, Kawakami K, Hirose S, (2007) Visualization in zebrafish larvae of Na^+ uptake in mitochondria-rich cells whose differentiation is dependent on foxi3a. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology,* 292(1):R470-R480.

Evans DH, (2008) Teleost fish osmoregulation: What have we learned since August Krogh, Homer Smith, and Ancel Keys. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, 295(4):R1359-R1359.

Evans DH, Piermarini PM, Choe KP, (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews,* 85(1):97-177.

Farmer GJ, (2000) Effects of low environmental pH on Atlantic salmon (*Salmo salar* L.) in Nova Scotia. *Canadian Stock Assessment Secretariat Research Document,* (2000/050)

Farmer GJ, Goff TR, Ashfield D, Samat HS, (1980) Some effects of the acidification of Atlantic salmon rivers in Nova Scotia. *Canadian Technical Report of Fisheries and Aquatic Sciences,* 972:13p.

Fenwick JC, Wendelaar Bonga SE, Flik G, (1999) In vivo bafilomycin-sensitive Na^+ uptake in young freshwater fish. *Journal of Experimental Biology,* 202(24):3659-3666.

Fjellheim A, Raddum GG, Sagen T, (1985) Effect of aluminum at low pH on the mortality of elvers (*Anguilla anguilla L.),* a laboratory experiment. *Proceedings International Association of Theoretical and Applied Limnology,* 22:2544-2547.

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

Freda J, Sanchez DA, Bergman HL, (1991) Shortening of branchial tight junctions in acid exposed rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences,* 48(10):2028-2033.

Fromm PO, (1980) Review of some physiological and toxicological responses of fresh-water fish to acid stress. *Environmental Biology of Fishes,* 5(1):79-93.

Galloway JN, Likens GE, Edgerton ES, (1976) Acid precipitation in northeastern United States - pH and acidity. *Science,* 194(4266):722-724.

Galvez F, Reid SD, Hawkings G, Goss GG, (2002) Isolation and characterization of mitochondria-rich cell types from the gill of freshwater rainbow trout. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology,* 282(3): R658-R668.

Gill TS, Epple A, (1993) Stress-related changes in the hematological profile of the American eel (*Anguilla rostrata)*. *Ecotoxicology and Environmental Safety,* 25:227-235.

Ginn BK, Cumming BF, Smol JP, (2007a) Long term lake acidification trends in high and low sulphate deposition regions from Nova Scotia, Canada. *Hydrobiologia,* 586: 261-275

Ginn BK, Cumming BF, Smol JP, (2007b) Assessing pH changes since pre-industrial times in 51 low-alkalinity lakes in Nova Scotia, Canada. *Canadian Journal of Fisheries and Aquatic Sciences,* 64(8):1043-1054.

Gonzalez RJ, Dunson WA, (1987) Adaptations of sodium balance to low pH in a sunfish (*Enneacanthus obesus*) from naturally acidic waters. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology,* 157(5):555-566.

Gonzalez RJ, Dunson WA, (1989) Differences in low pH tolerance among closely related sunfish of the genus *Enneacanthus*. *Environmental Biology of Fishes,* 26(4):303-310.

Gonzalez RJ, Preest MR, (1999) Ionoregulatory specializations for exceptional tolerance of ion-poor, acidic waters in the neon tetra (*Paracheirodon innesi*). *Physiological and Biochemical Zoology,* 72(2):156-163.

Gonzalez RJ, Wilson RW, (2001) Patterns of ion regulation in acidophilic fish native to the ion-poor, acidic Rio Negro. *Journal of Fish Biology,* 58(6):1680-1690.

Gonzalez RJ, Wilson RW, Wood CM, (2006) Ionoregulation in tropical fishes from ion-poor, acidic blackwaters. In A.L. Val, V.F. De Almeida-Val & D.J. Randall (Eds.), *The physiology of tropical fishes* (pp. 397-442). San Diego, CA: Academic Press.

Gorham E, (1957) The chemical composition of lake waters in Halifax county, Nova Scotia. *Limnology and Oceanography,* 2(1):12-21.

Gorham E, (1976) Acid precipitation and its influence upon aquatic ecosystems - overview. *Water Air and Soil Pollution,* 6(2-4):457-481.

Gorham E, Underwood JK, Janssens JA, Freedman B, Maass W, Waller DH, Ogden JG, (1998) The chemistry of streams in southwestern and central Nova Scotia, with particular reference to catchment vegetation and the influence of dissolved organic carbon primarily from wetlands. *Wetlands,* 18(1):115-132.

Gorham E, Underwood JK, Martin FB, Ogden JG, (1986) Natural and anthropogenic causes of lake acidification in Nova Scotia. *Nature,* 324(6096):451-453.

Goss GG, Adamia S, Galvez F, (2001) Peanut lectin binds to a subpopulation of mitochondria-rich cells in the rainbow trout gill epithelium. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology,* 281(5):R1718-R1725.

Goss GG, Perry SF, (1994) Different mechanisms of acid-base regulation in rainbow-trout *(Oncorhynchus mykiss*) and American eel (*Anguilla rostrata*) during $NaHCO_3$ infusion. *Physiological Zoology,* 67(2):381-406.

Goss GG, Perry SF, Laurent P, (1995) Ultrastructural and morphometric studies on ion and acid-base transport processes in freshwater fish. In C.M. Wood, & T.J. Shuttleworth (Eds.), *Cellular and molecular approaches to fish ionic regulation* (pp. 257-284). San Diego, CA: Academic Press.

Graham JH, (1993) Species diversity of fishes in naturally acidic lakes in new-jersey. *Transactions of the American Fisheries Society,* 122(6):1043-1057.

Graham JH, Hastings RW, (1984) Distributional patterns of sunfishes on the New Jersey coastal plain. *Environmental Biology of Fishes,* 10(3):137-148.

Grosell M, Hogstrand C, Wood CM, Hansen HJM, (2000) A nose-to-nose comparison of the physiological effects of exposure to ionic silver versus silver chloride in the European eel (*Anguilla anguilla*) and the rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology,* 48(2-3):327-342.

Grosell M, Wood CM, (2002) Copper uptake across rainbow trout gills: Mechanisms of apical entry. *Journal of Experimental Biology,* 205(8):1179-1188.

Gross MR, (1988) Evolution of diadromy in fishes. In M.J. Dadswell, R.L. Klauda, C.M. Moffitt, R.L. Saunders, R.A. Rulifson, & J.E. Cooper (Eds.) *Common Strategies of Anadromous and Catadromous Fishes.* American Fisheries Society, Symposium 1, Maryland. (pp. 14-25).

Haro AJ, Krueger WH, (1988) Pigmentation, size, and migration of elvers (*Anguilla rostrata*) in a coastal Rhode-island stream. *Canadian Journal of Zoology,* 66(11):2528-2533.

Haro A, Richkus W, Whalen K, Hoar A, Busch WD, Lary S, Brush T, Dixon D, (2000) Population decline of the American eel: Implications for research and management. *Fisheries,* 25(9):7-16.

Hayes FR, Anthony EH, (1958) Lake water and sediment. I. Characteristics and water chemistry of some Canadian east coast lakes. *Limnology and Oceanography,* 3(3):299-307.

Heibo E, Vollestad LA, (2002) Life-history variation in perch (*Perca. fluviatilis* L.) in five neighbouring Norwegian lakes. *Ecology of Freshwater Fish,* 11(4):270-280.

Helfman GS, Facey DE, Hales Jr. LS, Bozeman Jr. EL, (1987) Reproductive ecology of the American eel. In M.J. Dadswell, R.L. Klauda, C.M. Moffitt, R.L. Saunders, R.A. Rulifson, & J.E. Cooper (Eds.) *Common Strategies of Anadromous and*

Catadromous Fishes. American Fisheries Society, Symposium 1, Maryland. (pp. 42-56).

Hirata T, Kaneko T, Ono T, Nakazato T, Furukawa N, Hasegawa S, Wakabayashi S, Shigekawa M, Chang MH, Romero MF, Hirose S, (2003) Mechanism of acid adaptation of a fish living in a pH 3.5 lake. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology,* 284(5):R1199-R1212.

Hooper RP, Shoemaker CA, (1985) Aluminum mobilization in an acidic headwater stream: temporal variation and mineral dissolution disequilibria. *Science,* 229:463-464.

Horng J, Lin L, Huang C, Katoh F, Kaneko T, Hwang P, (2007) Knockdown of V-ATPase subunit A (atp6v1a) impairs acid secretion and ion balance in zebrafish (*Danio rerio*). *American Journal of Physiology-Regulatory Integrative and Comparative Physiology,* 292(5):R2068-R2076.

Howell GD, Brooksbank P, (1987) *An assessment of LRTAP acidification of surface waters in Atlantic Canada*. Ottawa: Environment Canada.

Hunn JB, (1985) Role of calcium in gill function in fresh-water fishes. *Comparative Biochemistry and Physiology A-Physiology,* 82(3):543-547.

Hunter ML, Gibbs JP, (2007) *Fundamentals of conservation biology*, 3rd edition. Blackwell Science. 516p.

Hwang P, Lee T, (2007) New insights into fish ion regulation and mitochondrion-rich cells. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology,* 148(3):479-497.

Hyde DA, Perry SF, (1987) Acid-base and ionic regulation in the American eel (*anguilla rostrata*) during and after prolonged aerial exposure - branchial and renal adjustments. *Journal of Experimental Biology,* 133:429-447.

Hyde DA, Perry SF, (1989) Differential approaches to blood acid-base regulation during exposure to prolonged hypercapnia in 2 fresh-water teleosts - the rainbow trout (*Salmo gairdneri*) and the American eel (*Anguilla rostrata*). *Physiological Zoology,* 62(6):1164-1186.

Jeffries DS, Clair TA, Couture S, Dillon PJ, Dupont J, Keller W, McNicol DK, Turner MA, Vet R, Weeber R, (2003) Assessing the recovery of lakes in southeastern Canada from the effects of acidic deposition. *Ambio,* 32(3):176-182.

Jelks HL, Walsh SJ, Burkhead NM, Conteras-Balderas S, Diaz-Pardo E, Hendrickson DA, Lyons J, Mandrak NE, McCormick F, Nelson JS, Plantania SP, Porter BA, Renaud CB, Schmitter-Soto JJ, Taylor EB, Warren Jr. ML, (2008) Conservation Status of

Imperiled North American Freshwater and Diadromous Fishes. *Fisheries,* 33(8):372-407.

Jessop BM, (1987) Migrating American eels in Nova Scotia. *Transactions of the American Fisheries Society,* 116(2):161-170.

Jessop BM, (1998a) Geographic and seasonal variation in biological characteristics of American eel elvers in the Bay of Fundy area and on the Atlantic coast of Nova Scotia. *Canadian Journal of Zoology,* 76(12):2172-2185.

Jessop BM, (1998b) The management of, and fishery for, American eel elvers in the maritime provinces, Canada. *Bulletin Francais De La Peche Et De La Pisciculture,* (349):103-116.

Jessop BM, (2000) Estimates of population size and in-stream mortality rate of American eel elvers in a Nova Scotia river. *Transactions of the American Fisheries Society,* 129(2):514-526.

Jessop BM, (2003) The run size and biological characteristics of American eel elvers in the East River, Chester, Nova Scotia. *Canadian Technical Report of Fisheries and Aquatic Sciences,* (2444):174p.

Jessop BM, Cairns DK, Thibault I, Tzeng WN (2008) Life history of American eel Anguilla rostrata: new insights from otolith microchemistry. *Aquatic Biology,* 1(3):205-216.

Jessop BM, Shiao JC, Iizuka Y, Tzeng WN, (2002) Migratory behaviour and habitat use by American eels *anguilla rostrata* as revealed by otolith microchemistry. *Marine Ecology-Progress Series,* 233:217-229.

Jessop BM, Shiao JC, Iizuka Y, Tzeng WN, (2006) Migration of juvenile American eels (*Anguilla rostrata)* between freshwater and estuary, as revelaed by otolith microchemistry. *Marine Ecology Progress Series*, 272:231-244.

Johansson-Sjobeck ML, Dave G, Larsson A, Lewander K, Lidman U, (1975) Metabolic and haematological effects of starvation in the European eel *Anguilla anguilla* L. II. Hematology. *Comparative Biochemistry and Physiology A. Comparative Physiology,* 52:431–434.

Katoh F, Hyodo S, Kaneko T, (2003) Vacuolar-type proton pump in the basolateral plasma membrane energizes ion uptake in branchial mitochondria-rich cells of killifish *Fundulus heteroclitus*, adapted to a low ion environment. *Journal of Experimental Biology,* 206(5):793-803.

Kerekes J, Beauchamp S, Tordon R, Tremblay C, Pollock T, (1986) Organic versus anthropogenic acidity in tributaries of the Kejimkujik watersheds in western Nova Scotia. *Water Air and Soil Pollution,* 31(1-2):165-173.

Kerekes J, Howell G, Beauchamp S, Pollock T, (1982) Characterization of 3 lake basins sensitive to acid precipitation in central Nova Scotia (June, 1979, to May, 1980). *Internationale Revue Der Gesamten Hydrobiologie,* 67(5):679-694.

Kerstetter TH, Kirschner LB, Rafuse DD, (1970) On mechanisms of sodium ion transport by irrigated gills of rainbow trout (*Salmo gairdneri*). *Journal of General Physiology,* 56(3):342-359.

Kleckner RC, McCleave JD, (1982) Entry of migrating American eel leptocephali into the gulf-stream system. *Helgolander Meeresuntersuchungen,* 35(3):329-339.

Kleckner RC, McCleave JD, (1985) Spatial and temporal distribution of American eel larvae in relation to North Atlantic Ocean current systems. *Dana*, 4:67-92.

Krogh A, (1937) Osmotic regulation by active absorption of ions in freshwater animals. *Skandinavisches Archiv Fur Physiologie,* 77:50-52.

Krueger WH, Oliveira K, (1999) Evidence for environmental sex determination in the American eel (A*nguilla rostrata)*. *Environmental Biology of Fishes,* 55(4):381-389.

Kwain W, Mccauley RW, Maclean JA (1984) Susceptibility of Starved, Juvenile Smallmouth Bass, *Micropterus dolomieui* to Low pH. *Journal of fish biology,* 25(4):501-504.

Lacroix GL, (1985) Plasma ionic composition of the Atlantic salmon (*Salmo salar*), white sucker (*Catostomus commersoni*), and alewife (*Alosa pseudoharengus*) in some acidic rivers of Nova Scotia. *Canadian Journal of Zoology,* 63(10):2254-2261.

Lacroix GL, (1987) Fish community structure in relation to acidity in 3 Nova Scotia rivers. *Canadian Journal of Zoology,* 65(12):2908-2915.

Lacroix GL, (1989) Ecological and physiological responses of Atlantic salmon in acidic organic rivers of Nova Scotia, Canada. *Water, Air, & Soil Pollution,* 46:375-386.

Lacroix GL, Hood DJ, Belfry CS, Rand TG, (1990) Plasma electrolytes, gill aluminum content, and gill morphology of juvenile Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) indigenous to acidic streams of Nova Scotia. *Canadian Journal of Zoology,* 68(6):1270-1280.

Lacroix GL, Kan KT, (1986) Speciation of aluminum in acidic rivers of Nova Scotia supporting Atlantic salmon: A methodological evaluation. *Canadian Technical Report of Fisheries and Aquatic Sciences,* 1501:12p.

Lacroix G L, Townsend DR, (1987) Responses of juvenile Atlantic salmon to episodic increases in acidity of Nova Scotia rivers. *Canadian Journal of Fisheries and Aquatic Sciences,* 44(8):1475-1484.

Lamson HM, Shiao J, Iizuka Y, Tzeng W, Cairns DK, (2006) Movement patterns of American eels (*Anguilla rostrata*) between salt and freshwater in a coastal watershed, based on otolith microchemistry. *Marine Biology,* 149(6):1567-1576.

Leivestad H, (1982) Physiological effects of acid stress on fish. In R. E. Johnson (Ed.), *Acid Rain/Fisheries, proceedings of an international symposium on acidic precipitation and fishery impacts in north-eastern North America.* (pp. 157-164). Maryland: AFS.

Leivestad H, Muniz IP, (1976) Fish kill at low pH in a Norwegian river. *Nature,* 259(5542):391-392.

Lin H, Pfeiffer DC, Vogl AW, Pan J, Randall DJ, (1994) Immunolocalization of H^+ ATP-ase in the gill epithelia of rainbow trout. *Journal of Experimental Biology,* 195:169-183.

Lin H, Randall D, (1991) Evidence for the presence of an electrogenic proton pump on the trout gill epithelium. *Journal of Experimental Biology,* 161:119-134.

Lin H, Randall D, (1995) Proton pumps in fish gills. In C. M. Wood, & T. J. Shuttleworth (Eds.), *Cellular and molecular approaches to fish ionic regulation* (pp. 229-255). San Diego, CA: Academic Press.

Lin H, Randall DJ, (1993) H^+ ATP-ase activity in crude homogenates of fish gill tissue - inhibitor sensitivity and environmental and hormonal regulation. *Journal of Experimental Biology,* 180:163-174.

Lin LY, Horng JL, Kunkel JG, Hwang PP, (2006) Proton pump-rich cell secretes acid in skin of zebrafish larvae. *American Journal of Physiology-Cell Physiology,* 290(2):C371-C378.

Madara JL, (1988) Tight junction dynamics - is paracellular transport regulated. *Cell,* 53(4):497-498.

Maetz J, Garcia Romeu F, (1964) Mechanism of sodium and chloride uptake by the gills of fresh-water fish, *Carassius auratus*. II. Evidence for NH_4^+/NA^+ and HCO_3^-/CL^- exchanges. *Journal of General Physiology,* 47:1209.

Magnuson JJ, Baker JP, Rahel EJ, (1984) A critical assessment of effects of acidification on fisheries in North America. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* 305(1124):501-516.

Marshall WS, (2002) Na^+, Cl^-, Ca^{2+} and Zn^{2+} transport by fish gills: Retrospective review and prospective synthesis. *Journal of Experimental Zoology,* 293(3):264-283.

Marshall WS, Grosell M, (2006) Ion transport, osmoregulation and acid-base balance. In D. H. Evans, & J. B. Claiborne (Eds.), *The physiology of fishes* (pp. 177-230). Boca Raton, FL: CRC.

Martin MH, (1995) The effects of temperature, river flow, and tidal cycles on the onset of glass eel and elver migration into fresh-water in the American eel. *Journal of Fish Biology,* 46(5):891-902.

McCleave JD, (1993) Physical and behavioral controls on the oceanic distribution and migration of leptocephali. *Journal of Fish Biology,* 43:243-273.

McCleave JD, Edeline E, (2009) Diadromy as a conditional strategy: patterns and drivers of eel movements in continental habitats. In Haro, A. J., Smith, K. L., Rulifson, R.A., Moffit, C. M., Klauda, R. J., Dadswell, M. J., Cunjack, R., Cooper, J. E., Beal, K.L.,& Avery, T. S., (Eds.), *Challenges for diadromous fishes in a dynamic global environment.* American Fisheries Scoiety, Symposium 69, Bethesda, Maryland. (pp. 97-119).

McCormick JH, Leino RL, (1999) Factors contributing to first-year recruitment failure of fishes in acidified waters with some implications for environmental research. *Transactions of the American Fisheries Society,* 128(2):265-277.

McDonald DG, (1983) The effects of H^+ upon the gills of fresh-water fish. *Canadian Journal of Zoology,* 61(4):691-703.

McDonald DG, Freda J, Cavdek V, Gonzalez R, Zia SH, (1991) Interspecific differences in gill morphology of fresh-water fish in relation to tolerance of low-pH environments. *Physiological Zoology*, 64(1):124-144.

McDonald DG, Reader JP, Dalziel TRK, (1989) The combined effects of pH and trace metals. In R. Morris, E. W. Taylor, D. J. A. Brown & J. A. Brown (Eds.), *Acid toxicity and aquatic animals, society for experimental biology seminar series.* (pp. 221-242). Cambridge: Cambridge University Press.

McDonald DG, Wood CM, (1981) Branchial and renal acid and ion fluxes in the rainbow trout (*Salmo gairdneri*), at low environmental pH. *Journal of Experimental Biology,* 93:101-118.

McGrath KJ, Bernier J, Ault S, Dutil JD, Reid K, (2003) Differentiating downstream migrating American eels *Anguilla rostrata* from resident eels in the St. Lawrence River. In D.A. Dixon (Ed.), *Biology, management, and Protection of Catadromous Eels.* American Fisheries Society, Symposium 33, Missouri. (pp. 315-327).

McWilliams PG, (1980) Effects of pH on sodium uptake in Norwegian brown trout (*Salmo trutta*) from an acid river. *Journal of Experimental Biology,* 88:259-267.

McWilliams PG, (1982) A comparison of physiological-characteristics in normal and acid exposed populations of the brown trout *Salmo trutta*. *Comparative Biochemistry and Physiology A-Physiology,* 72(3):515-522.

Milligan CL, Wood CM, (1982) Disturbances in hematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology,* 99:397-415.

Myers RA, Worm B, (2005) Extinction, survival or recovery of large predatory fishes. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 360(1453):13-20.

Naeem S, Li S, (1997) Biodiversity enhances ecosystem reliability. *Nature,* 390:507-509.

Neave FB, Mandrak NE, Docker MF, Noakes DL, (2006) Effects of preservation on pigmentation and length measurements in larval lampreys. *Journal of Fish Biology,* 68(4):991-1001.

Oliveira K, (1999) Life history characteristics and strategies of the American eel (*Anguilla rostrata*). *Canadian Journal of Fisheries and Aquatic Sciences,* 56(5):795-802.

Oliver BG, Thurman EM, Malcolm RL, (1983) The contribution of humic substances to the acidity of colored natural-waters. *Geochimica Et Cosmochimica Acta,* 47(11):2031-2035.

Orr HA, (2007) Absolute fitness, relative fitness, and utility. *Evolution,* 61:2997-3000.

Pankhurst NW, Sorensen PW, (1984) Degeneration of the alimentary-tract in sexually maturing European (*Anguilla anguilla*) and American eels (*Anguilla rostrata*). *Canadian Journal of Zoology,* 62(6):1143-1149.

Parks SK, Tresguerres M, Goss GG, (2007) Interactions between Na^+ channels and Na^+-HCO_3^- co-transporters in the freshwater fish gill MR cell: A model for transepithelial Na^+ uptake. *American Journal of Physiology-Cell Physiology,* 292(2):C935-C944.

Patrick ML, Wood CM, (1999) Ion and acid-base regulation in the freshwater mummichog (*Fundulus heteroclitus*): A departure from the standard model for freshwater teleosts. *Comparative Biochemistry and Physiology A-Molecular and Integrative Physiology,* 122(4):445-456.

Perry SF, (1997) The chloride cell: Structure and function in the gills of freshwater fishes. *Annual Review of Physiology,* 59:325-347.

Perry SF, Goss GG, Laurent P, (1992) The interrelationships between gill chloride cell morphology and ionic uptake in 4 fresh-water teleosts. *Canadian Journal of Zoology,* 70(9):1775-1786.

Perry SF, Shahsavarani A, Georgalis T, Bayaa M, Furimsky M, Thomas SLY, (2003) Channels, pumps, and exchangers in the gill and kidney of freshwater fishes: Their role in ionic and acid-base regulation. *Journal of Experimental Zoology Part A-Comparative Experimental Biology, 300A*(1), 53-62.

Pimentel D, Wilson C, McCullum C, Huang R, Dwen P, Flack J, Tran Q, Saltman T, Cliff B, (1997) Economic and environmental benefits of biodiversity. *BioScience,* 47(11):747-757.

Posey DA, (Ed.) (1999) *Cultural and Spiritual Values of Biodiversity*. London: United Nations Environmental Programme & Intermediate Technology Publications.

Powles PM, Warlen SM, (2002) Recruitment season, size, and age of young American eels (*Anguilla rostrata*) entering an estuary near Beaufort, North Carolina. *Fishery Bulletin,* 100(2):299-306.

Purvis A, Hector A, (2000) Getting the measure of biodiversity. *Nature*, 405:212-219.

Rask M, (1983) Differences in growth of perch (*perca fluviatilis* L) in 2 small forest lakes. *Hydrobiologia,* 101(1-2):139-143.

Reid S D, Hawkings GS, Galvez F, Goss GG, (2003) Localization and characterization of phenamil-sensitive Na^+ influx in isolated rainbow trout gill epithelial cells. *Journal of Experimental Biology,* 206(3):551-559.

Sadler K, Lynam S, (1986) Some effects of low pH and calcium on the growth and tissue mineral-content of yearling brown trout, *Salmo trutta*. *Journal of fish biology,* 29(3):313-324.

Salman NA, Eddy FB, (1987) Response of chloride cell numbers and gill Na^+/K^+ ATPase activity of freshwater rainbow trout (*Salmo gairdneri*) to Salt Feeding. *Aquaculture,* 61(1):41-48.

Schmidt J, (1922). The breeding places of the eel. *Philosophical Transactions of the Royal Society of London Series B,* 211:179-208.

Scott WB, Crossman EJ, (1973) Freshwater Fishes of Canada. Bulletin 184. *Fisheries Research Board of Canada*. 966 pp

Secor DH, (1999) Specifying divergent migrations in the concept of stock: the contingent hypothesis. *Fisheries Research,* 43:13-34

Secor DH, (2007) The year-class phenomenon and the storage effect in marine fishes. *Journal of Sea Research,* 57:91-103.

Shaw RW, (1979) Acid precipitation in Atlantic Canada. *Environmental Science & Technology,* 13(4):406-411.

Shilts WW, (1981) Sensitivity of bedrock to acid precipitation: Modification by glacial processes. *Geological Survey of Canada , Paper 81-14,* p.7.

Shuter BJ, Ihssen PE, (1991) Chemical and biological factors affecting acid tolerance of smallmouth bass. *Transactions of the Maerican Fisheries Society,* 120:23-33.

Smith NF, Talbot C, Eddy FB, (1989) Dietary salt intake and its relevance to ionic regulation in fresh-water salmonids. *Journal of Fish Biology,* 35(6):749-753.

Soule ME, (1985) What is conservation biology? *BioScience*, 35(11):727-734.

Spry DJ, Wood CM, Hodson PV, (1981) The effects of environmental acid on freshwater fish with particular reference to the softwater lakes in Ontario and the modifying effects of heavy metals. A literature review. *Canadian Technical Report of Fisheries and Aquatic Sciences*, 999, 145p.

SRPR (2010) Species at Risk Public Registry, Government of Canada. http://www.sararegistry.gc.ca

Sullivan GV, Fryer JN, Perry SF, (1995) Immunolocalization of proton pumps (H^+-ATPase) in pavement cells of rainbow-trout gill. *Journal of Experimental Biology,* 198(12):2619-2629.

Sullivan MC, Able KW, Hare JA, Walsh HJ, (2006) *Anguilla rostrata* glass eel ingress into two, US east coast estuaries: Patterns, processes and implications for adult abundance. *Journal of Fish Biology,* 69(4):1081-1101.

Summers PW, Whelpdale DM, (1976) Acid precipitation in Canada. *Water Air and Soil Pollution,* 6(2-4):447-455.

Tesch FW, (2003) In Thorpe J. E. (Ed.), *The eel* (fifth ed.). Oxford: Blackwell.

Tresguerres M, Katoh F, Orr E, Parks SK, Goss GG, (2006) Chloride uptake and base secretion in freshwater fish: A transepithelial ion-transport metabolon? *Physiological and Biochemical Zoology*, 79(6):981-996.

Tsukamoto K, Aoyama J, Miller MJ, (2002) Migration, speciation, and the evolution of diadromy in anguillid eels. *Canadian Journal of Fisheries and Aquatic Sciences,* 59(12):1989-1998.

Tsukamoto K, Nakai I, Tesch WV, (1998) Do all freshwater eels migrate? *Nature,* 396(6712):635-636.

van Ginneken V, Ballieux B, Willemze R, Coldenhoff K, Lentjes E, Antonissen E, Haenen O, van den Thillart G, (2005) Hematology patterns of migrating European eels and the role of EVER virus. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology,* 140(1):97-102.

van Ginneken VJT, van den Thillart G, (2000) Physiology - eel fat stores are enough to reach the Sargasso. *Nature,* 403(6766):156-157.

Verreault G, Dumont P, (2003) An estimation of American eel escapement from the Upper St. Lawrence River and Lake Ontario in 1996 and 1997. In D.A. Dixon (Ed.), *Biology, management, and Protection of Catadromous Eels.* American Fisheries Society, Symposium 33, Missouri. (pp. 243-251).

Vuorinen PJ, Keinanen M, Lappalainen A, Peuranen S, Rask M, (2004) Physiological status of whitefish (*Coregonus lavaretus pallasi*) prior to spawning in lakes of differing acidity. *Aquatic Sciences,* 66(3):305-314.

Watt WD, (1987) A summary of the impact of acid-rain on Atlantic salmon (*salmo salar*) in Canada. *Water Air and Soil Pollution,* 35(1-2):27-35.

Watt WD, Scott D, Ray S, (1979) Acidification and other chemical-changes in Halifax county lakes after 21 years. *Limnology and Oceanography,* 24(6):1154-1161.

Watt WD, Scott CD, White WJ, (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(4):462-473.

Watt WD, Scott CD, Zamora PJ, White WJ, (2000) Acid toxicity levels in Nova Scotian rivers have not declined in synchrony with the decline in sulfate levels. *Water Air and Soil Pollution,* 118(3-4):203-229.

Wilson JM, Laurent P, Tufts BL, Benos DJ, Donowitz M, Vogl AW, Randall DJ, (2000) NaCl uptake by the branchial epithelium in freshwater teleost fish: An immunological approach to ion-transport protein localization. *Journal of Experimental Biology,* 203(15):2279-2296.

Wilson EO, (1992) *The Diversity of Life*. Cambridge, MA: The Belknap Press of Harvard University Press.

Wilson RW, Bergman HL, Wood CM (1994) Metabolic Costs and Physiological Consequences of Acclimation to Aluminum in Juvenile Rainbow-Trout (Oncorhynchus-Mykiss) .1. Acclimation Specificity, Resting Physiology, Feeding, and Growth. *Canadian Journal of Fisheries and Aquatic Sciences,* 51(3):527-535.

Wirth T, Bernatchez L, (2003) Decline of north Atlantic eels: A fatal synergy? *Proceedings of the Royal Society of London Series B-Biological Sciences,* 270(1516):681-688.

Wood CM, (1989) The physiological problems of fish in acid waters. In R. Morris, E. W. Taylor, D. J. A. Brown & J. A. Brown (Eds.), *Acid toxicity and aquatic animals, society for experimental biology seminar series.* (pp. 125-148). Cambridge: Cambridge University Press.

Wood CM, (2001) Toxic responses of the gill. In D. W. Schlenk, & W. H. Benson (Eds.), *Target organ toxicity in marine and freshwater teleosts, volume 1 - organs.* (pp. 1-89). New York: CRC.

Wood CM, Kelly SP, Fletcher M, Zhou B, O'Donnell M, Eletti B, Part P, (2002) Cultured gill epithelia as models for the freshwater fish gill. *Biochimica Et Biophysica Acta-Biomembranes,* (1566):72-83.

Wood CM, McDonald DG, (1982) Physiological mechanisms of acid toxicity to fish. In R. E. Johnson (Ed.), *Acid Rain/Fisheries, proceedings of an international symposium on acidic precipitation and fishery impacts in north-eastern North America.* (pp. 197-226). Maryland: AFS.

Wood CM, Part P, (1997) Cultured branchial epithelia from freshwater fish gills. *Journal of Experimental Biology,* 200(6):1047-1059.

Wuenschel MJ, Able KW, (2008) Swimming ability of eels (*Anguilla rostrata*, *Conger oceanicus*) at estuarine ingress: Contrasting patterns of cross-shelf transport? *Marine Biology,* 154(5):775-786.