Evaluating the effects of sub-lethal acidification on wild populations of juvenile Atlantic salmon

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Abstract

Evaluating the effects of sub-lethal acidification on wild populations of juvenile Atlantic salmon

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One of the many environmentally deleterious effects of industrialization is the acidification of freshwater, which results in part from precipitative inputs of anthropogenic pollutants. Considerable effort has been allocated to the study and remediation of severely acidified aquatic systems, with the effects of weak levels of acidification (pH 6 – pH 7) on biological communities having received less attention. One effect that has been studied is the chemical mediation of predator-prey interactions in freshwater and marine fishes. Following mechanical damage to the epidermis, as would likely occur during a predation event, many taxa release chemical alarm cues which elicit antipredator responses from conspecifics and other opportunistic receivers subject to similar levels of predation risk and potentially enhance the survival of alarm cue receivers during subsequent interactions with predators. Under weakly acidic conditions (pH < 6.6), these chemical alarm cues are rendered non-functional and do not elicit alarm responses from conspecific or heterospecific receivers. Weak acidification effectively deprives prey fishes of one source of chemosensory information on ambient risk levels.

Here, I describe a series of field experiments designed to evaluate the effects of this environmentally-mediated loss of information on wild populations of juvenile Atlantic salmon (*Salmo salar*) in four acidic and five neutral streams in the Miramichi

River system, New Brunswick, Canada. Acid-impacted Atlantic salmon in these experiments demonstrated a loss of response to conspecific and heterospecific chemical alarm cues, as well as significantly greater responses to remaining (visual) threat cues than salmon under neutral conditions. Fish in neutral streams appeared to demonstrate additive responses to multiple risky cues consistent with dynamic threat-sensitivity and the sensory complementation hypothesis, whereas fish in acidic streams demonstrated non-threat-sensitive responses consistent with the absence of sensory complementation and greater value being assigned to information received through visual cues. Contrary to predictions, juvenile salmon do not appear to suffer increased mortality through predation as a result of this loss of information, nor do they experience negative growth effects resulting from temporal trade-offs between antipredator and foraging behaviours. Rather, acid-impacted Atlantic salmon demonstrated behavioural compensation through increased preference for complex habitats which offer greater abundance of physical refugia and limit line-of-sight for visually foraging predators, effectively mitigating the increased risk of predation associated with limited information.

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Contribution of Authors

The chapters of this thesis were designed as manuscripts for submission to peer-reviewed journals. I contributed to the conception, planning, collecting of data, data analyses and preparation of all manuscripts. Dr. G. E. Brown contributed to the conception, planning, data analyses and editing of all manuscripts. C. J. Macnaughton contributed to the collecting of data and editing the manuscript presented in Chapter 1 and to data collection for Experiment 3b.

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General Introduction

Behavioural mediation of predator-prey interactions

The ultimate costs incurred by prey following a successful predation attempt suggests that individuals should benefit from adopting any risk-aversive or antipredator strategies that increase the probability of survival following detection of either a foraging predator or some other public information indicating elevated ambient risk (Lima and Dill 1990; McNamara and Dall 2010). Freshwater fishes are subject to predation pressure from both aquatic and terrestrial predators. Due to gape size limitations, predatory fishes are typically able to handle and ingest prey up to two-thirds their own length (Popova 1978). Consequently, fishes are subject to predation risk from individuals ≥ 50% longer than themselves, resulting in relatively larger pools of potential fish predators during early life history stages and at smaller body sizes than during later stages at larger sizes. By contrast, much larger fish, terrestrial and avian predators might prefer larger size classes of prey in order to maximize the cost-benefit ratio between energy expenditure in capture and handling and energy gained from consuming individual prey (Marten 1973). For example, the predatory pike-cichlid (*Crenicichla alta*) demonstrated a consistent preference for larger Trinidadian guppies (*Poecilia reticulata*), reducing their daily consumption of guppies in proportion to manipulated size ratios of experimental guppy shoals (Mattingly and Butler 1994).

Two general survival enhancing behavioural strategies employed by prey species are characterized by the avoidance or deterrence of predators (Smith 1997) and evasion or escape from predators (Godin 1997). Predator avoidance behaviour in fishes may involve selective space use in heterogeneous habitats, resulting in individuals avoiding areas with relatively high rates of predation or where predators are more likely to be present (Chivers and Smith 1994; Brown *et al.* 1995; Friesen and Chivers 2006; Kim *et al.* 2011b). Prey may also preferentially occupy areas with more abundant or effective physical refugia (Allouche and Gaudin 2001; Dolinsek *et al.* 2007; Dupuch *et al.* 2009). In the presence of predators or in areas perceived to be of relatively high risk, avoidance strategies may also include behavioural modifications such as decreases in the size of defended territories (Kim *et al.* 2011a) and levels of activities that potentially increase the conspicuousness of prey to a predator, such as movement, foraging or mating displays (Smith 1997). These responses may reduce the level of risk experienced by potential prey as stationary individuals may be more difficult to detect by visually foraging predators (Cronin 1997). Following detection, prey fish may attempt to deter potential predators through overt visual displays (*e.g.*, fin-flicking; Brown *et al.* 1999) or evade them through escape or flight behaviours (Godin 1997). Potentially costly antipredator responses may represent indirect negative effects of predation on individuals and populations in the forms of decreased energy intake from lost foraging opportunities, increased investment in defense, loss of mating opportunities and cascading trophic effects (Preisser *et al.* 2005) as population depressions may provide opportunities for other species (Dill *et al.* 2003). Captive descendents of wild-caught populations of the killifish Hart's rivulus (*Rivulus hartii*) from relatively high risk environments have lower fitness (measured as growth rate) than fish from low-risk populations under conditions of food limitation, but have higher fitness when foraging opportunities are abundant (reviewed in Walsh 2013). The presence of this population difference in captive-reared killifish was attributed to predator mediated selection, whereby killifish are less abundant due to predation in high-risk environments and remaining individuals have access to relatively greater amounts of resources than under conditions of lower predation risk and greater abundance of conspecific competitors. A meta-analysis of literature on the magnitude of direct (via prey consumption) and indirect (via fear or intimidation) effects of predation in aquatic systems suggests that both contribute approximately equally to limiting the fitness and abundance of prey populations (Preisser *et al.* 2005).

Many species of fish, including Atlantic salmon (*Salmo salar*), produce chemicals in the epidermis which are released into the water following mechanical damage, as would likely occur during a predation event (Smith 1992). Detection of these damage-released chemical cues has been demonstrated to elicit a wide range of responses in receivers, largely influenced by the relatedness and ecological similarity between the receiver and the cue source. Detection of a sudden release of these chemicals by a conspecific of similar life history stage can elicit a suite of short-term behavioural changes consistent with antipredator or alarm responses (Brown and Smith 1997; Chivers and Smith 1998). Similar antipredator responses have also been documented in heterospecifics receivers, with evidence for taxonomic conservation of chemical alarm cues within families (Mirza and Chivers 2001a) and associative learning mechanisms in unrelated sympatric species subject to similar predation threats (Chivers *et al.* 1995b; Mathis *et al.* 1996; Chivers *et al.* 2002).

Certain antipredator behaviours, including taking refuge from perceived threats or avoiding high-risk areas (Wisenden *et al.* 1994; Brown *et al.* 1995), may limit the time spent engaging in other important fitness-related activities such as foraging. Consequently, optimized behavioural responses to particular threat cues should be proportionate to the degree of risk conveyed by a cue. Because the relative costs and benefits of different behaviours to an individual can vary considerably over time, for example as potential predators migrate through an area or as the abundance of foraging opportunities changes, the antipredator strategies demonstrated by individuals at any given time are likely the result of a perpetual series of temporal trade-offs mediated primarily by the immediate perceived level of predation risk (Lima and Dill 1990).

Threat-sensitive antipredator responses have been documented in several fish taxa, including cyprinids (Brown and Cowan 2000; Brown *et al.* 2001a), salmonids (Dionne and Dodson 2002; Leduc *et al.* 2008; Kim *et al.* 2009), poeciliids (Botham *et al.* 2008; Brown *et al.* 2009) centrarchids (Marcus and Brown 2003) and killifishes (Elvidge *et al.* 2010). The threat-sensitive responses of an individual to a particular cue are influenced not only by the magnitude or amount of a cue that is detected (Brown *et al.* 2004a), but also by the level of risk previously experienced (Brown *et al.* 2006) over both short (days: Ferrari *et al.* 2010b) and long (years: Brown *et al.* 2009) timescales. Freshwater fish populations have demonstrated the capability to rapidly learn about new predation threats and adopt appropriate behavioural strategies. Individual fathead minnows (*Pimephales promelas*) demonstrated fright responses to the chemical and visual cues of Northern pike (*Esox lucius*) in as little as two to four days and six to eight days, respectively, following the introduction of pike to an experimental pond (Chivers and Smith 1995; Brown *et al.* 1997).

In addition to short-term fright responses, minnows under laboratory conditions also demonstrated avoidance of areas labelled with the feces of minnow-fed pike (Brown *et al.* 1995). Brook sticklebacks (*Culaea inconstans*) have demonstrated acquired recognition of pike chemical cues through a releaser-induced learning mechanism (Suboski 1990), following a conditioning exposure to the novel pike cue paired with familiar risky chemical cues (conspecific alarm cues: Chivers *et al.* 1995a). Paired associative learning mechanisms and environmental constraints such as decreased reliance on visual cues in turbid or low-light environments (Ferrari *et al.* 2010c; Leduc *et al.* 2010b; Ranaker *et al.* 2012) and loss of chemical information (Leduc *et al.* 2007b; Smith *et al.* 2008; Brown *et al.* 2012) on learning have also been observed in wild and captive salmonids. Similarly, fishes have demonstrated a capacity for social learning, allowing recognition and response to novel cues when detected in close temporal proximity to foraging and fright responses by more experienced nearby conspecifics (Giraldeau *et al.* 1994; Reader *et al.* 2003; Lindeyer and Reader 2010). This capacity for learning to recognize and respond to novel cues has been exploited in the aquaculture industry, with predator-naïve hatchery-reared fishes, including salmonids, exposed to realistic risky cues with the intention of increasing survival following release (Brown and Smith 1998; Brown and Laland 2001; Berejikian *et al.* 2003). Logistic limitations preclude extensive tracking of released fishes, but experimental evidence demonstrates significant survival advantages as a result of learning to recognize risky cues regardless of the specific learning mechanism (Mirza and Chivers 2000; Chivers *et al.* 2002; Mirza and Chivers 2003; Manassa and McCormick 2012).

Detection of both conspecific and heterospecific damage-released chemical cues has been demonstrated to increase the probability of survival of individual prey fish receivers during staged encounters with predators (Mirza and Chivers 2001b; Chivers *et al.* 2002; Mirza and Chivers 2003). Conversely, both conspecific (Mirza and Chivers

2002; Harvey and Brown 2004) and heterospecific (Brown *et al.* 2001b; Elvidge *et al.* 2010; Lonnstedt *et al.* 2012) fish receivers have demonstrated the ability to determine the body condition and relative size of alarm cue donors, with larger receivers shifting responses from antipredator strategies to those consistent with increased levels of foraging (Harvey and Brown 2004; Elvidge *et al.* 2010) and exploratory behaviours (*e.g.*, predator inspection) under both laboratory (Brown *et al.* 2001b; Elvidge *et al.* 2010) and field (Wisenden and Thiel 2002; Elvidge and Brown 2012) conditions. These observations lend considerable support to the predator attraction hypothesis (Lima and Dill 1990; Smith 1992; Chivers *et al.* 1996), which predicts that prey may experience an increased probability of escape and survival if a primary predator experiences interference from another, secondarily attracted by the release of alarm cues. The occurrence of antipredator behavioural responses to these damage-released chemical cues in taxonomically distant receivers, as well as significant ontogenetic shifts in elicited responses, strongly suggest that the evolution of this chemical signaling system was driven by selective advantages accrued by a receiver through social eavesdropping (Stowe *et al.* 1995) and the ability to distinguish between different forms of publicly available chemical information (Danchin *et al.* 2004; Chivers *et al.* 2012), rather than on survival or physiological benefits accrued by the sender (Chivers *et al.* 2007).

Fishes can simultaneously detect and respond to multiple sources of public information received through different sensory modalities as both threat (Wisenden and Chivers 2006; Kim *et al.* 2009) and foraging (Elvidge and Brown 2012) cues. When multiple cues conveying complementary information are detected in close temporal proximity to each other, they may elicit additive or synergistic behavioural responses from a receiver (Ferrari *et al.* 2008; Kim *et al.* 2009; Ferrari *et al.* 2010b). Conversely, individuals temporarily deprived of one sensory modality may demonstrate increased responses to information gained through the remaining senses, including increased reliance on olfaction in the absence of visual cues (Hartman and Abrahams 2000; Leduc *et al.* 2010b) and increased reliance on visual information in the absence of chemical cues (Lemly and Smith 1985). In addition, temporary deprivation of some component of a common or familiar cue combination may result in decreased or absent responses to the remaining cues (Brown *et al.* 2010).

Atlantic salmon and juvenile survival

The Atlantic salmon is an anadromous species which spawns in cold, low-order tributary streams throughout the northern hemisphere (Froese and Pauly 2010). Canadian juvenile salmon spend several years growing in low-order nursery streams, after which they undergo smoltification in the spring and migrate seawards to continue growth and maturation, often off the western coast of Greenland (Hansen and Quinn 1998). Juveniles are present in nursery streams in at least three age classes: young of the year (YOY), also referred to as fry or 0^+ salmon, second year (1^+ parr) and third year (2^+ parr) fish. Depending on environmental conditions, juveniles can spend three to six years in freshwater before smolting (ASF 2010). Juvenile salmon experience fairly high mortality, with first-year recruitment averaging 58% (Froese and Pauly 2010) and between 1.5 - 30% of emergent fry surviving through smoltification (Cunjak and Therrien 1998; Hutchings and Jones 1998). Since major determinants of this pattern of early life history mortality are the size and condition of an individual at the end of the summer growth season (Hutchings and Jones 1998), any differences in behaviours (*e.g.*, decreased foraging rate) resulting from anthropogenic changes to the environment may have profound consequences on the growth rates or survival of juvenile salmon and decrease production at the population level. Additionally, survival during the marine phase of salmon life history may be as low as 5% and has been demonstrating a decline in response to changing sea-surface temperatures over the last several decades (Hansen and Quinn 1998; DFO and MRNF 2009).

Several aspects of the ecology and life history of Atlantic salmon make this species extremely attractive for study as a model organism. Stream-dwelling juvenile stages, for instance, typically forage on drift items (Keeley and Grant 1995), and can be strongly territorial in order to protect profitable foraging stations (Grant and Noakes 1987; Grant and Kramer 1990). Consequently, salmon have been used as a model to study factors influencing habitat selection (Metcalfe *et al.* 1997; Kim *et al.* 2011a), competitive interactions (Blanchet *et al.* 2008; Vehanen *et al.* 2009) and density effects on behaviours and population processes (Gibson *et al.* 2008; Finstad *et al.* 2009). The site preferences of salmon enable their observation in short-term studies on antipredator behaviours (*e.g.*, Leduc *et al.* 2006; Kim *et al.* 2009) and territoriality *in situ* (Kim *et al.* 2011a), as they typically return to their defended territory following disturbance and tolerate the proximity of stationary human observers. Salmon are extremely sensitive to environmental conditions, lending themselves to study of the effects of chemical and temperature changes on behaviour (Ikuta *et al.* 2001; Ikuta *et al.* 2003; Leduc *et al.* 2006) and physiology (Ikuta and Kitamura 1995; Kroglund *et al.* 2007b; Monette and McCormick 2008; McCormick *et al.* 2009) throughout their different life history stages.

The shift in osmoregulatory demands associated with differing levels of salinity between freshwater and marine environments provides a unique window into a physiologically-mediated survival bottleneck influenced by a number of biotic (Renkawitz and Sheehan 2011) and abiotic factors (Magee *et al.* 2003; Monette *et al.* 2008).

Atlantic salmon face several threats to the persistence of natural populations, most notably: the current and historical effects of recreational and commercial fishing including marine by-catch; freshwater habitat alteration and degradation resulting from land and water usage, including hydroelectric infrastructure; air and water pollution; competition, disease transmission and hybridization with aquaculture strains, resulting in the loss of endemic specialization; competition, disease transmission and predation on early life-history stages from invasive species; mechanical disturbances such as shipping noise; and effects of climate change, particularly of a warming nature, on individual survival, resource abundance and availability of suitable habitat in both freshwater and marine habitats (reviewed in DFO and MRNF 2009). As an aquaculture species, Canadian Atlantic salmon accounted for 8.2% of global salmon production, and at 1.2×10^5 t of fish, approximately two-thirds of all Canadian aquaculture production with an estimated market value of $750M in 2006 (Canadian Aquaculture Industry Alliance 2012). As a recreationally fished species, Atlantic salmon angling licenses, fishing guide services, outfitting and tackle generated $100M - $200M in revenue nationally and more than $30M in New Brunswick alone on an annual basis in the late 1990s (Whoriskey and Glebe 1998). In addition to the cultural and economic value associated with Atlantic salmon, many coastal First Nations communities are dependent to some extent on traditional methods of salmon harvesting, with an estimated 40 000 aboriginals in 50 communities in Atlantic Canada and Quebec alone benefiting from salmon as a natural resource. Consequently, Atlantic salmon are valuable as a model species to study large-scale environmental changes and the efficacy of remediation and restoration techniques (Sandoy and Langaker 2001; Clair *et al.* 2007; Einum *et al.* 2008; Hesthagen *et al.* 2011) in the context of biological conservation efforts.

Freshwater acidification

Increasing levels of freshwater acidification were observed as early as the 1930s (Schopp *et al.* 2003) but were only attributed to the deposition of acidic compounds, particularly those containing sulfur, produced by combustion during industrial processes decades later (Gorham *et al.* 1986; Schindler 1988; Evans *et al.* 2001). Atmospheric transportation of these anthropogenic pollutants generally results in their deposition during precipitation events at some distance down-wind from the source of emissions, typified by the acidification of Norwegian surface waters by inputs from the United Kingdom (Sandoy and Langaker 2001). Acidic precipitation may result in changes to the chemical profile of freshwater systems (Evans *et al.* 2001), eliciting changes in both the diversity and abundance of aquatic biota in affected waters (Schindler 1988; Muniz 1990; Herrmann *et al.* 1993). Any such biotic changes may in turn result in adverse effects on ecosystem functioning and various measures of ecosystem production, resulting in measurable economic losses to industries dependent on freshwater systems (Holland 1995). Although acidification also occurs in the absence of anthropogenic inputs, natural acidification typically develops and persists over much longer timescales and consequently, impacted organisms are better able to adapt to changing chemical conditions. While some naturally acidic streams have been found to contain diverse and functional faunal communities (Dangles *et al.* 2004), anthropogenically acidified streams typically experience reduced diversity and abundance of invertebrate and vertebrate species and altered composition of invertebrate communities which may result in decreased productivity and ecosystem functioning (Schindler 1988; Muniz 1990; Herrmann *et al.* 1993) A recent meta-analysis (Petrin *et al.* 2008) comparing the effects of natural and anthropogenic acidification found decreasing macroinvertebrate abundance and rates of leaf litter decomposition with increasing acidity under both regimes, although macroinvertebrate diversity decreased three times more rapidly when acidification was anthropogenic. Naturally acidic streams, in contrast to anthropogenically acidic streams, may not experience decreased ecosystem functioning as a result of greater abundance and activity of any remaining acid-tolerant invertebrate species (Petrin *et al.* 2008). Some measures have been adopted in the industrialized world to reduce both the volume and the sulfurous content of industrial emissions (Clair *et al.* 2007) and remediate certain acidified freshwater systems (*e.g.*, by liming: Henrikson *et al.* 1995) with the intention of restoring historical chemical and biotic profiles. These measures have achieved varying degrees of success, depending primarily on the interaction between the severity of historical acidification and the magnitude of monetary and temporal investment in any remediation efforts (Alewell *et al.* 2001; Evans *et al.* 2001; Monteith *et al.* 2001; Clair *et al.* 2002; Tipping *et al.* 2002; Ferrier *et al.* 2003).

Aquatic systems demonstrate considerable spatial and temporal variation in their chemical and physical properties, as well as their responses to acidic inputs. The degree to which any aquatic system becomes acidified is influenced by a number of factors, notably the amount of acidic input and the acid neutralizing capacity (ANC) or buffering strength of the substrate in the catchment area or drainage basin (Kalff 2003). Although remediation efforts have succeeded at restoring historic conditions in certain areas (Evans *et al.* 2001), many impacted streams and lakes remain acidified due to the depletion of the ANC of their catchments and are expected to remain acidified for decades (Alewell *et al.* 2001; Monteith *et al.* 2001; Ferrier *et al.* 2003) under current reduced input levels and remediation measures (Clair *et al.* 2007). Additionally, recovery and return to historic conditions in heavily or moderately acidified waterbodies are gradual processes and recovering systems may spend considerable amounts of time at intermediate, weakly acidic levels.

Different levels of acidity have been found to exert particular effects on freshwater biota. At strongly acidic levels ($pH < 5.5$), many organisms experience direct acid-mediated mortality (Herrmann *et al.* 1993), potentially resulting in pronounced decreases in abundance or the complete extirpation of acid-intolerant species from impacted waterbodies and resultant alterations in the composition of freshwater communities (Lemly and Smith 1987; Xu *et al.* 1999; Holt *et al.* 2003). Fishes, amphibians and invertebrates are each susceptible to toxic effects of dissolved metals in strongly acidic waters, as the solubilities of certain metals such as copper and aluminum increase with acidity. At sufficient concentrations, these metals form precipitates on the gill epithelia of fishes and impair respiration to the point of asphyxia (Wren and Stephenson 1991; Horne and Dunson 1995; Kalff 2003; Pettersen *et al.* 2006; Kroglund *et al.* 2007b).

Exposure to moderate (pH 5.5 – pH 6.0) levels of acidity may result in non-lethal stresses including decreased growth and reproduction in fishes (Ikuta and Kitamura 1995; Kitamura and Ikuta 2001; Ogawa *et al.* 2001; Ikuta *et al.* 2003), invertebrates (Chan *et al.* 2008), submerged macrophytes (Titus and Hoover 1993) and algae (Kinross *et al.* 2000). Exposure to moderate levels of acidity for extended periods may impair future physiological functions such as ionoregulation in invertebrates (Dennis and Bulger 1995; Zalizniak *et al.* 2009) and smoltification in Atlantic salmon (Kroglund *et al.* 2007b; Monette *et al.* 2008; McCormick *et al.* 2009). Moderate levels of acidity decrease the rate of decomposition and nutrient cycling in freshwater habitats through the inhibition of fungal activity (Dangles and Chauvet 2003). Species composition, trophic structure and diversity of invertebrate communities may change as acid-tolerant species become more abundant (Dangles and Guerold 1999; Holt *et al.* 2003), potentially causing cascading effects in the composition, diversity and productivity of associated riparian and terrestrial ecosystems (Desgranges and Gagnon 1994).

At weak levels of acidity (pH < 6.6), as commonly occurs in nursery streams throughout the distribution of Atlantic salmon (Leduc *et al.* 2006), sensory impairment and differences in antipredator behaviours have been observed in different groups of fishes, including salmonids (Leduc *et al.* 2004a; Leduc *et al.* 2006; Leduc *et al.* 2008), centrarchids (Leduc *et al.* 2003) and cyprinids (Lemly and Smith 1985; Brown *et al.* 2002). Recent studies conducted under both laboratory (Brown *et al.* 2002) and field (Leduc *et al.* 2010a) conditions have shown that under these weak levels of acidity, damage-released chemical alarm cues from the epidermis of prey fishes are altered such that they do not elicit an antipredator response in conspecific receivers. Earlier studies demonstrated that under laboratory conditions, alarm cue extract titrated to below pH 6.0 does not elicit the antipredator responses in receivers that are observed following exposure to untreated alarm cues (Brown *et al.* 2002; Leduc *et al.* 2004a; Brown *et al.* 2012). Under natural conditions, alarm cues do not elicit the antipredator responses in populations of salmonids in weakly acidic streams that are demonstrated by conspecifics in neutral streams (Leduc *et al.* 2004a; Elvidge *et al.* 2013). Cross-population transplant experiments using wild Atlantic salmon between streams of different pH classes confirm that the loss of response under acidic conditions is due to some functional degradation of the alarm cues themselves, rather than the result of differences in provenance or physiological impairment resulting from exposure to weak acidification (Leduc *et al.* 2010a; Elvidge *et al.* 2013). This is indicative of sensory deprivation arising from the loss of one source of public information on the immediate level of predation risk, and results in different antipredator behavioural patterns between fish populations inhabiting streams differing in acidity. More recently, this acid-mediated information loss has been demonstrated to negatively impact the abilities of prey fish to generalize the learned recognition of taxonomically related predators (Leduc *et al.* 2004b; Brown *et al.* 2012).

Experimental questions and design

The dynamic relationship between multiple complementary sensory cues and the behavioural responses they elicit (Ferrari *et al.* 2008; Leduc *et al.* 2010b) has typically been investigated with regard to the synergistic effects of multiple sources of information available to receivers over brief timescales (Kim *et al.* 2009), or to the effects on receivers temporarily deprived of information conveyed through one particular sense

(Hartman and Abrahams 2000; Leduc *et al.* 2010b). To date, few studies have attempted to examine the consequences of long-term deprivation of particular sensory modalities in freshwater fishes. By rendering chemical alarm cues non-functional, weak anthropogenic acidification effectively deprives freshwater fishes of one important source of information concerning their immediate risk of predation (Leduc *et al.* 2004a; Leduc *et al.* 2006). The goal of this thesis is to identify and quantify changes in behavioural patterns and strategies, and resulting differences in population processes of Atlantic salmon in weakly acidic and neutral streams in the Miramichi River system, Northumberland County, New Brunswick Canada.

Given that fish in weakly acidic streams are deprived of chemical information on risk, I exposed fish in neutral and acidic streams to different combinations of chemical and visual threat cues to quantify differences in antipredator responses. This experiment was conducted over multiple consecutive years (2008 – 2010), during which time the pH levels in a subset of the acidic study streams increased to above the neutral threshold (pH 6.6), effectively controlling for stream-derived differences and allowing interannual comparisons within populations. This experiment was further augmented by reciprocal cross-population transplants between an acidic and a neutral stream, again with the intent of demonstrating that the observed behavioural differences are the result of differences in stream chemistry as opposed to provenance of the test fish. Individual 0^+ Atlantic salmon in a neutral stream were also exposed to neutral and acidified damage-released chemical cues of two sympatric heterospecifics, juvenile brook trout (*Salvelinus fontinalis*) and blacknose dace (*Rhinichthys atratulus*) roughly size-matched to the salmon, in order to quantify the responses of juvenile salmon to heterospecific cues and facilitate a comparison of how ambient acidification influences the responses of fish to conspecific and heterospecific cues.

Direct growth and survival consequences of the acid-mediated loss of chemical alarm cues were estimated through tethering experiments (after Dupuch *et al.* 2009) involving hatchery-reared 0^+ Atlantic salmon of wild provenance to compare relative levels of predation pressure on ecologically naïve fish in neutral and weakly acidic streams. Differences in growth rate, another potential consequence of threat-sensitive antipredator strategies, were evaluated through captive growth trials involving wild-caught fish from a neutral stream transplanted into enclosures in acidic and neutral streams and exposed to different levels of chemically simulated risk for seven day periods. Drift samples were collected in a subset of the study streams to provide relative estimates of forage abundance for comparison with any observed differences in growth rate.

The diversity and composition of fish communities were measured through multiple electrofishing and visual (snorkeling) surveys. While snorkeling surveys tend to underestimate the abundance of stream-dwelling salmonids (Cunjak *et al.* 1988) as well as the overall diversity of local communities and the age-size structure within populations (Thurow and Schill 1996) relative to electrofishing, snorkeling provides the advantage of day-night comparisons facilitated by the independence of this survey method from the photoperiod as electrofishing at night is not allowed under the safety regulations set by Fisheries & Oceans Canada. Based on earlier work demonstrating increased responses to chemical alarm cues in the absence of visual cues at night (Leduc *et al.* 2010b) and equal levels of activity during day and night observation periods (Imre and Boisclair 2004) in

0^+ Atlantic salmon under neutral conditions, snorkeling surveys were conducted at midday and midnight (with the assistance of an underwater flashlight) to determine whether acid-impacted juvenile salmon differ in their activity partitioning in order to maximize the availability of remaining threat cues. Finally, central foraging stations of free-swimming 0^+ salmon were identified and several physical properties of these preferred habitats were compared to those of uninhabited, nearby sites. Collectively, diel shifts in activity patterning and selective avoidance of riskier microhabitat types may serve as compensatory mechanisms which offset acid-mediated loss of chemical information independently of direct fitness-related (growth or survival) consequences.

Chapter 1: Sensory complementation and antipredator behavioural compensation in acid impacted juvenile Atlantic salmon

Introduction

The potential costs incurred by prey following a predation attempt suggest that individuals should benefit from adopting behavioural strategies which may increase their chances of survival. Potential strategies range from avoiding initial detection by predators (Smith 1997) to taking flight and escaping risky situations (Godin 1997) following detection. Potential prey are faced with the need to balance their time and energy expenditures devoted to antipredator behaviours with other fitness-related activities such as foraging (Lima and Dill 1990), likely constraining behavioural responses in proportion to the degree of risk conveyed by a threat cue. The availability of threat cues in turn is determined by the amount released from a cue source as well as environmental conditions that may serve to limit either their transmission through the environment or their detection by receivers. In aquatic environments, visual cues are limited by light availability and other factors such as turbidity (Cronin 1997; Ferrari *et al.* 2010c), while chemical cues may be degraded under particular conditions such as acidity (Leduc *et al.* 2006) and be rendered unavailable to potential receivers. Prey fishes have demonstrated sophisticated abilities both in their ability to detect and discriminate between olfactory cues (reviewed in Ferrari *et al.* 2010a) and other forms (*e.g.*, visual) of publicly available information (Danchin *et al.* 2004) conveying information on risk through different sensory modalities, as well as adopting appropriate responses to complementary forms of information. For example, paired chemical and visual risk cues have been demonstrated to elicit flight responses at significantly greater distances from model predators than visual cues alone in crustaceans (Dalesman and Inchley 2008) and fishes (Kim *et al.* 2009).

Many aquatic taxa, including fishes (Smith 1992), amphibians (Mirza *et al.* 2006) and crustaceans (Hazlett and McLay 2005) produce chemicals which are released into the water following mechanical damage, as would likely occur during a predation event. In fishes, including juvenile Atlantic salmon (*Salmo salar*), these damage-released chemicals are localized in the epidermis and elicit antipredator or alarm responses from conspecifics following detection (Chivers and Smith 1998; Wisenden 2000). The presence of chemical alarm cues and any behavioural responses they may elicit remain undocumented in adult salmon. Upon release, these chemical cues become publicly available and convey qualitatively and quantitatively different information to different receivers, acting as alarm cues to conspecifics and heterospecifics subject to similar predation pressures (Smith 1992) and confer survival advantages during predator encounters (Mirza and Chivers 2000). Recently, acidification has been demonstrated to inhibit the learning (Leduc *et al.* 2004b) and generalization (Brown *et al.* 2012) of novel predator odours. There is also evidence that damage-released chemicals serve as attractant or foraging cues indicating the availability of prey to nearby predators (Elvidge and Brown 2012). Under weakly acidic conditions (pH < 6.4-6.6: Leduc *et al.* 2006), these damage-released chemical cues are rendered non-functional and do not elicit antipredator responses in conspecific receivers, while the effects of acidification on heterospecific transmission remain largely unexplored. This threshold range (pH 6.4 - 6.6) may be chronically exceeded in acidified waterbodies, or temporarily exceeded following acidic precipitation inputs (Leduc *et al.* 2009) in streams supplied by catchment areas with poor buffering or acid neutralizing capacity (ANC). By rendering chemical alarm cues non-functional, weak acidification effectively deprives affected freshwater fishes of an important source of information on their immediate risk of predation.

Whereas previous work on sensory complementation has investigated the additive effects multiple forms of information exert on the behavioural responses of prey receivers (Dalesman and Inchley 2008; Kim *et al.* 2009), changes in information use with cue intensity (Hartman and Abrahams 2000) or the effects of temporary deprivation of particular sensory modalities (Leduc *et al.* 2010b), little remains known about the consequences of long-term sensory deprivation under natural conditions. Chronic weak acidification provides opportunities to examine the long-term ecological effects of effective sensory deprivation on both the usage of remaining public information and the resulting behavioural patterns in the context of predation risk demonstrated between fish populations under natural conditions. To address these questions, I: (1) compared the antipredator responses of individual, free-swimming wild 0^+ Atlantic salmon to familiar and unfamiliar visual threat cues paired with either chemical alarm cues or stream water in four weakly acidic and four neutral nursery streams; (2) compared the interannual variation in antipredator responses of 0^+ salmon in a subset of the study streams after an acidic stream returned to neutral levels; and (3) conducted a paired cross-population transplant experiment under semi-natural conditions between one weakly acidic and one neutral stream in order to eliminate both population differences and acid-mediated physiological impairment as the sources of the observed differences in antipredator behavioural responses between salmon populations. In addition, (4) free-swimming salmon were exposed to neutral and acidified damage-released chemical cues of blacknose dace (*Rhinichthys atratulus*) and brook trout (*Salvelinus fontinalis*), in a neutral stream to directly assess the responses of salmon to heterospecific cues and the effects of acidification on responses to damage-released heterospecific cues.

Materials and Methods

Study sites

All experimental work was carried out during consecutive summers (July and August) in 2009 and 2010 in eight low-order Atlantic salmon nursery streams in the Northwest and Southwest branches of the Miramichi River system, Northumberland Country, New Brunswick, Canada (Figure 1.1, Table 1.1). Study streams were selected based on stream chemistry, the abundance of wild populations of juvenile salmon and involvement in previous studies (*e.g.*, Leduc *et al.* 2006). In all streams, the visual field of the observer was limited by physical barriers such as boulders and not water properties such as turbidity. Streams were classified as either weakly acidic ($pH < 6.6$) or neutral ($pH \geq 6.6$) with each class comprising four streams. Mean physical habitat variables measured at each observation site in 2009 are presented in Table 1.1. This region is characterized geologically by non-carbonate bedrock and shallow soils with low to moderate acid neutralizing capacity (ANC: Environment Canada 2012), resulting in fluctuations in the acidity levels of streams with catchment areas of particularly low ANC in response to precipitation inputs. During the summer of 2010, Northumberland County received relatively little precipitation and experienced unusually high temperatures, including a record 20 year high water temperature (approximately 31° C) recorded in the Little Southwest Miramichi River by the Catamaran Brook Habitat Research Project which resulted in aggregations of 1^+ and 2^+ salmon parr at densities above $100 \cdot m^{-2}$ in thermal refugia created by coldwater seepages and tributaries and outright stress-derived mortality of multi-sea-winter adult Atlantic salmon (R. Cunjak, personal communication).

Visual and chemical threat cues

In order to present the 0^+ salmon with an unfamiliar but realistic model predator, we removed the treble hooks from a weighted rubber fishing lure styled after a juvenile (L_S 140 mm) Northern pike (*Esox lucius*) which was then attached to a thin metal rod by 1 m of clear monofilament fishing line. Northern pike was selected as an unfamiliar predator as it does not occur in the Miramichi River drainage (Government of New Brunswick 2011). An orange rubber ball (Ball; 12 cm diameter) was mounted on one end of a 1 m metal rod to serve as a novel, conspicuously coloured visual cue after Kim *et al.* (2009). Visual threat cues were moved towards focal fish a rate of $\sim 20\ cm \cdot s^{-1}$ from their points of introduction approximately 1.5 m away at either 45° or 315° to the direction of orientation of the fish until a flight response was observed.

Damage-released chemical cues were extracted from hatchery-reared 1^+ salmon donors in 2009 and 0^+ salmon donors in 2010 spawned from wild-caught adults from the Little Southwest Miramichi as part of the restocking efforts of the Miramichi Salmon Association (South Esk, NB, Canada). Previous studies have found no effect of these donor age classes on the antipredator responses of 0^+ salmon (Leduc *et al.* 2006; Kim *et* *al.* 2009). Alarm cue donors were euthanized via cervical dislocation as per Concordia University Animal Care Committee Protocol AREC-2008-BROW. Lateral skin fillets were removed from the donors with a scalpel and placed immediately into chilled distilled water. The fillets were then homogenized, filtered through polyester floss and diluted to a final concentration of 0.1 $cm^2 \cdot ml^{-1}$. Alarm cue samples were packaged in 20 ml aliquots and kept frozen until immediately prior to use. This method and concentration has been shown to reliably induce antipredator responses in a wide range of fish species, including Atlantic salmon, under both natural (Kim *et al.* 2009) and laboratory (Leduc *et al.* 2004a) conditions. Chemical cues were introduced by injecting 10 ml volumes 20 - 50 cm upstream of a focal fish.

Experiment 1a: Complementary responses to risky cues

Free-swimming 0^+ salmon were located visually by snorkeling in an upstream direction, approached to a distance of approximately 1 m at 90° or 270° to the orientation of the subject, and allowed to acclimate to the presence of the observer. Following the resumption of normal foraging activity (typically ~ 2 min), subjects were observed for 5 min, exposed to one of the threat cue treatments, and observed for an additional 5 min. Cue combinations consisted of paired chemical and visual risk cues (AC+Lure), risky visual cues paired with control injections of stream water (Lure), and a novel visual cue paired with a water control (Ball). Behavioural measures recorded during the observation periods were: (1) the number of foraging attempts, directed at the surface, an object in the water column or on the substrate; (2) time spent in contact with the substrate (seconds); and (3) time spent moving (seconds), where moving involved a displacement of at least one body length. These measures were subsequently examined as post- minus pre-stimulus differences; although more complex statistical approaches such as repeated measures (ANOVAR) were explored, comparisons of different models revealed no significant improvement over simple ANOVAs. We also measured the flight initiation distance (FID, cm) from the approaching visual cue and recorded the latency to resume foraging behaviour following stimulus exposure (seconds).

At the end of each trial, the preferred station occupied by the subject was marked with a flagged rock to measure several physical parameters at the observation sites. Flow rate and depth were measured with a Marsh-McBirney Flo-Mate 2000 flow meter (Hach Company, Loveland, CO, USA) and wading rod while pH and temperature were measured with a portable Multiline P4 digital meter (WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). We visually assessed the proportion of visible sky directly over an observation site covered by tree canopy and measured substrate complexity by averaging the deviations from 1 m of a length of steel chain laid across the observation site both perpendicular and parallel to the direction of stream flow (after Leduc *et al.* 2007b). Due to the visual nature of the threat cues, as well as the absence of an assistant during some trial observations, the observer was not blind to treatment.

Experiment 1b: Interannual variation in acidity and behaviour

Despite having been weakly acidic during July and August for several years (2004 - 2007: Leduc 2008; 2008 - 2009: Elvidge *et al.* 2013), Lower Devil's Brook was neutral during the low precipitation year (2010). To evaluate the importance of provenance and environmental acidification under natural conditions, behavioural observations were again conducted on free-swimming 0^+ salmon in Catamaran Brook and Lower Devil's

Brook following the protocol outlined in Experiment 1. In this second experiment, two threat cue combinations were presented consisting of the realistic Northern pike model paired with either stream water or alarm cues (AC+Lure or Lure). Interannual physical habitat measurements recorded at observation sites during this experiment are presented in Table 1.2.

Experiment 1c: Cross-population transplants

During August 2009, wild 0^+ salmon were captured via dipnet from neutral Catamaran Brook and weakly acidic Upper Devil's Brook (*n* = 32 per population). Half of the captured fish were placed into flow-through holding bins (99 l volume; 0.8 m length × 0.46 m width × 0.34 m height) exposed to ambient temperature, current and drift (after Jackson and Brown 2011) in their home stream, while the other half were placed in identical bins in the other stream to achieve a balanced cross-population transplant. Captive fish were allowed to acclimate to ambient stream chemistry and conditions within the holding bins for a period of seven days, after which subjects were placed individually into trial arenas identical to the holding bins for behavioural observations. Observations were conducted with a protocol and experimental treatments similar to those presented in Experiment 2. Physical habitat measurements recorded inside the trial arenas during this experiment are presented in Table 1.3.

Experiment 1d: Heterospecific alarm signaling

During August 2012, additional behavioural observations were conducted on free-swimming 0^+ salmon in Catamaran Brook following the general protocol outlined in

Experiment 1. Alarm cues were extracted from wild-caught blacknose dace and juvenile brook trout donors, diluted with well water and stored as described above. The alarm cues were each divided into two fractions, one of which was left untreated at neutral pH (dace and trout: pH 7.815), while the other fraction was titrated to weakly acidic levels with 9×10^{-3} mol·l^{-1} H_2S0_4 (dace: pH 6.27; trout: pH 6.28). Individual salmon were exposed to 10 ml injections of one of five chemical stimuli: neutral or acidic alarm cues from either donor species or a stream water control (n = 14 for each treatment). Behavioural measures recorded were the number of foraging attempts, time spent moving, latency to resume foraging and the number of aggressive interactions. Aggressive interactions involved some combination of charging, chasing or nipping (Keenleyside and Yamamoto 1962; Grant and Noakes 1988) by the focal fish directed at nearby juvenile salmon, brook trout or blacknose dace, typically followed by a return to its starting location. As this experiment did not involve a visual threat cue, flight initiation distance was not a relevant behavioural measure.

Statistical analyses

Each of the behavioural measures recorded in the first three experiments met the assumptions of normality (homogeneity of error variances, skewness, kurtosis) and were significantly correlated with at least one other measure (Pearson's correlation coefficient, r; $P < 0.05$), justifying their collective treatment as multivariate responses. Preliminary testing found a significant relationship between the multivariate response and pH ($F_{5,182} = 3.109$, $P = 0.017$), but no other physical variables, in all three experiments. Since the alarm response to damage-released chemical cues in 0^+ salmon is only observed above a certain threshold ($pH \geq 6.6$), the streams were divided into weakly acidic or neutral classes. Subsequent analyses focused on the main effects of stream class and experimental stimulus as well as their interaction. In the first three experiments, the multivariate responses were examined in two-way MANOVAs against experimental treatment and stream class to identify which behavioural measures would be retained for further analyses. In all cases, one behavioural measure, time spent on substrate, did not vary with any main effects factor and was excluded from all analyses. The retained measures were then examined as general linear models with ANOVA using a significance level of $\alpha = 0.1$ to avoid committing a potential Type II error by failing to reject a biologically false null hypothesis based on overly rigorous statistical criteria, given the relatively high levels of noise and low sample sizes in these datasets. The data from the first experiment were examined with a two-way ANOVA with treatment and stream class as main effects. In the second experiment, the multivariate response was examined in a one-way MANOVA with year (2009 or 2010) as main factors; the data were then divided by year, and the significant behavioural measures were analyzed in two-way MANOVAs against stream and treatment. In the third experiment, the multivariate response was examined in a three-way MANOVA against treatment, stream of origin and transplanted stream. Individually significant behavioural measures were examined in three-way ANOVAs against treatment, transplant stream and stream of origin. In the fourth experiment, two of the four behavioural measures, difference in foraging and latency to resume foraging, demonstrated significant positive skew and/or leptokurtosis, while difference in time moving displayed significant error heterogeneity between treatments. Accordingly, these data were ranked for one-way parametric analyses against treatment as per the Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf 1995; Brown *et al.* 2009). The fourth measure, change in the number of aggressive interactions, met the assumptions of normality and was not transformed. For all experiments, any behavioural measure which varied significantly with one or more main factor was retained and examined using *post-hoc* Tukey's HSD tests. All analyses were conducted using R version 2.13.1 (R Development Core Team 2011) and figures were generated using the *gplots* package (Warnes 2011).

Results

Experiment 1a: Complementary responses to risky cues

Free-swimming 0^+ Atlantic salmon in the two classes of study streams did not demonstrate any significant differences in their baseline (pre-stimulus) behavioural measures (*i.e.* number of foraging attempts, time spent moving, and time spent on the substrate; Table 1.4). The multivariate behavioural responses of subjects were significantly influenced by experimental treatment and the interaction between treatment and stream class, but not stream class as a main effect (Table 1.5). Interaction plots showing the relationships between four of the behavioural measures, treatment and stream class, as well as pairwise differences between treatments from *post-hoc* Tukey's tests are shown in Figure 1.2. In three of the responses (number of foraging attempts: Figure 1.2a,b; time spent moving: Figure 1.2c,d; FID: Figure 1e,f), there is an apparent treatment × class interaction driven by differences in response to the paired chemical and visual cues (AC+Lure) between classes, although this interaction only has a statistically significant effect on FID (Table 1.5). Mean responses to the three threat cue combinations do not differ significantly from each other in acidic streams, whereas fish in neutral streams demonstrated significantly different mean responses to the different cue combinations in their foraging activity (Figure 1.2b), FID (Figure 1.2f) and latency to resume foraging (Figure 1.2h).

Mean responses to the paired visual and chemical cues (AC+Lure) differed significantly from the responses to the other cues in the observed number of foraging attempts (Figure 1.2b), FID (Figure 1.2f) and latency to resume foraging (Figure 1.2h) in the neutral but not the acidic streams, suggesting that this particular treatment (AC+Lure) is driving the significant interaction between stream class and treatment in the multivariate model. With the removal of the paired chemical and visual cue (AC+Lure) treatment from the analysis, fish in acidic streams displayed significantly greater antipredator responses to the visual cues than fish in neutral streams in their foraging behaviour, FID and latency to resume foraging following cue exposure. Mean responses to the visual cues in the absence of chemical cues (Ball and Lure) in each stream class are shown in Figure 1.3. These results suggest that acid-impacted fish assign greater importance to remaining threat cues, whereas fish under neutral conditions are able to mediate their responses to solitary cues relative to multiple cues in a threat-sensitive manner (Chivers *et al.* 2001; Ferrari *et al.* 2005; Brown *et al.* 2006).

Experiment 1b: Interannual variation in acidity

The three-way factorial MANOVA revealed significant main effects of treatment and year on the multivariate response (Table 1.6). Since the three-way interaction did not have significant effects on either the multivariate or univariate responses, it was excluded from all analyses. As individual behavioural responses, the amount of time spent moving did not differ significantly, while the number of foraging attempts differed between treatments, as did the amount of time spent on the substrate to both treatment and year, although their interaction was not significant. FID differed significantly with treatment, year, and the two-way interactions between treatment and year and treatment and stream. Latency to resume foraging differed between treatment and stream, with a significant two-way interaction between treatment and year (Table 1.6). The interactions between treatment, year and stream in the latter two behavioural measures as revealed by *post-hoc* Tukey's tests on the interannual data from each stream are presented in Figure 1.4. Whereas the responses demonstrated by fish to the two treatments in perennially neutral Catamaran Brook do not differ between years, fish under the fluctuating acidic conditions in Lower Devil's Brook demonstrated significantly greater responses to the paired chemical and visual cues (AC+Lure) during the neutral year (2009) than they did during the acidic year (2010).

Experiment 1c: Cross-population transplants

In the full three-way factorial MANOVA model, each of the three main factors significantly influenced the multivariate response, as did the interaction between test stream and treatment (Table 1.7). The three-way interaction again did not significantly influence either the multivariate or univariate responses and was excluded from all analyses as in Experiment 1b. The number of foraging attempts did not differ significantly with any main factor or interaction. Time spent moving differed significantly with provenance of the test fish as did time spent on the substrate. Latency to resume foraging differed with both test stream and provenance, while FID differed with treatment, test stream and the two-way interaction between test stream and treatment (Table 1.7).

Mean behavioural responses of subjects from both populations to the experimental treatments appear to be qualitatively similar between the stream of testing (Figure 1.5a,b), with complementary (additive) effects of paired visual and chemical cues (AC+Lure) compared to the visual cue alone under neutral conditions in Catamaran Brook as evidenced by significantly greater FIDs (Figure 1.5a). Similar but non-significant trends are evident in the difference in number of foraging attempts and time spent moving. Subjects demonstrated significantly greater latencies to resume foraging in the acidic Upper Devil's Brook (Figure 1.5b), and the absence of significant differences in response to either cue combination suggests that regardless of provenance, subjects are not detecting or responding to the chemical cue under acidic conditions. Divided by provenance, transplanted Upper Devil's Brook and resident fish demonstrated significantly greater FIDs in Catamaran Brook than in Upper Devil's Brook (Figure 1.5c,e), with neither population demonstrating differences in their flight responses to the cue combinations. Both populations, however, showed significantly greater latencies to resume foraging in the acidic Upper Devil's Brook than in the neutral Catamaran Brook (Figure 1.5d,f).

Experiment 1d: Heterospecific alarm signaling

The overall MANOVA comparing the four behavioural measures against chemical treatment was not significant (P = 0.226; Table 1.8), although the differences in number of aggressive interactions (Figure 1.6c) and the latency to resume foraging (Figure 1.6d) both showed significant effects of treatment (P = 0.028 and P = 0.073, respectively). *Post-hoc* Tukey's HSD tests on these two measures in one-way ANOVAs indicates significant differences between the neutral trout and acidified dace cues on change in aggression (P = 0.031) and between the neutral trout and the control on latency to resume foraging (P = 0.094). Despite the absence of statistically significant differences between treatment effects, there appears to be a consistent trend towards neutral cues eliciting responses consistent with antipredator behaviours, and responses to acidified cues more closely resembling responses to the control treatment (Figure 1.6). A secondary analysis on a subset of the data with the control treatment removed consisted of a two-way MANOVA against cue donor species (trout or dace) and cue class (neutral or acidic). In the overall factorial test, cue class significantly influenced the multivariate response ($F_{4,49}$ = 2.98, P = 0.028). As individual responses, aggressive interactions ($F_{1,52}$ = 8.25, P = 0.006) and latency to resume foraging ($F_{1,52}$ = 3.42, P = 0.07) both varied significantly with cue class, with neutral cues eliciting responses consistent with antipredator behaviours and acidified cues eliciting responses qualitatively similar to the control (Figure 1.6, closed bars).

Discussion

Fishes, including juvenile Atlantic salmon, living under weakly acidic conditions are deprived of an important source of chemosensory information from both conspecific and heterospecific senders on predation risk that is publicly available under neutral (pH ≥ 6.6) conditions. This study provides evidence that chemosensory deprivation as a result of weak acidification results in significantly greater antipredator responses to remaining visual threat cues compared to fishes in neutral streams. Additionally, under neutral conditions fishes demonstrate complimentary responses to paired threat cues when multiple cues are available. Interannual variation in antipredator responses of juvenile salmon in Devil's Brook, which was weakly acidic during the summer of 2009 but not during 2010, follows this pattern, with fish demonstrating both the ability to detect chemical cues as well as complimentary responses to paired cues under neutral conditions during the summer of 2010. Similarly, fish involved in the cross-population transplant demonstrated both chemosensory and complimentary responses to paired risky cues in the neutral stream, and greater mean responses to the threat cues presented in the acidic stream, regardless of their own provenance. Collectively, these results provide strong evidence for environmental mediation of antipredator behaviours in freshwater fishes, with environmental constraints potentially eliminating particular forms of sensory information and limiting the ability of prey to adopt threat-sensitive responses to different levels of risk conveyed by remaining cues.

The relatively greater responses demonstrated to the remaining cues under acidic conditions are consistent with compensatory behavioural responses arising from the acid-mediated loss of information and are representative of indirect or non-lethal effects of predation on prey populations (Preisser *et al.* 2005). Different levels of actual or perceived predation risk between fish populations have been found to exert significant influences on fitness, with high-predation populations demonstrating greater fitness under conditions of high resource abundance than low-predation populations. Conversely, the high-predation population demonstrated lower fitness than the low-predation population under conditions of limited resource availability (Walsh and Reznick 2008; Walsh 2013). Walsh (2013) attributed this difference to altered foraging activity in response to cascading trophic effects, as greater resource abundance may be common in high-predation environments which are likely to be characterized by relatively lower density or abundance of a prey (consumer) species than are low-risk habitats. Under conditions of resource limitation, *e.g.*, as a result of habitat degradation or through time-lagged trophic cascade effects, high-predation populations may be more vulnerable to stochastic effects than their low-predation counterparts.

Optimization theory applied to predator-prey interactions predicts that prey individuals adopt appropriate or threat-sensitive responses to available public cues indicating elevated risk in order to minimize any fitness costs associated with antipredator behaviours (Lima and Dill 1990). Detection of novel cues concurrently with familiar risky cues may contribute to future assessment of predation risk through associative learning (releaser-induced recognition learning: Suboski 1990). In fishes, this learning mechanism has been demonstrated in response to learned visual (Hall and Suboski 1995) and chemical (Leduc *et al.* 2007b) cues following their paired association with familiar cues (*e.g.*, damage-released conspecific chemical alarm cues) indicating elevated risk. Repeated previous instances of paired learning may be the mechanism enabling individuals living under neutral conditions and experienced with both visual and chemical threat cues to respond appropriately to different risk levels. Whereas fish under neutral conditions demonstrate complementary (synergistic or additive) antipredator behavioural effects (Kim *et al.* 2009) upon detection of paired chemical and visual cues consistent with responses proportional to the perceived threat level, fish under acidic conditions are unable to develop similarly modulated responses and consequently demonstrate significantly greater responses to any remaining cues. These apparent compensatory behavioural responses to visual cues under acidic conditions may result from the absence of previous learned-association opportunities resulting from environmental deprivation of an important form of public chemosensory information. Prior learning, conversely, may have limited or decreased the level of threat conveyed by visual cues in the absence of both conspecific and heterospecific chemical cues under neutral conditions.

The detection of multiple learned or familiar complementary cues in close temporal proximity to each other may elicit behavioural responses from a receiver which differ significantly from those displayed in response to individual cues (Mikheev *et al.* 2006) in additive, non-additive (Schmidt 2006) or complementary fashion (Ferrari *et al.* 2008). The sensory compensation model proposed by Hartman & Abrahams (2000) suggests that the importance of the information conveyed by chemical alarm cues is mediated by the risk level conveyed by visual cues such that under optimal visual conditions, chemical cues do not elicit antipredator or alarm responses. Such a unidirectional compensatory mechanism makes the implicit assumption that chemical information is of secondary importance to visual information. Subsequent studies have provided some support for this by demonstrating the inverse effect, *i.e.* greater antipredator responses to chemical cues under sub-optimal visual conditions at night (Leduc *et al.* 2010b) and with increasing turbidity (Ferrari *et al.* 2010c) in aquatic systems. The findings of the present study, in demonstrating significantly greater responses to visual cues when chemical cues are unavailable, strongly imply that there is no general hierarchy of information in aquatic conditions and instead the relative importance of different sensory modalities and the information thereby received is determined by extrinsic environmental constraints. This could have considerable ecological relevance for acid-impacted fishes, as chemical cues are potentially available over greater spatio-temporal scales than visual cues. Fishes relying on visual information in the absence of chemical cues are likely to detect risky visual cues in closer spatial proximity to the threat source, decreasing the likelihood of avoiding a predation attempt and potentially incurring increased survival consequences (Mirza and Chivers 2000).

The sensory complementation hypothesis (Ferrari *et al.* 2008) has thus far been applied to the additive, non-additive or synergistic responses elicited by multiple cues. While different sensory modalities provide quantitatively different information varying in quality and reliability, selection pressure is predicted to strongly shape the ability to respond to any available information. Threat-sensitive or complementary responses to multiple cues, when available, would greatly enhance receiver survival while concurrently reducing the likelihood of making costly errors by responding in hypersensitive fashion to less risky cues (Amo *et al.* 2004). The present findings indicating that prey individuals can compensate behaviourally for the loss or impairment of a sensory modality by adopting significantly greater responses to remaining sources of information are in direct contrast to the sensory compensation model, and it is therefore proposed that information use be considered in the context of sensory complementation eliciting compensatory behaviours amongst impacted individuals.

It remains unknown whether wild, acid-impacted juvenile salmon suffer indirect fitness or survival consequences (*sensu* Preisser *et al.* 2005) resulting from this loss of chemical information, although chemical alarm cue usage has been demonstrated to enhance survival of predator encounters under both natural (Mirza and Chivers 2000) and laboratory conditions (Mirza and Chivers 2001b; Mirza and Chivers 2003). Future studies examining the effects of public information on predator-prey interactions under natural conditions would benefit from greater attention to environmental influences, including the effects of anthropogenic stressors, and limitations on information availability and use by receivers. Given the widespread occurrence of natural and anthropogenically acidified waterbodies (Muniz 1990) and recent predictions that acidified waters with low or depleted acid neutralizing capacities will remain acidic for decades even in the absence of continued acid inputs (Clair *et al.* 2007), this loss of information and threat-sensitivity to multiple cues may exert deleterious effects on populations of aquatic species throughout affected areas (Preisser *et al.* 2005). The occurrence of such behavioural stressors in wild fish at acidity levels well above the thresholds for physiological impairment (Kroglund *et al.* 2007a) suggests that conservation and remediation efforts may benefit from adopting more conservative acidification thresholds, particularly in the context of cost-intensive restocking efforts, as are widespread throughout historical Atlantic salmon distributions. Due to the high initial mortality through predation incurred by newly released, hatchery-reared fishes (Brown and Laland 2001), such restocking efforts should seek to exclude recipient streams which limit the amount and types of information on predation risk available to inexperienced fish.

Table 1.1: GPS coordinates and physical habitat measurements at sites preferred by 0^+ Atlantic salmon (*Salmo salar*) in eight study streams in Northumberland County, New Brunswick, Canada in 2009.

Stream	**Class**	**Location**		**pH**	**Temperature (°C)**	**Depth (m)**	**Flow** $(\mathbf{m\ s^{-1}})$	**Substrate Complexity**	**Canopy Cover**
Little Southwest	Neutral	46°52.807’ N	66°06.418’ W	**7.06**	19.24	0.35	0.406	0.732	**0.167**
Catamaran Brook	Neutral	46°52.747’ N	66°06.235’ W	**7.29**	18.96	0.33	0.341	0.862	**0.045**
Renous River	Neutral	46°47.681’ N	66°11.820’ W	**6.96**	16.70	0.39	0.183	0.828	**0.000**
North Pole Stream	Neutral	49°59.071’ N	66°31.167’ W	**6.89**	20.35	0.37	0.166	0.802	**0.010**
Rocky Brook	Acidic	46°47.490’ N	66°28.658’ W	**6.56**	21.67	0.48	0.306	0.773	**0.063**
Lower Devil’s Brook	Acidic	46°52.377’ N	66°13.545’ W	**6.38**	19.15	0.26	0.239	0.799	**0.323**
Upper Devil’s Brook	Acidic	46°52.386’ N	66°13.610’ W	**6.12**	16.44	0.23	0.305	0.775	**0.932**
Correy Creek	Acidic	46°52.424’ N	66°13.603’ W	**6.36**	18.89	0.34	0.170	0.799	**0.521**

All trials were conducted within 200 m of the specified locations. Habitat variables are mean values for $n = 24$ measurements per stream. Bold-face values indicate Bonferroni-corrected significant differences ($P < 0.008$) in that measure between acidic (pH < 6.6) and neutral stream classes.

Table 1.2: Interannual variation in mean physical habitat measurements at sites preferred by 0^+ Atlantic salmon (*Salmo salar*) in two study streams.

Stream	**Year**	**pH**	**Temperature (°C)**	**Depth (m)**	**Flow (m/s)**	**Substrate Complexity**	**Canopy Cover**
Catamaran Brook	2009	**7.29**	18.72	**0.34**	0.35*	**0.867**	**0.055**
	2010	**7.54**	18.69	**0.28**	0.08*	**0.866**	**0.070**
Devil's Brook	2009	**6.38**	19.15	**0.27**	0.25*	**0.797***	**0.336***
	2010	**6.79**	18.88	**0.27**	0.07*	**0.684***	**0.102***

Bold font indicates significant differences between streams, asterisks indicate significant differences ($P < 0.05$) between years within streams; $n = 16$ for each.

Table 1.3: Mean physical habitat variables measured inside trial arenas in two streams used for a cross-population transplant experiment.

Stream	**pH**	**Temperature (°C)**	**Depth (m)**	**Flow (m/s)**	**Canopy Cover**
Catamaran Brook	**7.37**	**17.95**	0.12	0.10	0.75
Upper Devil's Brook	**6.30**	**12.16**	0.12	0.10	0.75

Bold font indicates significant differences ($P < 0.05$) between streams; $n = 32$ for each.

Table 1.4: Comparison of baseline behavioural measures in free-swimming 0^+ Atlantic salmon (*Salmo salar*) in the four neutral and four weakly acidic streams in Experiment 1a.

Response	**Stream class**				**Treatment**				**Class:Treatment**			
	Pillai's Trace	*F*	df	*P*	Pillai's Trace	*F*	df	*P*	Pillai's Trace	*F*	df	P
Multi-variate	0.016	1.02	3,184	0.39	0.011	0.35	6,370	0.91	0.038	1.20	6,370	0.31
Foraging		0.21	1,186	0.65		0.49	2,186	0.61		1.37	2,186	0.26
Time moving		0.34	1,186	0.56		0.23	2,186	0.79		0.39	2,186	0.68
Time on substrate		0.51	1,186	0.48		0.15	2,186	0.87		1.13	2,186	0.33

Table 1.5: Results of two-way MANOVA on the effects of stream class and treatment on behavioural measures demonstrated by free-swimming 0^+ Atlantic salmon (*Salmo salar*).

Response	**Treatment**			**Class**			**Treatment: Class**		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
Multivariate	2.20	8, 368	**0.027**	1.57	4, 183	0.18	2.25	8, 368	**0.023**
Foraging	3.07	2, 168	**0.049**	3.85	1, 168	*0.051*	1.64	2, 168	0.19
Time moving	1.20	2, 168	0.30	3.31	1, 168	*0.071*	0.46	2, 168	0.64
Latency	2.95	2, 168	*0.055*	0.32	1, 168	0.57	0.73	2, 168	0.49
FID	3.24	2, 168	**0.041**	0.07	1, 168	0.80	7.49	2, 186	**<0.001**

Bold type indicate significance at $\alpha = 0.05$, italics indicate significance at $0.1 > \alpha > 0.05$. Pillai's trace < 0.13 for multivariate tests.

Table 1.6: Results of three-way MANOVA on the interannual variation in response by free-swimming 0^+ Atlantic salmon (*Salmo salar*) to threat cues in Catamaran Brook (neutral in 2009 & 2010) and Devil's Brook (weakly acidic in 2009 & neutral in 2010).

Response	**Treatment**		**Stream**		**Year**		**Treatment:Stream**		**Year:Treatment**		**Stream:Year**	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Multivariate	6.11	**<0.0001**	2.45	**0.04**	10.83	**<0.0001**	1.78	0.13	3.27	**0.0095**	1.47	0.21
Foraging	18.79	**<0.0001**	0.25	0.62	0.63	0.43	0.34	0.56	1.26	0.27	1.79	0.18
Time moving	1.54	0.22	0.78	0.38	0.89	0.35	0.01	0.99	2.34	0.13	0.24	0.62
Time on substrate	5.33	**0.023**	3.01	*0.086*	8.87	**0.0037**	4.29	**0.041**	1.58	0.21	1.89	0.17
Latency	2.39	0.13	8.74	**0.004**	0.26	0.61	0.77	0.38	4.28	**0.041**	1.68	0.19
FID	13.19	**<0.001**	1.73	0.19	32.19	**<0.0001**	4.92	**0.029**	13.12	**<0.001**	2.05	0.16

Bold type indicate significance at $\alpha = 0.05$, italics indicate significance at $0.1 > \alpha > 0.05$. Pillai's trace < 0.38 for all multivariate tests.

Table 1.7: Results of three-way MANOVA on the effects of treatment, origin and test stream on the variation in response by captive 0^+ Atlantic salmon (*Salmo salar*) in a cross-population transplant between Catamaran Brook (neutral) and Devil's Brook (weakly acidic).

Response	**Test Stream**		**Origin**		**Treatment**		**Stream:Origin**		**Stream:Treatment**		**Origin:Treatment**	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	P
Multivariate	2.91	**0.022**	2.48	**0.043**	5.97	**<0.001**	0.64	0.67	8.04	**<0.0001**	0.79	0.56
Foraging	0.04	0.84	0.03	0.87	0.23	0.63	0.28	0.59	0.23	0.63	0.09	0.77
Time moving	0.13	0.72	3.61	*0.062*	0.03	0.86	0.01	0.94	0.48	0.49	0.03	0.86
Time on substrate	0.12	0.73	3.22	*0.078*	0.01	0.94	0.06	0.80	0.04	0.84	1.11	0.29
Latency	8.76	**0.0045**	6.83	**0.011**	0.01	0.93	<0.01	0.97	0.07	0.78	0.02	0.88
FID	6.80	**0.012**	0.02	0.89	31.68	**<0.0001**	3.18	*0.079*	41.62	**<0.0001**	1.53	0.22

Bold type indicate significance at α = 0.05, italics indicate significance at 0.1> α > 0.05. Pillai's trace < 0.44 for all multivariate tests.

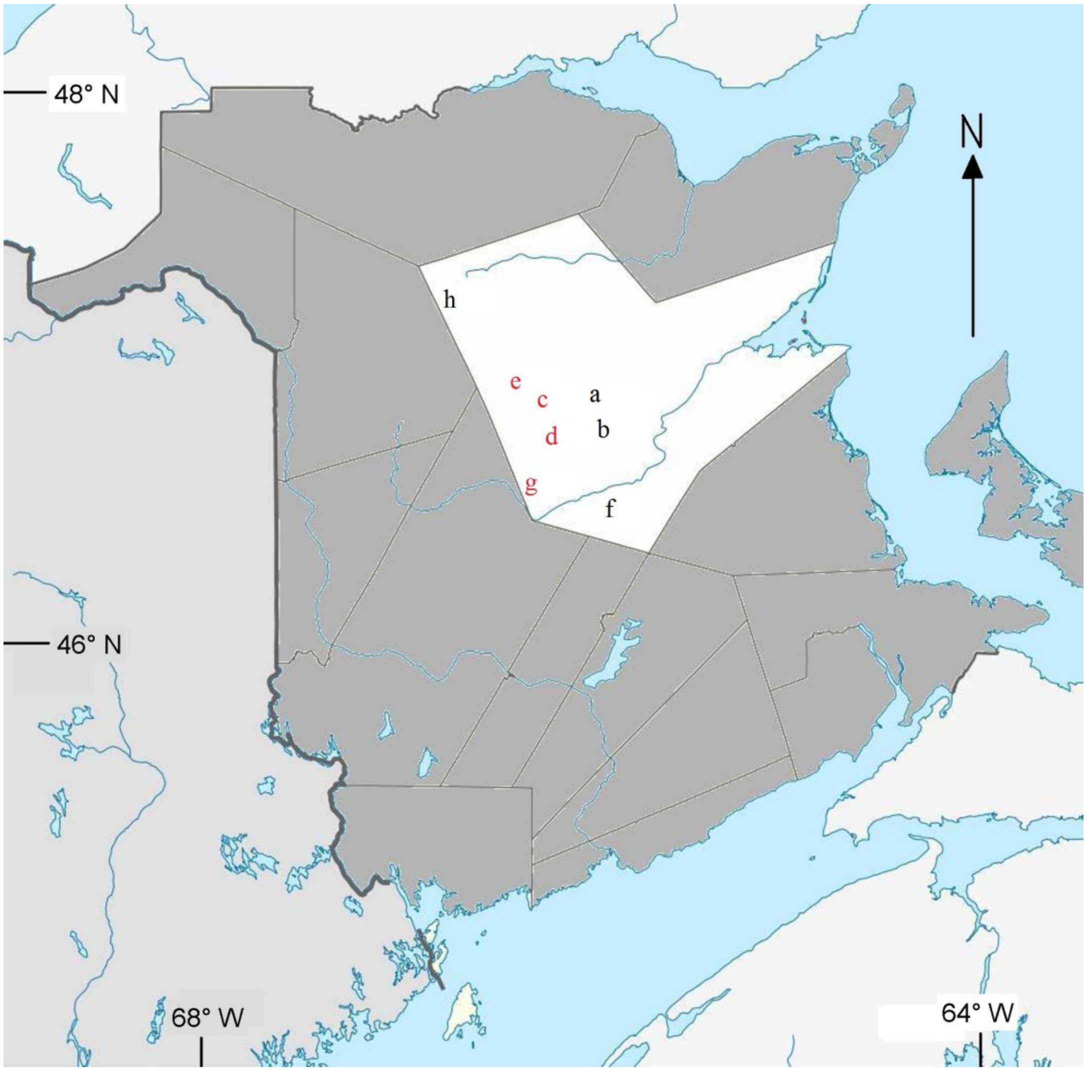

Figure 1.1: Map of the study area in Northumberland County, NB, Canada (white section), adjacent to the Gulf of St. Lawrence, showing the neutral (black letters) and acidic (red letters) Atlantic salmon (*Salmo salar*) nursery streams. (a) Little Southwest Miramichi (neutral); (b) Catamaran Brook (neutral); (c) Lower Devil's Brook (acidic); (d) Upper Devil's Brook (acidic); (e) Correy Creek (acidic); (f) North branch Renous River (neutral); (g) Rocky Brook (acidic); (h) North Pole Stream (neutral). GPS coordinates and physical attributes of the study sites are presented in Table 1.1. Image modified from http://en.wikipedia.org/wiki/File:Map_of_New_Brunswick_highlighting_Northumberland_County.png

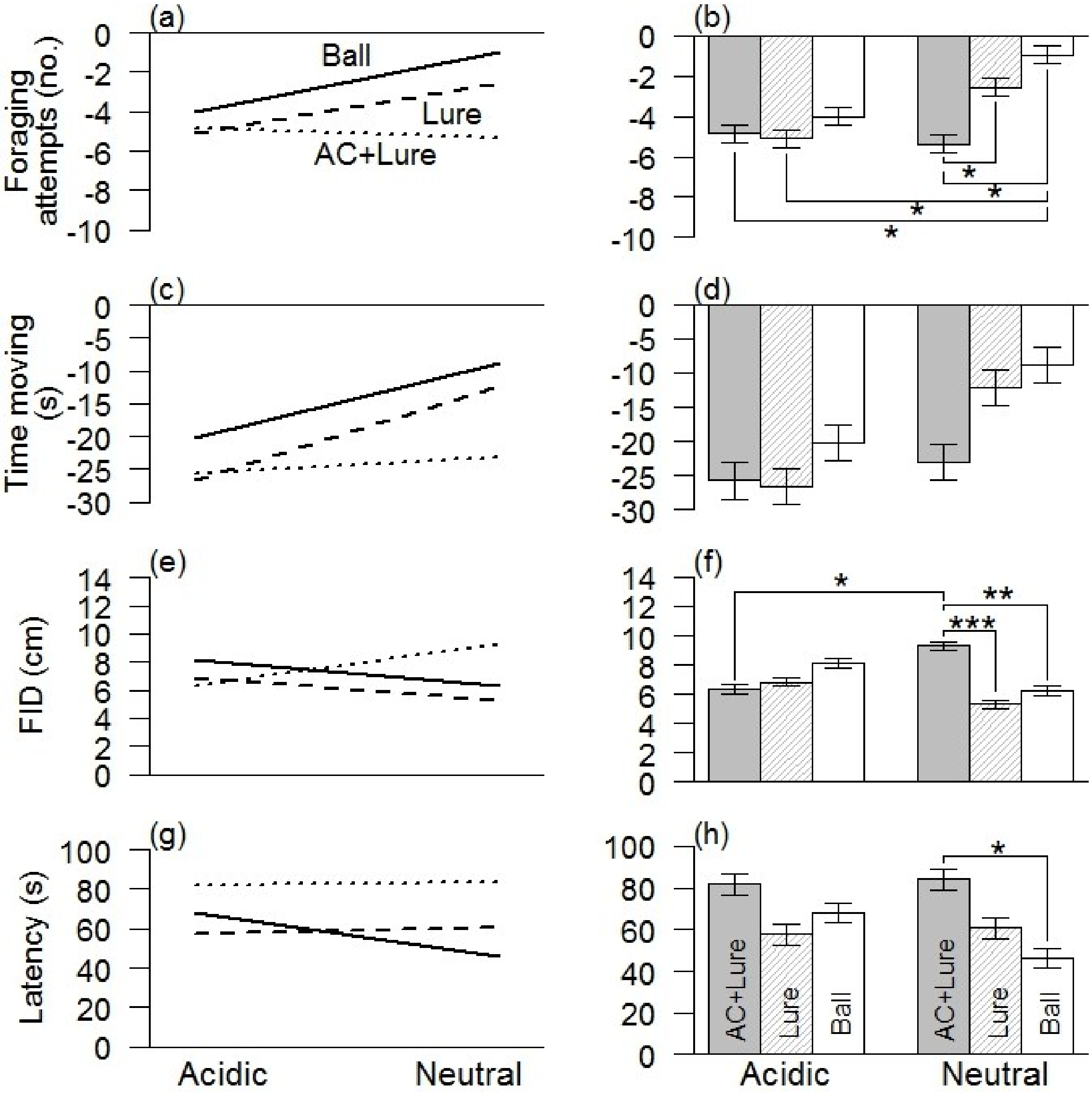

Figure 1.2: Behavioural responses of free-swimming 0^+ Atlantic salmon (*Salmo salar*) in weakly acidic or neutral streams and interactions between these stream classes and three predatory threat cue treatments. Cue treatments consisted of paired realistic chemical and visual cues (AC+Lure), realistic visual cues paired with a chemical control (Lure) and a novel visual cue paired with the chemical control (Ball). Mean differences in response between stream classes are illustrated in the left column while standard errors of the means and significant differences between treatments are illustrated in the right column.

Responses represent post- minus pre-stimulus differences of individual *S. salar* in (a,b) number of foraging attempts and (c,d) time spent moving (seconds), (e,f) flight initiation distance (FID, cm) from the visual cues and (g,h) latency to resume foraging (seconds) following cue exposure. Asterisks denote significant differences between treatment combinations as revealed by *post-hoc* Tukey's HSD tests (*** = $P < 0.001$, * = $P < 0.01$, * = $P < 0.1$; $n = 32$ for each bar).

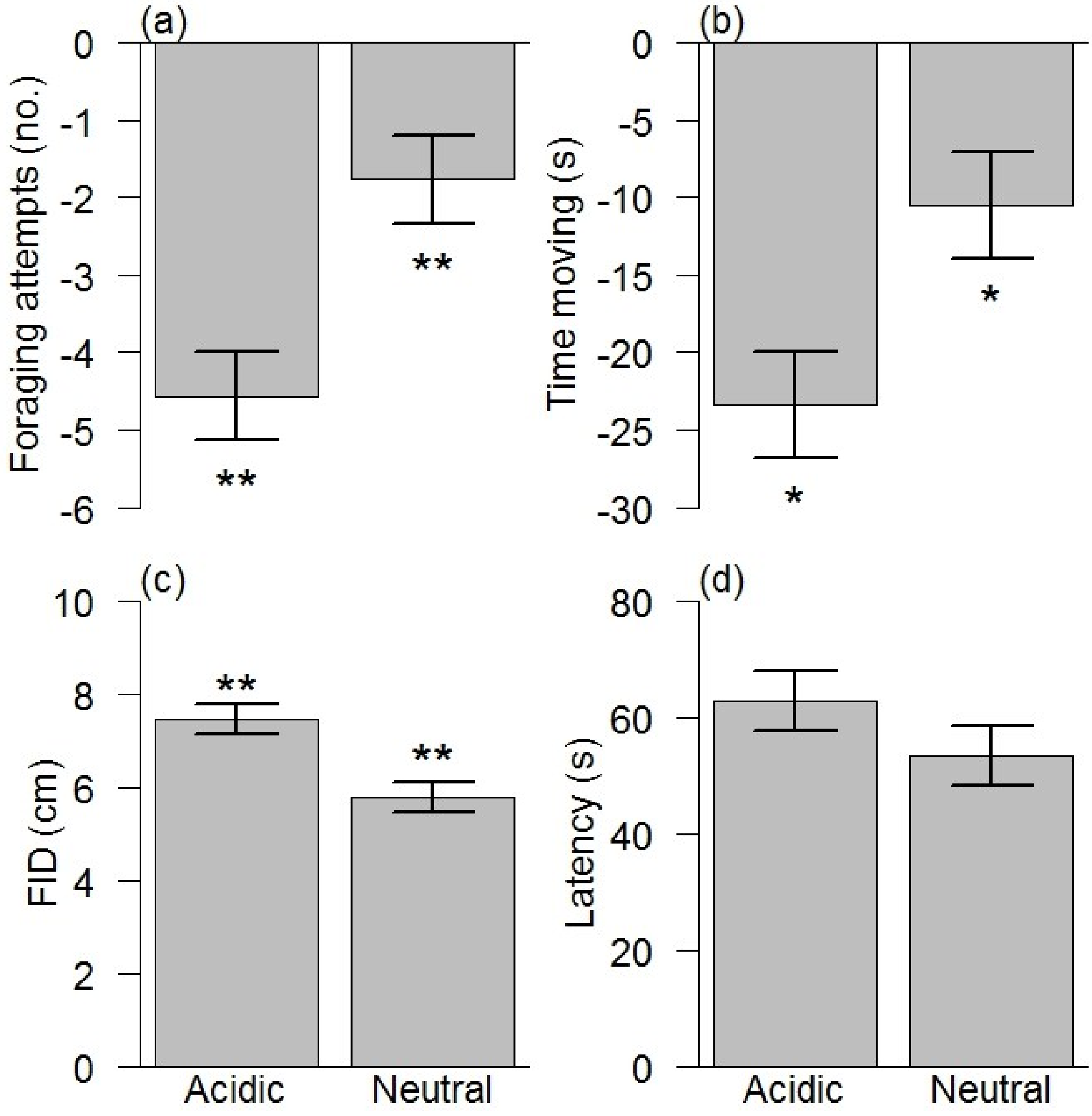

Figure 1.3: Behavioural responses (mean ± SE) of 0^+ Atlantic salmon (*Salmo salar*) to the visual threat cues (Lure and Ball together) in the acidic and neutral streams. Post- minus pre-stimulus differences in (a) number of foraging attempts and (b) time spent moving (seconds); (c) flight initiation distance from the visual cue (FID, cm) and (d) latency to resume foraging following cue exposure (seconds). Asterisks denote significant differences between treatment combinations as revealed by *post-hoc* Tukey's HSD tests (* = $P < 0.1$, ** = $P < 0.05$; $n = 64$ for each bar).

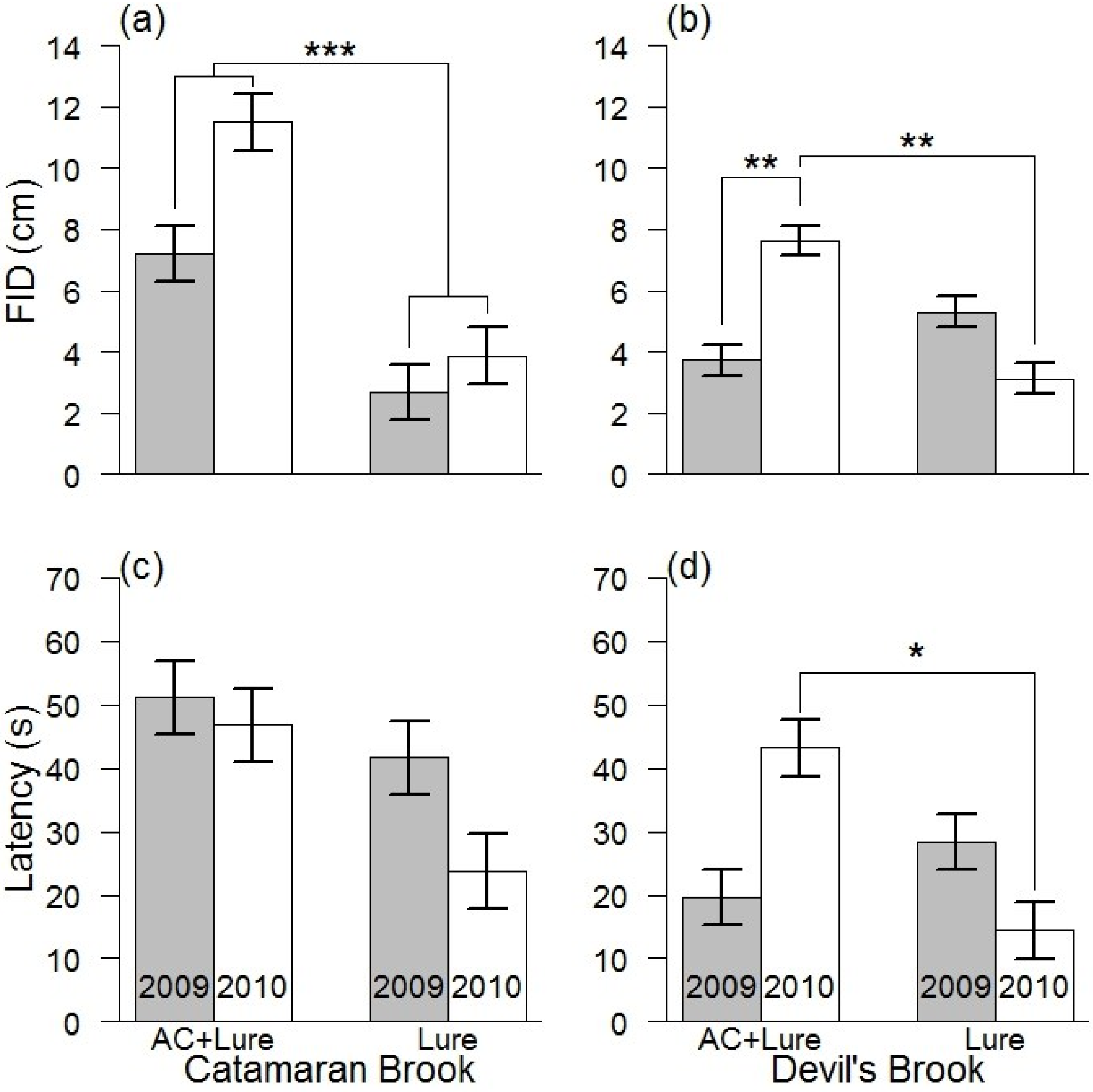

Figure 1.4: Interannual variation in antipredator behaviour of 0^+ Atlantic salmon (*Salmo salar*) from 2009 to 2010 exposed to a combination of visual and chemical threat cues (AC+Lure) or visual threat cues combined with a chemical control (Lure) in Catamaran Brook (neutral both years) and Lower Devil's Brook (acidic in 2009, neutral in 2010). Post- minus pre-stimulus differences in (a,b) flight initiation distance from the visual cue (FID, cm) and (c,d) latency to resume foraging following cue exposure (seconds).

Asterisks indicate significant differences between year × treatment combinations within each stream as revealed by *post-hoc* Tukey's HSD tests (* = $P < 0.1$, ** = $P < 0.05$, *** = $P < 0.001$; $n = 8$ for each bar).

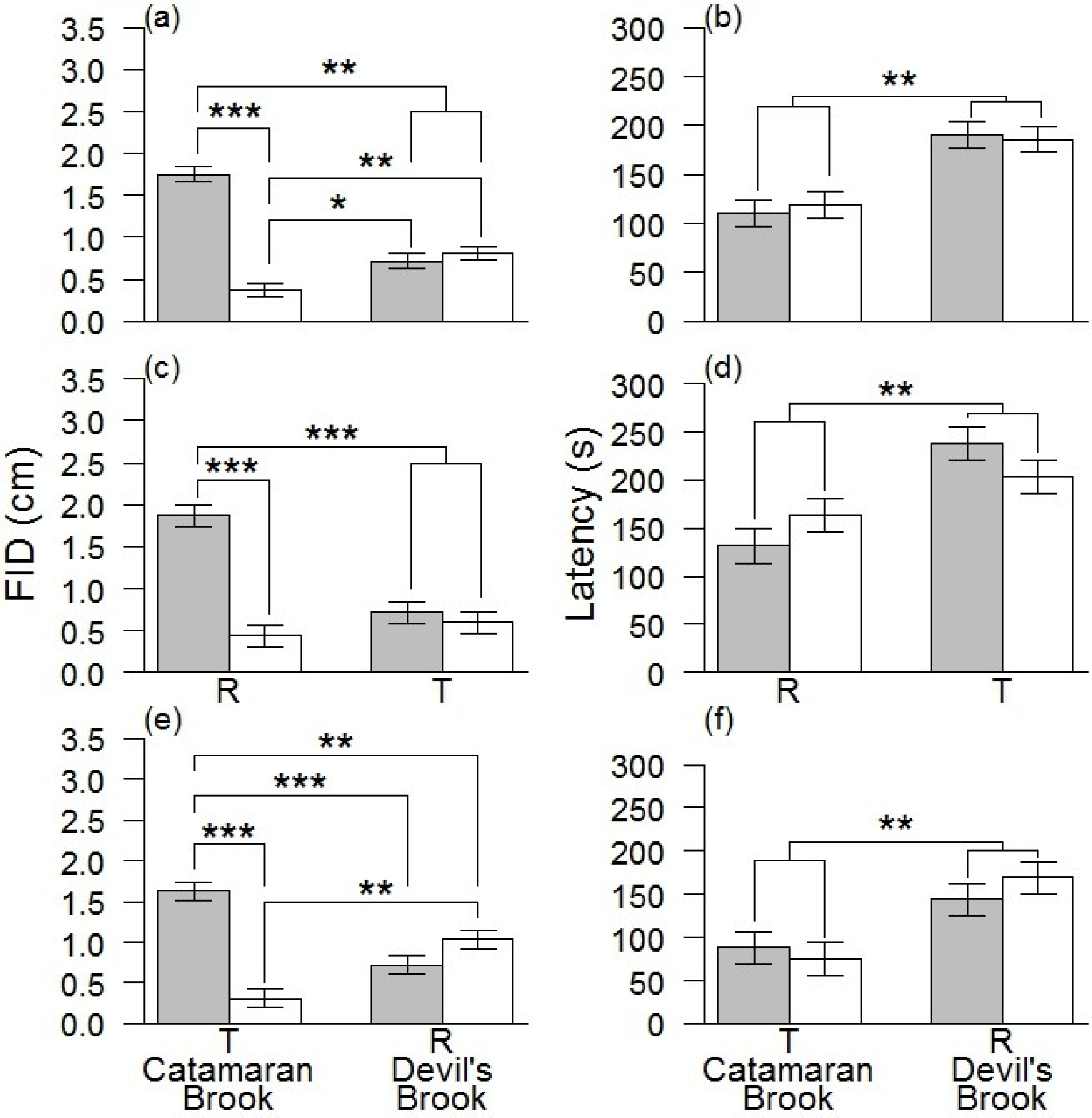

Figure 1.5: Behavioural responses (mean ± SE) of 0^+ Atlantic salmon (*Salmo salar*) to a realistic predator model paired with chemical alarm cues (shaded bars) or a realistic predator model paired with a chemical control (open bars) in a cross-population transplant experiment under semi-natural conditions. Flight initiation distance (FID, cm, left column) and latency to resume foraging (seconds, right column) following stimulus exposure in (a,b) all test fish in both test streams (*n* = 32 for each bar); (c,d) fish from neutral Catamaran Brook in each stream (R = Resident, T = Transplant; *n* = 8 for each

bar); (e,f) fish from acidic Devil's Brook in each stream (R = Resident, T = Transplant; n = 8 for each bar). Asterisks denote significant differences between treatments as revealed by *post-hoc* Tukey's HSD tests (*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.1$).

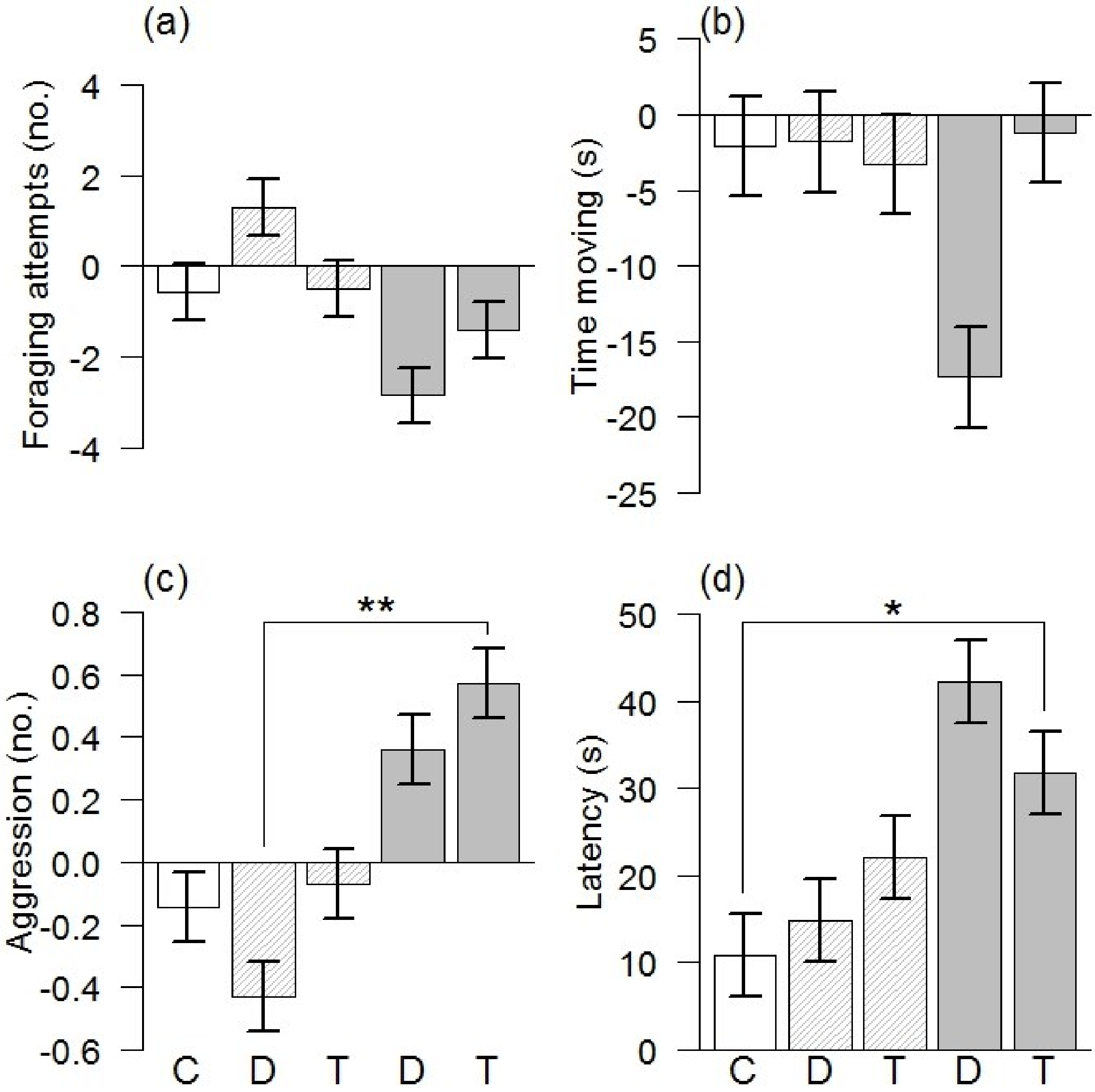

Figure 1.6: Behavioural responses (mean ± SE) of 0^+ Atlantic salmon (*Salmo salar*) to neutral (pH > 6.5, closed bars) or acidified (pH < 6.5, shaded bars) damage-released alarm cues from heterospecifics (D, blacknose dace [*Rhinichthys atratulus*]; T, brook trout [*Salvelinus fontinalis*]) or a stream water control (open bars, C) in Catamaran Brook. Differences in (a) number of foraging attempts, (b) time spent moving and (c) number of aggressive interactions between pre- and post-stimulus observation periods, and (d) latency to resume foraging following stimulus exposure. Asterisks denote

significant differences between treatments as revealed by *post-hoc* Tukey's HSD tests (** = $P < 0.05$, * = $P < 0.1$; $n = 14$ for each bar).

Chapter 2: Direct predation costs of impaired chemosensory communication

Introduction

Predator-prey interactions are one of the most studied phenomena in ecology, particularly with regard to the prey who face greater potential fitness costs as a result of 'losing' during a predation event than do predators (Lima and Dill 1990). Prey species, including freshwater vertebrates like fishes, have demonstrated considerable sophistication in their abilities to detect and respond to different public (Danchin *et al.* 2004) olfactory signals or cues conveying information on the ambient level or type of predation risk present (reviewed in Wisenden 2000; Ferrari *et al.* 2010a). For example, shoals of fathead minnows (*Pimephales promelas*, 21 mm ± 4.1 mm standard length L_S, mean ± SD) showed significantly more frequent antipredator or alarm responses to the odour (kairomone) of smaller (22.8 cm – 25 cm L_S) Northern pike (*Esox lucius*) than they did to larger (63 cm – 66 cm L_S) pike, given equal biomasses of kairomone (predator odour) donors (*i.e.*, two small pike for one large: Kusch *et al.* 2004). Due to the small size of fathead minnows, smaller pike are likely to pose greater risk than larger pike as the relative energetic value of a minnow would be considerably less to a larger pike. A similar size-based preference for prey fishes has been demonstrated in the pike-cichlid (*Crenicichla alta*), which reduced its daily intake when presented with shoals of smaller Trinidadian guppies (*Poecilia reticulata*), although this was not true for all pike-cichlids tested (Mattingly and Butler 1994). Antipredator responses to the damage-released chemical cues of heterospecific prey have also received considerable attention, with magnitude of response influenced by differences in population (Brown *et al.* 2009), phylogenetic relatedness (Mirza and Chivers 2001a; Brown *et al.* 2003; Mirza *et al.* 2003), body size (Mirza and Chivers 2002) and donor condition (Brown *et al.* 2004b). Similarly, predatory fishes have demonstrated the ability to distinguish between the damage-released chemical cues of different prey species (Elvidge and Brown 2012) and individual size and condition of the cue donors (Lonnstedt *et al.* 2012) through different levels of exploratory and/or foraging behaviours.

There has been some debate over the main role played and extent of the benefits conferred by chemical alarm cues with respect to the cue sender. Three main hypotheses addressing these issues have been explored: 1) kin selection, whereby an individual accrues fitness benefits from warning nearby related conspecifics of danger; 2) secondary predator attraction, in which alarm cues serve to recruit additional predators which may then create escape opportunities for the prey through interference with the primary predator (Mathis *et al.* 1995); and 3) various immune functions within the Ostariophysan superorder of fishes (*e.g.*, UV protection and antiparasite function: Chivers *et al.* 2007), which does not include salmonids (reviewed in Chivers *et al.* 2012). Of these three, kin selection is arguably the most obvious mechanism due to the pronounced antipredator responses demonstrated by conspecific alarm cue receivers; however, the occurrence of heterospecific responses in conjunction with limited or conflicting data on the extent of kin associations under natural conditions (Naish *et al.* 1993; Gerlach *et al.* 2001; Brodeur *et al.* 2008) suggest severe limitations on any kin-related benefits accrued by the sender. The predator attraction hypothesis, by contrast, is supported by a number of field-based studies demonstrating some degree of localized recruitment of free-swimming predatory fishes (Wisenden and Thiel 2002; Elvidge and Brown 2012; Lonnstedt *et al.* 2012) and suggests that chemical alarm signaling in fishes is functionally analogous to predator distress calls in birds and mammals (Chivers *et al.* 1996). However, behavioural responses to these chemical cues by both prey (conspecifics and heterospecifics) and predators strongly implies that the establishment and persistence of this signaling mechanism are driven by advantages accrued by signal receivers, as opposed to any survival benefits gained by signal senders (Chivers *et al.* 2012). As such, this chemical signaling mechanism more closely resembles social eavesdropping (Stowe *et al.* 1995; Earley 2010) on publicly available information (Danchin *et al.* 2004), as was recently demonstrated in a similar eavesdropping response to acoustic distress cues between heterospecific rodents (Schmidt *et al.* 2008).

The antipredator responses to chemical alarm cues demonstrated by receivers have received the most attention by researchers thus far (reviewed in Ferrari *et al.* 2010a), while surprisingly few studies have examined whether these behavioural strategies actually serve to enhance survival of prey during encounters with a predator under natural conditions. Notable exceptions have, for the most part, been staged under laboratory conditions or in enclosures, which constrain the movements of both predators and prey. For example, brook trout (*Salvelinus fontinalis*) that were exposed to conspecific alarm cues were better able to avoid predatory chain pickerel (*Esox niger*) than unexposed trout (Mirza and Chivers 2001b). Trout that had been pre-conditioned to recognize pickerel odour via paired exposure to conspecific alarm cues through a releaser-induced learning mechanism (Suboski 1990) were also better able to avoid pickerel during staged encounters than pickerel-naïve trout (Mirza and Chivers 2000; Chivers *et al.* 2002).

Fathead minnows conditioned to recognize the chemical alarm cues of brook stickleback (*Culaea inconstans*) were less likely to be attacked by predatory rainbow trout (*Onchorhynchus mykiss*) which had been fed on a diet of stickleback and were consequently “labeled” with stickleback cues than were unconditioned minnows (Chivers *et al.* 2002). Collectively, these experiments provide evidence that there are, in fact, some tangible survival benefits associated with antipredator behavioural responses to chemical alarm cues, and that these benefits can be accrued from responding to cues from both conspecific and heterospecific sources. Additional evidence supporting this explanation has been documented in related populations of the Trinidadian guppy. The Aripo River in the Northern Range of Trinidad is divided into “Upper” and “Lower” reaches by a series of barrier waterfalls which preclude the upstream migration of predatory fishes (Magurran 2005). Consequently, guppies in the Upper Aripo experience relatively low levels of predation, whilst guppies in the Lower Aripo experience relatively high levels of predation. The functional value of damage-released chemical cues is evident in the responses of guppies from either population, with Lower Aripo guppies demonstrating significantly greater magnitudes of antipredator responses to alarm cues of a standard concentration than did Upper Aripo guppies. Guppies from high predation sites also demonstrate graded or threat-sensitive responses to different concentrations of alarm cues, whereas guppies from low predation sites demonstrated a non-graded, all-or-nothing response to only the highest concentrations tested (Brown *et al.* 2009).

Despite the survival benefits demonstrated under laboratory conditions of detecting and responding to damage-released chemical cues in freshwater fishes, the chemical cue itself is degraded under weakly acidic conditions (pH < 6.6: Leduc *et al.*

2004a; Elvidge *et al.* 2013) and does not elicit an alarm response in conspecific receivers. Similarly, weak acidification interferes with both the acquisition of additional risky chemical cues (Leduc *et al.* 2004b; Smith *et al.* 2008) and the ability to generalize learned risky cues across predator taxa (Brown *et al.* 2012). The widespread occurrence of weak acidification, both chronic (Clair *et al.* 2002; Clair *et al.* 2011) and episodic following precipitation inputs and spring melts (Baker *et al.* 1996; Laudon and Bishop 2002; Leduc *et al.* 2009) strongly suggests that many populations of freshwater fishes are deprived of an important source of information on predation risk perceived through this chemical signaling mechanism over different timescales. The coral reef-inhabiting lemon damselfish (*Pomacentrus moluccensis*) has demonstrated diel patterning in its foraging behaviours such that levels of activity are inversely proportional to ambient risk in a multi-predator system (Bosiger *et al.* 2012). Wild common bullies (*Gobiomorphus cotidianus*) exposed to chemical cues of a novel predator (Eurasian perch *Perca fluviatilis*) during the day spent significantly more time under cover at night, when additional complementary sources of information on risk (*e.g.*, visual cues) are absent (Vanderpham *et al.* 2012). Similarly, free-swimming juvenile Atlantic salmon (*Salmo salar*) have demonstrated significantly greater responses to conspecific chemical alarm cues at night, in the absence of complementary visual cues, than during the day (Leduc *et al.* 2010b). These results indicate that under fully natural conditions, fishes are able to recognize and learn different predictable, temporal patterns in levels of ambient risk and adjust their behavioural responses in threat-sensitive fashions. Unpredictable periods of acid-mediated loss of chemical information throughout a growth season due to precipitation, as well as intermittent seasonal pulses (*e.g.*, spring snow melts), may preclude the development of these temporal compensatory behavioural mechanisms in affected fish populations.

Here, I describe a series of experiments designed to address whether, as a result of acid-mediated loss of chemical information on the immediate level of predation risk, juvenile Atlantic salmon in neutral and acidic nursery streams: 1) are subject to predation by similar fish species; 2) do in fact suffer greater risk of predation under acidic conditions than under neutral conditions; and 3) are subject to different levels of extra-stream (terrestrial or avian) predation pressure between stream classes within the study area in the Miramichi River system, Northumberland County, NB.

Materials and Methods

Study sites

The eight study streams described in Chapter 1 were electrofished in 2009, concurrent with the behavioural observations (Elvidge *et al.* 2013). A subset of those streams were selected based on the physical similarities of their substrates for further study, consisting of one neutral (pH ≥ 6.6: Little Southwest Miramichi River) and two acidic (pH < 6.6: Upper Devil's Brook and Correy Creek) streams, plus one which fluctuated between classes (Lower Devil's Brook: Elvidge *et al.* 2013). These streams have also served as the sites of previous studies on the antipredator behaviours of juvenile Atlantic salmon (*e.g.*, Leduc *et al.* 2006).

Experiment 2a: Determination of fish community composition and predator diversity

During July – August of 2009, two sections of approximately 100 m^2 in each of the eight study streams (4 neutral, 4 acidic, as Lower Devil's Brook remained acidic during this year; Table 2.1) presented in Chapter 1, including the four streams used for tethering in Experiment 2b, were electrofished three times at ≥ 2 week intervals in order to describe the composition of the resident fish communities. GPS coordinates and mean physical habitat variables during sampling in each stream are presented in Table 1.1. Sampling was conducted under license from Fisheries & Oceans Canada (license no. SG-NBT-09-050) using a Smith-Root® (Vancouver, WA) Model 12B backpack electrofisher operated by C.K. Elvidge, with either one or two assistants capturing fish with some combination of a 2 m length × 1m height lip seine (3 mm mesh size) and pole-mounted dip nets of varying aperture sizes. Each section was fished twiced by moving upstream in a rough zig-zag pattern with all captured fish retained for identification, measurement (standard length L_S) and weighing using a portable (though, ironically, neither submersible nor water resistant) digital scale. This protocol was intended to allow for the quantification of fish community diversity, as well as changes in abundance and differences in species-specific growth rates between stream classes.

Experiment 2b: Tethering to estimate relative predation pressure between stream classes

Tethering experiments have previously been used to estimate the relative levels of predation risk experienced by aquatic prey species between habitat and microhabitat types, for the purposes of both ecological comparisons and to inform the selection of stocking or release sites for aquaculture species. From an ecological perspective, predation levels experienced by prey fishes have been compared between littoral and pelagic habitats (Dupuch *et al.* 2009), and distance from physical refugia in mangrove swamps (Jaxion-Harm and Speight 2012) and on coral reefs (Dorenbosch *et al.* 2009; Grol *et al.* 2011). Aside from fishes, previous tethering experiments have involved, among others, sea urchins (Urriago *et al.* 2012), commercially valuable crustaceans including spiny lobster (*Panulirus argus*: (Smith and Herrnkind 1992), blue crab (*Callinectes sapidus*: (Hines and Ruiz 1995) and grass shrimp (*Palaemonetes pugio:* (Kneib and Scheele 2000), and bivalve molluscs (bay scallops Argopecten irradians: Pohle *et al.* 1991; Bologna and Heck 1999; sea scallops Placopecten magellanicus: ; Nadeau *et al.* 2009). In addition to the aquatic stages of the Odonata (Remsburg and Turner 2009), terrestrial insects (field crickets *Gryllus integer*) have also been used in tethering studies (Hedrick and Kortet 2006). Tethering experiments involving terrestrial vertebrates, by contrast, are practically non-existent, likely due to perceived ethical issues arising from the use of more charismatic fauna.

Hatchery-reared young-of-the-year (0^+) Atlantic salmon spawned from wild-caught Rocky Brook adults (Jackson and Brown 2011) in 2010 and Little Southwest adults in 2011 were obtained from the Miramichi Salmon Association (MSA), South Esk, NB, and transported under permit from Fisheries and Oceans Canada (permit nos. NBI&T – 10-077 and NB 2011-085) to flow-through holding bins in the Little Southwest Miramichi River (46°52.807’ N, 66°06.418’ W). Holding bins were transparent 99 l plastic containers (described in Chapter 1) with opposing ends cut out and replaced with steel mesh (3.175 mm) (Jackson and Brown 2011). The bins were provided with natural substrate including large rocks (≤ 20 cm diameter on the longest axis) in order to acclimate naive fish to a semi-natural environment including drift forage items, physical refugia and flow variability. The salmon were allowed to acclimate to the bins for at least 48 hrs prior to testing.

Prior to each trial (~ 09:00), fish were removed from the acclimation bins and transported to the study sites. Individual salmon were measured (L_S, mm) and then attached to the tethering apparatus using blunt-tipped tapestry needles used to pass black nylon sewing thread through the mouth and then out between the gill arches and the opercular plate on one side (Figure 2.2). One end of each tethering thread (~ 1 m length) was secured to the fish using double overhand knots, while the other end ran through a small cork floater (2.5 cm length × 1.5 cm width) approximately 0.75 m from the fish and tied to a metal washer (5 cm diameter, 25 g) which acted as an anchor. This configuration, based loosely on the methods of Dupuch *et al.* (2009), allowed fish freedom of movement within a ~ 1 m radius and generally prevented tangling of the tether line around the substrate. In each trial, 21 tethered fish were placed into the marked sites in a loose grid pattern to achieve uniform densities of 0.7 fish $\cdot$ m^{-2}, which is well below the typical density at which 0^+ salmon have been observed in this area (~2·m-2: Keeley and Grant 1995) and is unlikely to increase predator recruitment in the study sites (Barbeau and Scheibling 1994; Barbeau *et al.* 1994). Tethered fish were left in place for 6 hours, after which the individual tethers were retrieved in the order of placement and the state of the fish (alive, dead, missing, confirmed predation event) was recorded. Predation events were confirmed to have occurred on several occasions, when brook trout or partially digested salmon were discovered attached to the ends of the tether lines. In 2010, 12 replicates were collected in each of Little Southwest (neutral), Upper Devil's

Brook (acidic) and Lower Devil's Brook (variable) for a total of 36 trials, while in 2011, 12 replicates were collected in each of Little Southwest (neutral), Lower Devil's Brook (neutral), Upper Devil's Brook (acidic) and Correy Creek (acidic) for a total of 48 trials. In all, there were $n = 47$ replicates for the acidic and $n = 37$ for the neutral stream classes (11 replicates under acidic conditions, and 13 under neutral conditions in Lower Devil's Brook), involving a total of 1764 fish (L_S = 26 – 57 mm, 42.6 ± 5.62 mm, mean ± SD). After each trial, all surviving salmon were released into the Little Southwest Miramichi River as originally intended by the population enhancement program of the MSA, in which adult salmon are collected via seining prior to the fall spawn, transported to the hatchery and held until they are ready to be milted and then released at their site of capture. Fertilized eggs are incubated over the winter in natural stream water diverted to flow-through stream channels, and surviving fish are released into their streams of parental origin (M. Hambrook, MSA President, personal communication).

Experiment 2c: Estimating relative extra-stream predation by prey depletion

In order to estimate the relative differences in predation imposed by terrestrial predators (*e.g.*, mustelids, birds) on stream fishes within the study area, two green, inflatable nylon pools (2 m diameter) were placed on flat ground with ~ 50% canopy cover adjacent to study sites at both Catamaran Brook and Upper Devil's Brook. These sites were chosen as they were situated next to one neutral and one acidic stream separated by ~ 15 km, representing the opposing geographic limits of the tethering (although not the behavioural; Chapter 1) study area.

Each pool was filled to a depth of 0.15 m with stream water and stocked with wild-caught blacknose dace (*Rhinichthys atratulus*, *n* = 9 per pool; L_S 52 mm ± 66 mm, weight 2.21 g ± 0.55 g, mean ± SD) roughly size-matched to 0^+ Atlantic salmon. Blacknose dace were selected for use in this experiment because they are more tolerant to environmental extremes, including high temperatures and hypoxia, than are juvenile salmon or the sympatric brook trout (*Salvelinus fontinalis*). Following the introduction of dace to the pools, each pool and surrounding 2 m wide band were carefully examined once every 24 hours at either 11:00 or 18:00 in order to count the number of remaining fish and discount individual escapes as a mechanism of prey depletion. All fish carcasses found outside of the pools were counted as present to distinguish between confirmed escapes and possible removal by a predator. Dace were restocked daily as necessary to maintain the original density of nine fish per pool. Following each daily census, approximately one third of the water was drained and replaced with fresh stream water in order to re-oxygenate and reduce ambient temperature. Each trial was run for 10 days for a total of *n* = 4 replicates per stream.

Statistical analysis

Tethering data (Experiment 2b) were transformed into the proportion of fish missing (*i.e.* the number of tethers which did not have a salmon attached upon retrieval added to the number of attached brook trout, divided by the total number of tethers) at the end of each trial. Lower Devil's Brook was examined as two different streams based on its pH class at the time of each trial. The proportions of fish missing were analyzed as a one-way ANOVA against stream class, as were the sizes of tethered fish against stream, class and status of individual fish (present or absent) at the end of each trial. The proportion data was not analyzed as a binomial distribution as model comparison demonstrated no advantage to this approach over a simple ANOVA. The ANOVAs were then examined with *post-hoc* Tukey's HSD tests to identify pairwise differences between individual treatment levels. These analyses and all figures were generated using R version 2.15.2 (R Development Core Team 2011). In addition, Ryan-Einot-Gabriel-Welch F and Tukey's B tests were used to identify homogeneous subsets of streams in the proportion data using PASW version 18 (SPSS Inc. 2009). All tests were conducted with a level of significance $\alpha = 0.1$ in order to minimize the occurrence of ecological Type II errors by failing to reject a false statistical null hypothesis at the expense of identifying an ecologically or biologically significant trend in the data. Prey depletion data were examined using non-parametric Kaplan-Meier survival estimates (Kaplan and Meier 1958) to compare the rates of depletion attributed to predation between the two study areas using the *survival* package (Therneau 2013) in R.

Results

Experiment 2a: Determination of fish community composition and predator diversity

All species recorded in the study streams, excluding Atlantic salmon, are presented in Table 2.2. Due to systematic differences in sampling technique between repeated sampling efforts within and across streams, including different numbers of assistants, area and time spent electrofishing, the data on species abundance and individual size between streams and stream classes are not suitable for statistical comparisons, as originally intended. Furthermore, two species present in Catamaran Brook (brook stickleback *Gasterosteus aculeatus* and sea lamprey *Petromyzon marinus*) were not collected via electrofishing, but were instead sighted in the river during snorkeling activities. Similarly, brook trout and American eels (*Anguilla rostrata*) were observed visually in Little Southwest but not sampled during electrofishing. Despite acting as the migration corridor connecting some of the other study streams which both contained a particular species (*e.g.*, brook trout in Catamaran Brook and Lower Devil's Brook: Figure 2.1), no brook tout were collected in Little Southwest during electrofishing, and very few were observed during snorkeling. Adult brook trout are highly temperature-sensitive, preferentially inhabiting waters below 19° C (Brett 1956) and are typically found in deep pools with ample shading in cool, low-order tributaries during the warm summer months. Large brook trout inhabit higher-order streams and estuaries during the colder winter months, as there is a positive relationship between abundances of foraging opportunities and preferred deeper habitats (Cunjak and Power 1986; Cunjak 1996) and stream size, particularly when ice cover is present. These seasonal shifts in habitat preference and resulting migration patterns of adult brook trout represent a temporal confound to the electrofishing effort in Little Southwest. Similarly, the absence of American eels from most stream samples is more likely due to random sampling effects than an indication that this highly motile and dispersive species is absent from the study streams altogether. The electrofishing data are accordingly treated as approximate fish community surveys which only describe diversity at the time of sampling, and are not necessarily inclusive of all fish species, predatory or otherwise, which resident Atlantic salmon may encounter.

In the current electrofishing surveys, the most likely fish predators of juvenile Atlantic salmon are brook trout and American eels. The neutral streams appear to have greater overall fish species diversity, despite Catamaran Brook, Lower Devil's Brook and North Pole Stream each being indirectly connected as tributaries of Little Southwest on the Northwest branch of the Miramichi River. Upper Devil's Brook and Correy Creek are both tributaries of Lower Devil's Brook which are not fed by headwater lakes (Figure 2.1) as is the case with the other streams, but are each separated by a series of barrier waterfalls which appear to preclude the upstream dispersal of non-salmonid species as only brook trout and juvenile Atlantic salmon were observed in these streams. These two physically isolated streams were also the only streams that did not contain populations of blacknose dace (Table 2.2). Rocky Brook is a tributary of the Renous River on the Southwest branch of the Miramichi River, and also contained fewer fish species than the larger stream during the present sampling period. Generally, acidic streams in this dataset are smaller than neutral streams in terms of depth, flow rate and discharge (flow rate × channel depth × channel width), and appear to generally be of lower stream order, as evidenced by their status of tributaries to larger, neutral streams. This is in keeping with the general positive relationship between catchment area or size of a drainage basin and its acid neutralizing capacity, ANC (Kalff 2003; Petrin *et al.* 2007).

Experiment 2b: Tethering to estimate relative predation pressure between stream classes

Overall, 1094 of the 1764 fish remained attached to the tethers at the end of the trials, consisting of 870 living and 224 dead. Of the 670 fish considered absent or missing at the end of the trials, 655 were absent and 15 predation events were witnessed. A summary of these results divided by stream and stream class are presented in Table 2.3 and mean physical habitat variables within the tethering areas are presented in Table 2.4. A predation event typically involved the discovery of a brook trout at the end of the tether line upon retrieval. In most cases, the line failed, but on three separate occasions, the trout regurgitated a partially-digested salmon prior to escaping. No predatory species other than brook trout was observed at or near a tether site throughout the experiment. In one instance in Little Southwest, a severed tether was discovered resting on top of a large rock above the water line, suggesting that in this instance the test salmon was consumed either by a brook trout, which then jumped, or by an avian or terrestrial predator. On one occasion in Lower Devil's Brook in 2011 during the 6 hour trial period, an apparent demonic intrusion was incurred as all of the tethering equipment had been removed. On another occasion in 2011, a single large predator evidently moved downstream through the tethering site in Correy Creek and consumed most of the test salmon in rapid succession, as the anchors were fSyriSyound in a group at the downstream margin of the site with the tether lines entangled with bits of opercula. While it is tempting to attribute this lacuna to a predatory fish like a large brook trout, black bears (*Ursus americanus*) are also abundant in this area and several individuals and familial groups were sighted unexpectedly and in close physical proximity during the course of Experiment 3c. While a juvenile Atlantic salmon is unlikely to attract the attention of a bear, one could have simply trampled through the site. Similarly, there is abundant physical evidence and several personal sightings of moose (*Alces alces*), white tailed deer (*Odocoileus virginianus*), red fox (*Vulpes vulpes*), coyote (*Canis latrans*), beaver (*Castor canadensis*) and mink (*Neovison vison*) throughout the study area, and any of these could have potentially been responsible for the disturbance.

The proportion of fish missing varied significantly between streams ($F_{4,79} = 3.68$, $P = 0.0085$; Figure 2.3) and stream classes ($F_{1,82} = 13.76$, $P = 0.00038$; Figure 2.4), with significant differences between acidic and neutral conditions within Lower Devil's Brook ($P = 0.0491$) and between acidic Lower Devil's Brook and Little Southwest ($P = 0.0448$; Figure 2.3). The results of the Tukey's B tests indicate the presence of two stream subsets, with the second subset containing the three acidic streams while the first subset contains all streams excluding Lower Devil's Brook under acidic conditions (Table 2.5). The results of the R-E-G-W F test are similar, with three subsets identified. The third grouping is identical to the second subset in the Tukey's B test including all streams except acidic Lower Devil's Brook, although this grouping falls considerably short of statistical significance ($P = 0.757$). The other two subsets, although both statistically significant, are difficult to interpret as they group acidic and neutral streams together (Table 2.5). Two 'streams' which were never grouped by Tukey's B or the R-E-G-W F tests were the acidic and neutral Lower Devil's Brook replicates, in accordance with the significant pairwise difference between them exposed in the Tukey's HSD test. Of the two neutral streams, Little Southwest was grouped with the acidic streams in one of the subsets from Tukey's B test and the two significant subsets from the R-E-G-W F test. Consequently, the tethering results from Little Southwest appear to be more similar to the results from the acidic streams than from the neutral Lower Devil's Brook, and the separation of results within the identical site within Lower Devil's Brook based on pH class suggests that while there may be an interaction between acidity and ambient predation level, given similar levels of predation tethered fish experience greater mortality under acidic conditions.

Despite indiscriminately selecting juvenile salmon from a common stock on a daily basis and systematically rotating the order in which streams were stocked with tethered fish, there was a significant difference in the size of fish against both stream class ($F_{1,1762} = 53.5$, $P < 10^{-13}$; Figure 2.5) and individual stream ($F_{4,1759}= 39.95$, $P < 10^{-16}$; Figure 2.6), with significant pairwise differences ($P < 0.01$) between every combination except Upper Devil's Brook and Little Southwest (Figure 2.5). This trend is likely the result of trials conducted under neutral conditions being over-represented later in the summer, particular in Lower Devil's Brook as there tends to be less precipitation in August than in July in this area. Size of the focal fish also varied significantly with its status (present/absent) at the end of a trial ($F_{1,1762} = 38.83$, $P < 10^{-9}$), with larger fish (mean $L_S = 43.25$ mm) present and smaller fish (mean $L_S = 41.55$ mm) absent (Figure 2.7a). Considering all four possible states (present and alive, present and dead, missing or predation event), there is again a significant effect of size ($F_{3,1760} = 15.35$, $P < 10^{-9}$), with significant pairwise differences between alive and missing ($P < 10^{-6}$) and alive and confirmed predation events ($P = 0.0185$; Figure 2.7b). Despite these significant differences in size between stream classes and individual status, there was no significant interaction between class and status, suggesting that salmon in both stream classes were experiencing similar size-selective mortality and predation. In both comparisons, the "alive" fish were larger than their missing counterparts, suggesting some selectivity for smaller prey sizes by resident predators. It should be noted that despite the presence of statistically significant size differences, the magnitudes of the differences are ≤ 2 mm L_S

(Figure 2.5). Mean sizes of tethered fish by stream, class and status are listed in Table 2.6. Despite a trend towards larger fish being over-represented in the neutral streams, further *post-hoc* testing did not identify any significant homogeneous subsets based on fish size, although both Tukey's B and the R-E-G-W F test generated the same four groupings (Table 2.7). The sole subset containing more than one stream paired neutral Little Southwest and acidic Upper Devil's Brook, which differed qualitatively in the proportion of fish missing. Despite the potential confound to observed higher proportions of missing fish in acidic streams posed by the prevalence of smaller fish in these trials, the overall similarity of means given these large sample sizes (Table 2.7) suggests that the observed patterns are the result of inter-stream and inter-class differences, as opposed to systematic errors in sampling.

Experiment 2c: Estimating relative extra-stream predation by prey depletion

Overall, more focal dace absent from the pools at the time of sampling were accounted for following visual inspection of the surrounding areas than were not. On any given day, no more than two dace from any pool remained unaccounted for and were recorded as missing. By the end of the two 10 day trials, equal numbers were missing from both stream sites (Figure 2.8). Kaplan-Meier estimates (Figure 2.9) indicate that there was no difference in dace survival between streams ($\chi^2_1 = 0.1$, $P = 0.734$). These results are unsurprising, as the sites at Upper Devil's Brook and Catamaran Brook are not separated by any physical barriers (Figure 2.1), anthropogenic or otherwise, to the dispersal of terrestrial or avian predators such as roads (Forman and Alexander 1998; St Clair 2003; Rico *et al.* 2007) or fencing (Jaeger and Fahrig 2004), although there is some potentially disruptive recreational usage and forestry activity in the area. On one occasion during August 2011, a solitary male common loon (*Gavia immer*) spent several hours swimming and diving along the length of a pool in Catamaran Brook. Before its appearance, several dozen adult brook trout and hundreds of white suckers had been observed in the pool via snorkeling; following the departure of the loon that evening, the aggregation had almost entirely dissipated with fish presumably having emigrated or been consumed. Similarly, rafts of ducks (likely a species of merganser) are occasionally observed traveling through stream sections. Earlier work suggests that mergansers are the main predators on larger juvenile Atlantic salmon (parr and/or smolts) in New Brunswick (Elson 1962). While these stochastic events likely impose major short-term predation pressure on resident fish populations, the results of the prey depletion experiment suggest that predation on 0^+ salmon by other fish species such as brook trout may be the greatest limiting factor over longer time scales.

Discussion

Due to a combination of the loose sampling technique and relatively small sampling areas (Table 2.1), predator species diversity within the study streams was very likely underestimated. Both brook stickleback and sea lamprey have been visually identified in Catamaran Brook, despite their absence from the electrofishing samples. American eels are also likely to occur incidentally throughout the study area, but were only captured or observed in Catamaran Brook, Lower Devil's Brook and Little Southwest. Similar distributions of the less abundant sea lamprey are also predicted to occur over longer timescales. With the exception of the barrier waterfall-separated Upper

Devil’s Brook and Correy Creek, the study sections in Little Southwest and its tributaries (North Pole Stream, Lower Devil’s Brook, Catamaran Brook) on the Northwest Miramichi branch are not subject to any natural or anthropogenic barriers to fish movement, and observed differences in the estimates of fish community composition are more likely indicative of biases arising from sampling technique and non-uniform (Poisson) spatial and temporal distributions of non-Atlantic salmon species than of actual differences in species distributions. This is likely the case for the highly temperature-sensitive brook trout, which avoids wider rivers with less canopy cover and higher temperatures or preferentially occupies deeper, colder pools avoided by juvenile Atlantic salmon during the warm summer months.

Of the remaining two streams, Rocky Brook is tributary to the Renous River on the Southwest Miramichi branch, and while fish diversity may differ between branches, a similar inverse relationship between stream order and diversity during the warmest annual period appears to be present in the current limited dataset (Table 2.2). The geographic relationship between the study streams, free from anthropogenic barriers between tributaries within the Northwest and Southwest branches of the Miramichi River, with the two branches separated by one paved road (Highway 108), suggests that avian and terrestrial predators are unlikely to be excluded from particular streams within the study area. This prediction receives some support from the results of Experiment 2c, in which blacknose dace were depleted at similar rates from stock pools placed adjacent to the stream banks. Collectively, these findings indicate that juvenile Atlantic salmon throughout the study area are likely subject to similar levels of predation pressure by similar suites of aquatic, avian and terrestrial predators. The results of the tethering experiment, in which fish under acidic conditions were more likely to be missing from their tethers after the 6 hour trial periods, is indicative of greater actual predation pressure in physically smaller acidic streams containing less diverse predator guilds.

One common criticism of tethering experiments is that estimates of predation pressure may be artificially inflated as a result of either increased encounter rates between predators and prey, or through decreased escape ability on the part of prey (Barbeau and Scheibling 1994; Aronson *et al.* 2001). Furthermore, the rate of depletion of tethered, motile prey individuals has been demonstrated to be heavily influenced by predator density (Kneib and Scheele 2000), although more recent findings indicate that knowledge of predator diversity and species abundance within and between tethering sites can effectively inform the selection of correction factors to control for inter-site differences (Mills *et al.* 2008). The selection of tethering sites in the current experiment, based on the similarity of physical characteristics, in combination with the inference of Experiment 2a, namely that brook trout are similarly abundant and likely to pose the greatest predation risk to juvenile salmon in three of the four tethering streams during the periods of experimentation, are likely to have exerted a standardizing effect on the observed rates of tethered prey depletion between study stream classes. This standardizing effect is particularly noteworthy given that the fourth stream, the neutral Little Southwest, likely has the greatest diversity of aquatic predators and due its size (~ 100 m width) and lack of canopy cover resident fish may also be subject to the greatest levels of risk posed by avian predators. Earlier work involving population reduction of predatory mergansers strongly suggests that avian predators are the greatest limiter of Atlantic salmon smolt production (Elson 1962), while more recent studies have continued to examine the effects of predation primarily on smolt abundance (reviewed in Mather 1998). The present suggestion that brook trout are the most important predators of 0^+ salmon is therefore not necessarily in disagreement with earlier findings, as the difference in size between these age classes may result in fry being relatively unattractive prey items to foraging birds.

Experimental work conducted in Catamaran Brook under fully natural conditions has demonstrated that both juvenile Atlantic salmon and blacknose dace are found at significantly lower densities in stream reaches which were artificially labeled as riskier through regular injections of Atlantic salmon alarm cues than in control reaches. In fact, densities increased in control reaches as risk-averse fishes emigrated from the experimental reaches (Kim *et al.* 2011b). Similarly, individuals at risk of predation in several taxa preferentially occupy less risky habitats, which are often characterized by greater abundance of refugia compared to riskier habitats. Fish abundance in seagrass habitats in the Gulf of Mexico were estimated to be 760% greater than in sand flats, with this difference attributed to risk-averse habitat selection by the estuarine pinfish (*Lagodon rhomboids*). When predators were experimentally excluded from each type of habitat, pinfish showed no preference for seagrass (Jordan *et al.* 1997). Similarly, juvenile Atlantic cod (*Gadus morhua*) demonstrated a preference for fine gravel and sandy substrate in the absence of predation risk, but switched their preference to larger cobbles following exposure to a predator with the duration of the alteration generally inversely proportional to body size (Gotceitas and Brown 1993). A similar study to Experiments 2a and 2b involving Northern redbelly dace (*Phoxinus eos*) in Canadian shield lakes confirmed that dace were at greater risk of predation in pelagic zones relative to littoral zones, and that dace in more structured habitats within the littoral zones experienced less predation pressure than in unstructured littoral zones. In addition, the likelihood of dace occupying the littoral zone in any of the studied lakes was directly proportional to the level of risk in the pelagic zone (Dupuch *et al.* 2009).

Using hatchery-reared juvenile Atlantic salmon in Experiment 2b which were placed into tethering sites with similar availabilities of refugia, with identical constraints on the movement and site preferences of individual salmon, effectively eliminated any potential individual or population-derived differences in predator avoidance described above and provided a clear estimate of the relative differences in ambient pressure between study stream classes. The observed differences in the diversity of predatory fish species indicates that prey fish living in acidic streams are subject to greater predation pressure from other fishes than their counterparts in neutral streams, with this difference arising not from the abundances of predators but from acid-mediated impairment of chemosensory risk assessment.

Table 2.1: Area of stream sections electrofished during July –August 2009.

Class			
Neutral		**Acidic**	
	Area (m^2)		
Catamaran Brook	126	152.5	**Lower Devil's Brook**
Little Southwest	90	139.2	**Upper Devil's Brook**
North Pole Stream	120	137.5	**Correy Creek**
Renous River	90	90	**Rocky Brook**

Table 2.2: Summary of fish species collected via electrofishing in each study stream. Streams used for the tethering experiment are indicated with asterisks (*). Potential predators of 0^+ Atlantic salmon (*Salmo salar*) are printed in bold type.

	Class		
	Neutral	**Acidic**	
	Species		
Catamaran Brook	***Salvelinus fontinalis*** *Gasterosteus aculeatus* *Couesius plumbeus* *Rhinichthys atratulus* ***Anguilla rostrata*** *Petromyzon marinus* *Cottus cognatus* *Catostomus commersonii*	***Salvelinus fontinalis*** *Couesius plumbeus* *Rhinichthys atratulus* ***Anguilla rostrata*** *Cottus cognatus*	***Lower Devil's Brook**
***Little Southwest**	*Couesius plumbeus* ***Anguilla rostrata*** *Gasterosteus aculeatus* *Rhinichthys atratulus* *Catostomus commersonii* *Cottus cognatus* ***Salvelinus fontinalis***	*Couesius plumbeus* *Rhinichthys atratulus*	**Rocky Brook**
Renous River	*Catostomus commersonii* *Petromyzon marinus* *Rhinichthys atratulus*	***Salvelinus fontinalis***	***Upper Devil's Brook**
North Pole Stream	*Rhinichthys atratulus*	***Salvelinus fontinalis***	***Correy Creek**

Brook trout (*Salvelinus fontinalis*); threespine stickleback (*Gasterosteus aculeatus*); creek chub (*Couesius plumbeus*); blacknose dace (*Rhinichthys atratulus*); American eel (*Anguilla rostrata*); sea lamprey (*Petromyzon marinus*); slimy sculpin (*Cottus cognatus*); white sucker (*Catostomus commersonii*).

Table 2.3: Summary of all tethering results for individual 0^+ Atlantic salmon (*Salmo salar*) by stream and stream class in 2010 and 2011.

				Present				**Absent**		
Class	**Stream**	**Total**	**Alive**	**Dead**	**Total**	**Percentage**	**Missing**	**Predation Event**	**Total**	**Percentage**
Acidic		924	432	75	507	**54.9%**	407	10	417	**45.1%**
	CC	252	118	19	137	**54.4%**	115	0	115	**45.6%**
	UDB	504	256	33	289	**57.3%**	207	8	215	**42.7%**
	LDB	168	58	23	81	**48.2%**	85	2	87	**51.8%**
Neutral		840	438	149	587	**69.9%**	248	5	253	**30.1%**
	LSW	504	248	104	352	**69.8%**	151	1	152	**30.2%**
	LDB	336	190	45	235	**69.9%**	97	4	101	**30.1%**
Total		1764	870	224	1094	**62.0%**	655	15	670	**37.9%**

CC = Correy Creek; UDB = Upper Devil's Brook; LDB = Lower Devil's Brook; LSW = Little Southwest Miramichi River.

Table 2.4: GPS coordinates and mean physical measurements of the tethering study sites shown in Figure 2.1. Mean annual pH values for Lower Devil's Brook do not represent its classification as neutral or acidic due to high intra-annual variation in pH.

Stream	**GPS Coordinates**			**Depth (m)**		**Flow (m · s^{-1})**		**Temperature (° C)**		**pH**	
			Year		Mean		Mean		Mean		Mean
Little Southwest	46°52.807' N	66°06.418' W	**2010**	0.25	**0.29**	0.21	**0.22**	20.8	**20.5**	7.29	**7.07**
			2011	0.33		0.23		20.2		6.84	
Lower Devil's Brook	46°52.377' N	66°13.545' W	**2010**	0.17	**0.21**	0.11	**0.18**	17.5	**16.9**	6.74	**6.57**
			2011	0.25		0.25		16.3		6.4	
Upper Devil's Brook	46°52.386' N	66°13.610' W	**2010**	0.14	**0.16**	0.13	**0.19**	16.4	**15.8**	6.38	**6.32**
			2011	0.18		0.25		15.2		6.26	
Correy Creek	46°52.424' N	66°13.603' W	**2010**	NA	**0.29**	NA	**0.11**	NA	**17.3**	NA	**6.21**
			2011	0.29		0.11		17.3		6.21	

Table 2.5: Homogenous stream subsets by proportion of tethered fish missing as revealed by *post-hoc* Ryan-Einot-Gabriel-Welch F test ($\alpha = 0.1$).

***Post-hoc* Test**	**Stream**	***N***	**Subset 1 (mean)**	**Subset 2 (mean)**	**Subset 3 (mean)**
Tukey's B	LDBN	13	0.2784		
	LSW	24	0.3016		
	UDB	24	0.4325	0.4325	
	CC	12	0.4563	0.4564	
	LDBA	11		0.5108	
R-E-G-W F	LDBN	13	**0.2784**		
	LSW	24	**0.3016**	**0.3016**	
	UDB	24	**0.4325**	**0.4325**	0.4325
	CC	12		**0.4564**	0.4564
	LDBA	11			0.5109
	P		**0.059**	**0.061**	0.757

Values are the mean proportions of fish missing in each stream. LDBN = Lower Devil's Brook (neutral); LSW = Little Southwest; UDB = Upper Devil's Brook; CC = Correy Creek; LDBA = Lower Devil's Brook (acidic). Abbreviations of acidic streams are printed in italics. Bold type indicates statistically significant stream groupings, italics indicate acidic streams.

Table 2.6: Standard lengths of tethered 0^+ Atlantic salmon (*Salmo salar*) in each stream, stream class, and individual status at the end of each trial.

Grouping		**Standard Length (mm) (mean ± SD)**
Stream	Lower Devil's Brook (neutral)	44.84 ± 5.08
	Little Southwest	43.06 ± 5.43
	Lower Devil's Brook (acidic)	41.29 ± 5.37
	Upper Devil's Brook	43.14 ± 5.48
	Correy Creek	39.38 ± 5.45
Class	Neutral	43.69 ± 5.38
	Acidic	41.74 ± 5.66
Status	Alive	43.42 ± 5.46
	Dead	42.55 ± 5.16
	Missing	41.6 ± 5.77
	Predation event	39.2 ± 6.49
	Present	43.25 ± 5.41
	Absent	41.55 ± 5.79

"Present" includes "Alive" and "Dead"; "Absent" includes "Missing" and "Predation Event".

Table 2.7: Homogenous stream subsets by tethered fish length (L_S, mm) as revealed by *post-hoc* Ryan-Einot-Gabriel-Welch *F* test ($\alpha = 0.1$).

***Post-hoc* test**	**Stream**	***n***	**Subset 1** (mean)	**Subset 2** (mean)	**Subset 3** (mean)	**Subset 4** (mean)
Tukey's B	*CC*	252	39.98			
	LDBA	231		41.29		
	LSW	504			43.06	
	UDB	504			43.14	
	LDBN	273				44.84
R-E-G-W F	*CC*	252	39.38			
	LDBA	231		41.29		
	LSW	504			43.06	
	UDB	504			43.14	
	LDBN	273				44.84
	P		1	1	.987	1

Group means are equivalent to mean proportions of fish missing in each stream. LDBN = Lower Devil's Brook (neutral); LSW = Little Southwest; UDB = Upper Devil's Brook; CC = Correy Creek; LDBA = Lower Devil's Brook (acidic). Abbreviations of acidic streams are printed in italics.

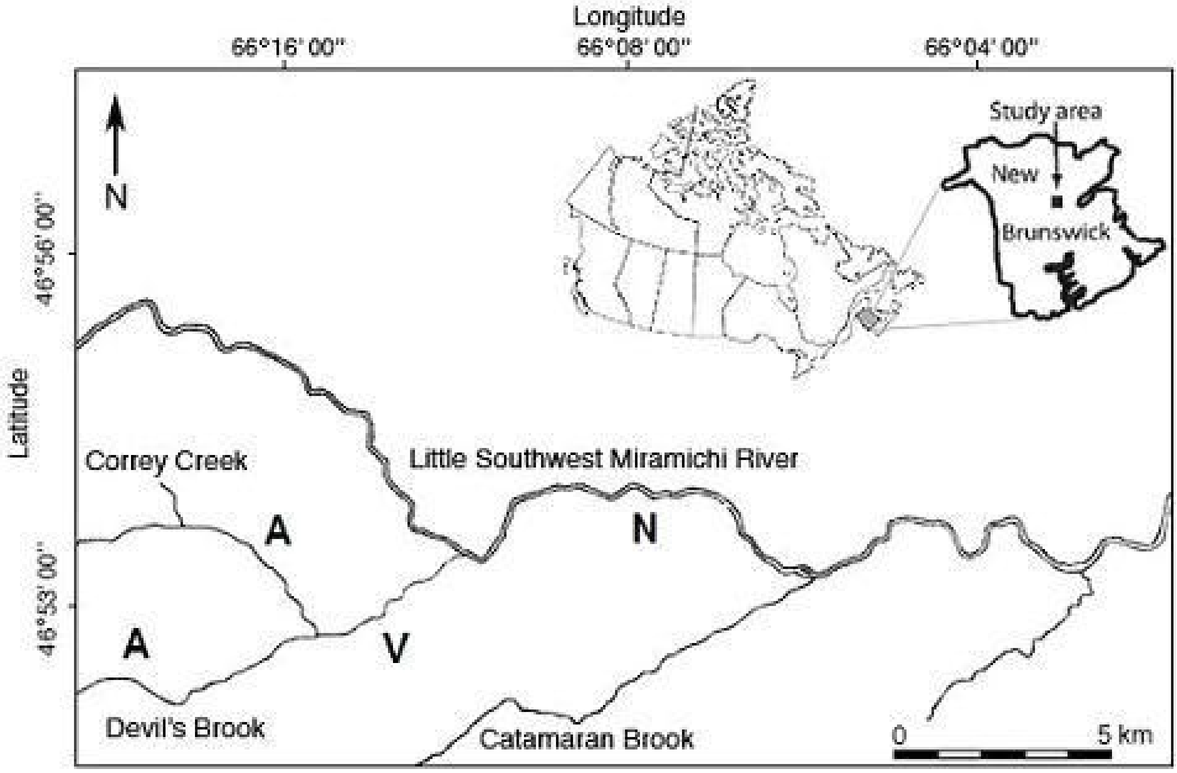

Figure 2.1: Map of the tethering study area in Northumberland County, New Brunswick, Canada. Letters indicate the approximate locations of study sites in the neutral (N, Little Southwest), acidic (A, Upper Devil's Brook and Correy Creek) and variable (V, Lower Devil's Brook) streams. Image modified from Leduc *et al.* (2006).

Figure 2.2: Wild-caught young-of-year (0^+) Atlantic salmon (*Salmo salar*) attached to the tethering apparatus for illustrative purposes in Catamaran Brook in August 2010.

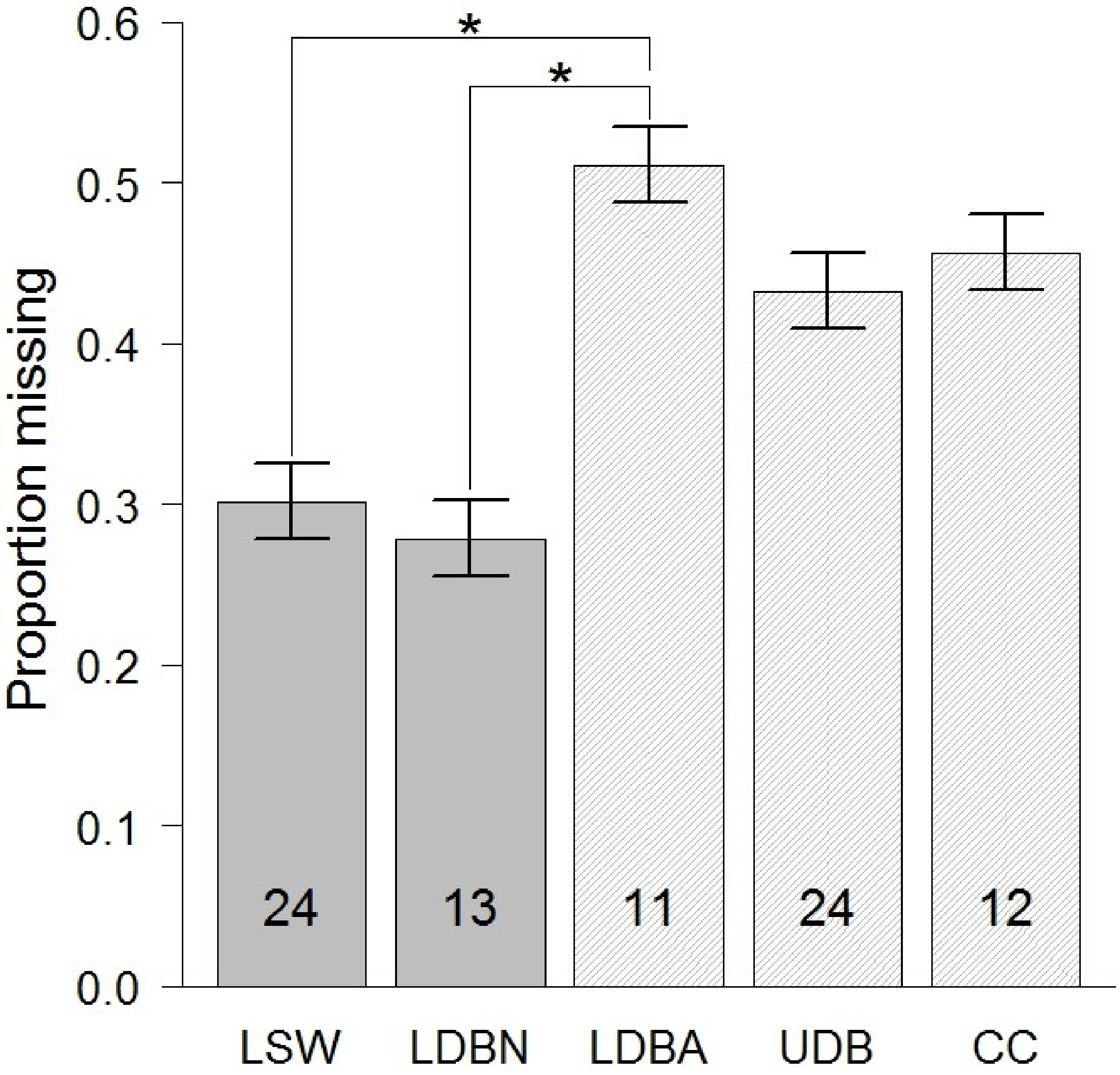

Figure 2.3: Mean (± SE) proportion of tethered fish missing at the end of each trial in the study streams. Closed bars represent neutral streams, hatched bars represent acidic streams. Asterisks denote significant differences ($P < 0.05$) between streams as revealed by *post-hoc* Tukey's HSD test. LSW = Little Southwest; LDBN = Lower Devil's Brook under neutral conditions; LDBA = Lower Devil's Brook under acidic conditions; UDB = Upper Devil's Brook; CC = Correy Creek. Numbers indicate sample size (trial replicates) for each bar.

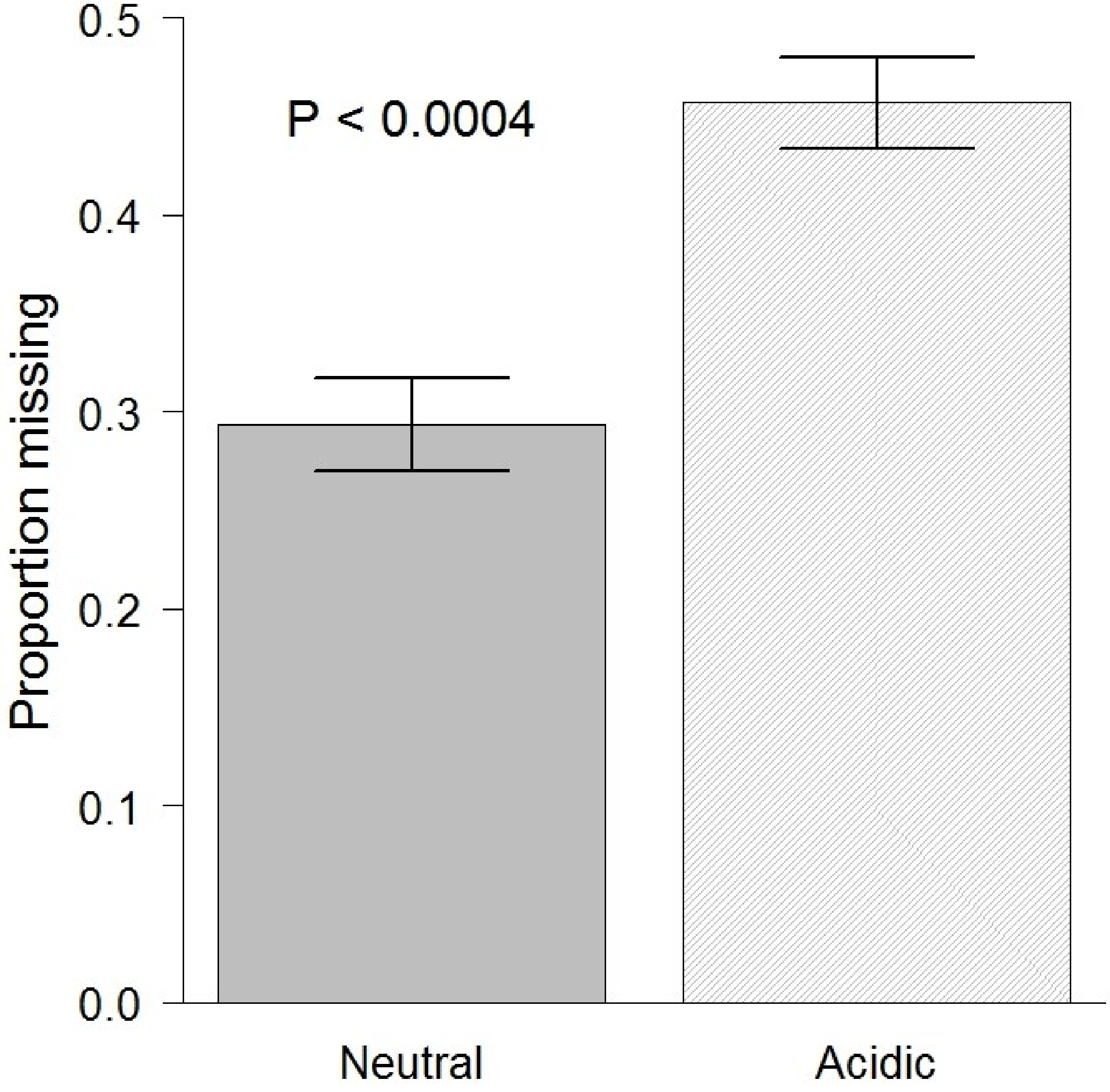

Figure 2.4: Mean (± SE) proportion of tethered fish missing at the end of each trial in each stream class. n = 37 trials under Neutral conditions; n = 47 trials under acidic conditions.

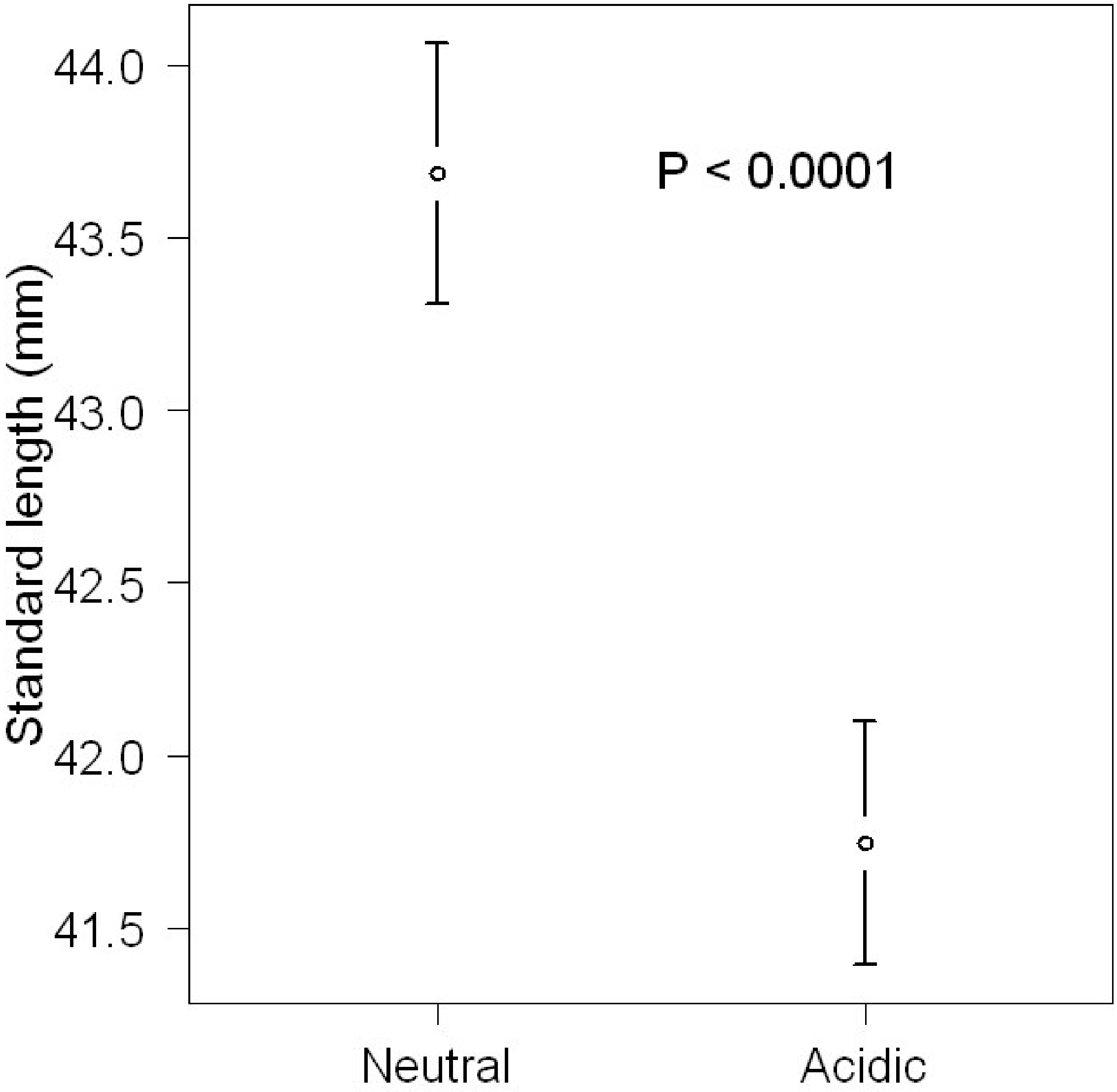

Figure 2.5: Mean (± 95% CI) size (standard length, L_S) of all tethered fish in each stream class (*n* = 777 for neutral streams; *n* = 987 for acidic streams).

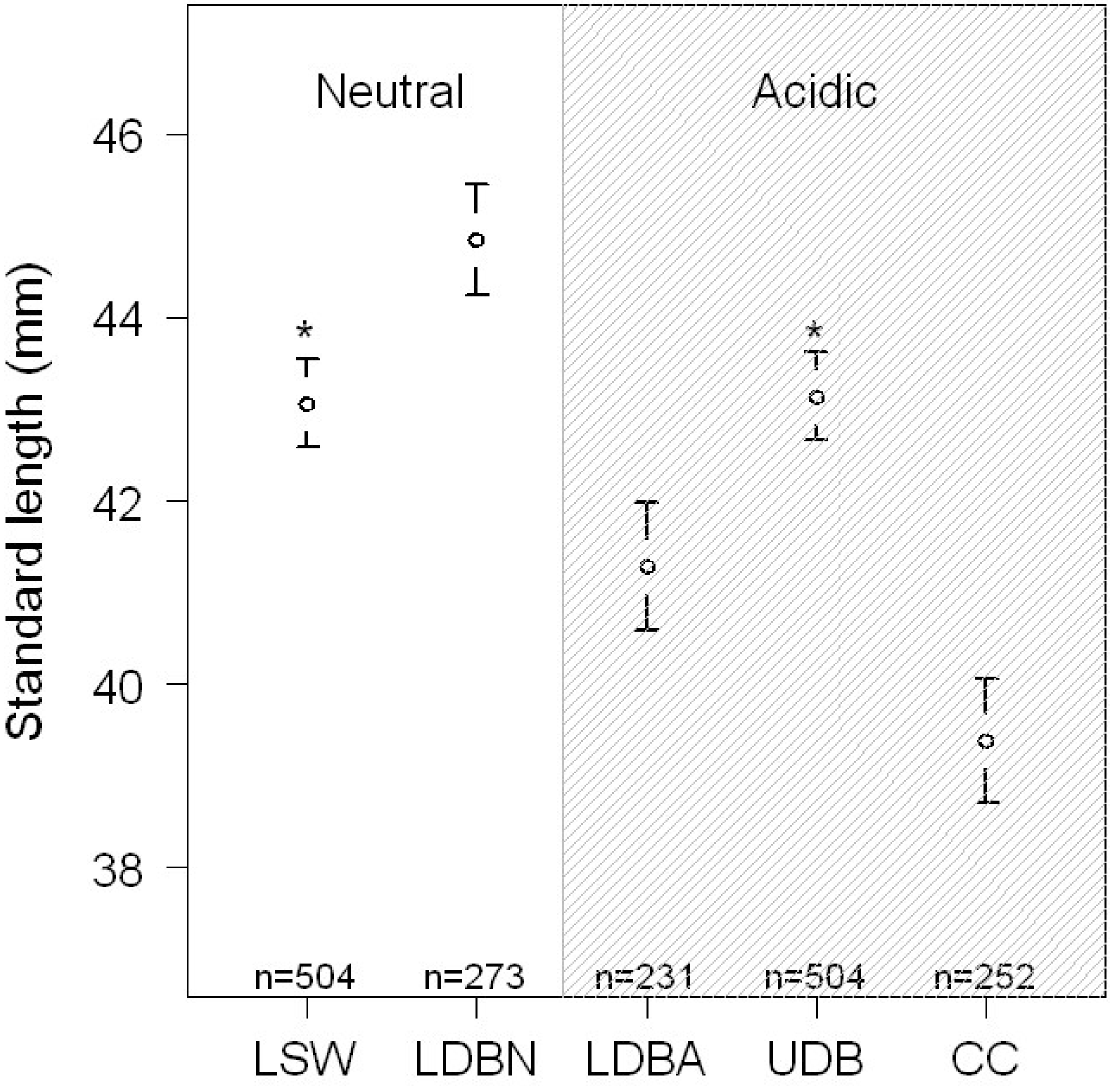

Figure 2.6: Mean (± 95% CI) size (standard length, L_S) of all tethered fish in each stream. All pairwise comparisons of sample means except those indicated by asterisks (*: $P > 0.05$) were significantly different ($P < 0.001$) in a *post-hoc* Tukey's HSD test. LSW = Little Southwest; LDBN = Lower Devil's Brook under neutral conditions; LDBA = Lower Devil's Brook under acidic conditions; UDB = Upper Devil's Brook; CC = Correy Creek.

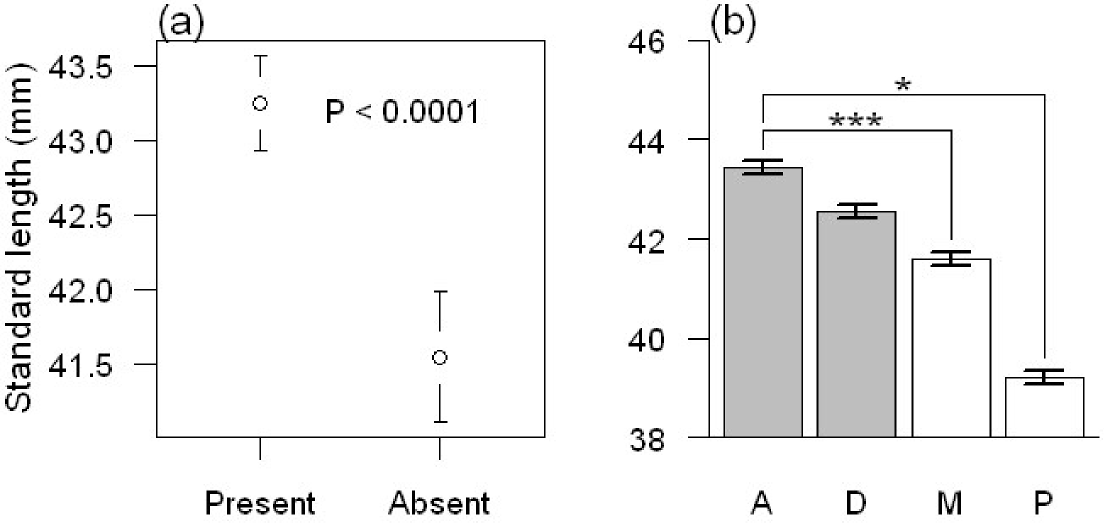

Figure 2.7: (a) Mean (± 95% CI) size (standard length, L_S) of all tethered fish by status class (present or absent) and (b) mean (± SE) size by each possible status (A = alive; D = dead; M = missing; P = predation event). Closed bars represent fish counted as present, open bars represent fish counted as missing. Asterisks denote significant differences in size (*: $P < 0.05$; ***: $P < 0.0001$) between individual status as revealed by a *post-hoc* Tukey's HSD test.

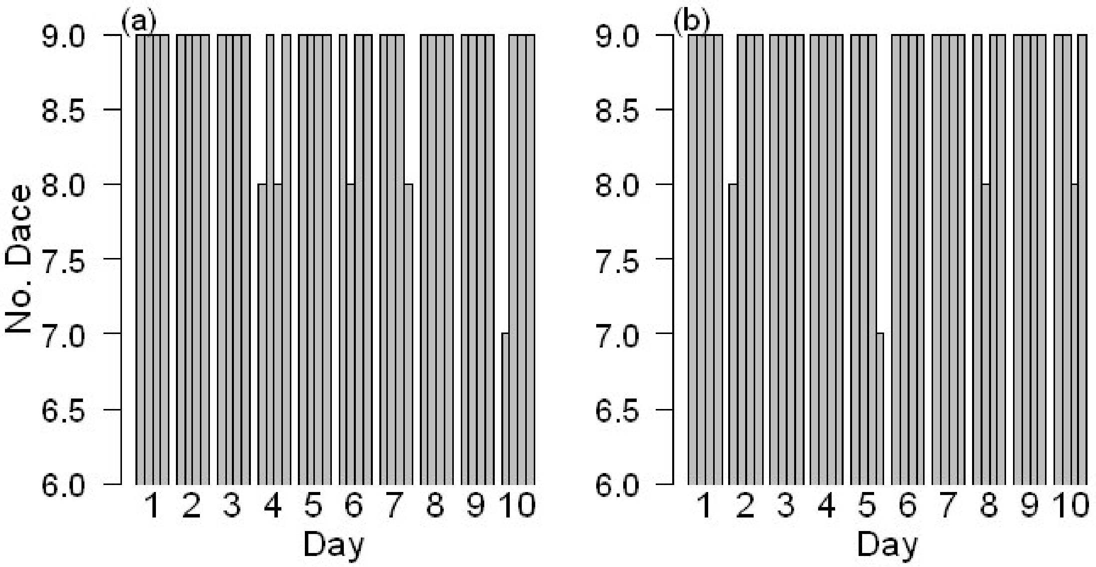

Figure 2.8: Daily depletion of blacknose dace (*Rhinichthys atratulus*) from 4 replicate pools at (a) Catamaran Brook and (b) Devil's Brook over 10 day trial periods. Dace were restocked daily to the original density of $9 \cdot \text{pool}^{-1}$.

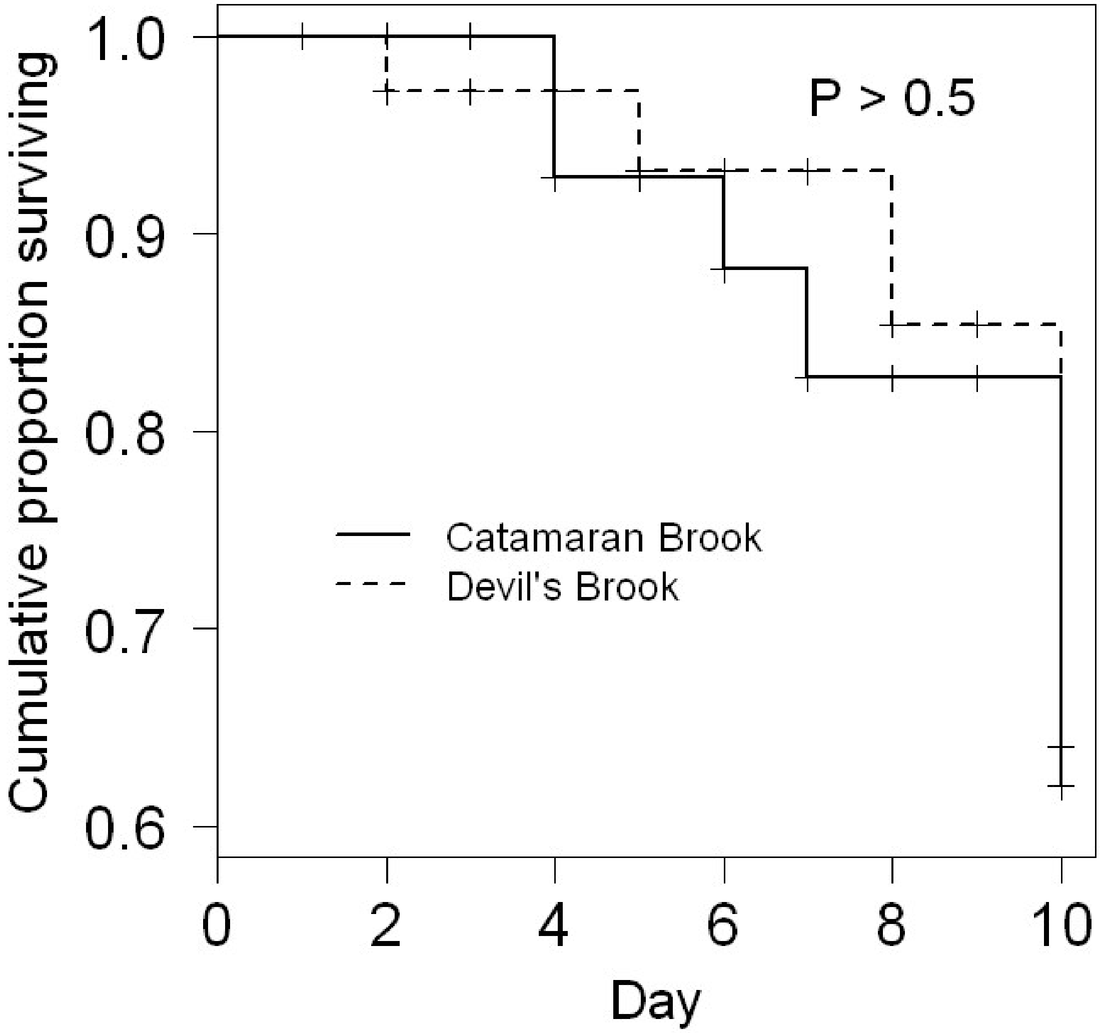

Figure 2.9: Kaplan-Meier survival estimates for blacknose dace (*Rhinichthys atratulus*) in experimental pools at Catamaran Brook (solid line, neutral pH) and Upper Devil's Brook (dashed line, acidic pH) in August 2009. Plotted data represent estimated cumulative probability of survival for an individual over the experimental period of 10 days. Significance level taken from χ^2 testing of the estimated curves ($\chi^2_1 = 0.1$, $P = 0.734$).

Chapter 3: Indirect effects of predation risk and impaired chemosensory communication

Introduction

In addition to the direct lethal costs explored in Chapter 2, predators and the perception of predation risk may also exert numerous indirect or non-lethal effects on prey populations (Cresswell 2008). Two major effects can include depressed rates of reproduction and growth, both of which were demonstrated by Hart's rivulus (*Rivulus hartii*) in pools with an introduced predator, the wolf fish (*Hoplias malabaricus*). Adult rivulus experienced significant decreases in growth rate relative to rivulus unexposed to wolf fish, while there was no difference in juvenile growth rate. The presence of wolf fish also resulted in a ~ 50% reduction in rivulus egg production, and led to increased temporal and spatial patchiness in egg production (Fraser and Gilliam 1992). Reduced clutch size has also been demonstrated in the collared flycatcher (*Ficedula albicollis*) in areas subject to greater rates of nest predation (Doligez and Clobert 2003). Conversely, exposure of mouthbrooding cichlids (*Eretmodus cyanostictus*) to predator cues as juveniles results in the production of significantly larger eggs, but no difference in clutch size (Segers and Taborsky 2012). A comparison of life-history traits of captive, second-generation Hart's rivulus from high- and low-risk populations found that descendants of high-predation populations achieved sexual maturity at smaller size and produced larger clutches of smaller eggs (Walsh and Reznick 2008), indicating that predation on different life-history stages exerts selective pressures resulting in divergent effects on reproductive investment.

An interesting reciprocal relationship in predation on early life-history stages has been well studied in sympatric population of Hart's rivulus and the Trinidadian guppy (*Poecilia reticulata*). Rivulus prey opportunistically on smaller guppies (Seghers 1973; Magurran 2005), while adult guppies prey opportunistically on early life-history stages of rivulus (Fraser and Lamphere 2013). In accordance with a general principle limiting predation risk to gape-limited fish predators > 50% larger than their prey (Popova 1978), rivulus switch from alarm to foraging responses following exposure to damage-released chemical cues from guppies when they exceed the maximum size of wild guppies (Elvidge *et al.* 2010). This reciprocal predation results in larger size at emergence from the eggs in rivulus and larger size at parturition in guppies, as well as increased growth rates leading to younger age of maturity (Walsh and Reznick 2010) and decreased abundance of adults in both species. Interestingly, the indirect effects of guppy predation on rivulus seem to be mediated by food abundance, with rivulus from populations exposed to guppies demonstrating earlier maturation and larger clutches of smaller eggs under conditions of high food abundance, consistent with increased reproductive allocation. Rivulus from the same population reared under conditions of low food abundance demonstrated the opposite pattern of later maturation at larger size and smaller clutches of larger eggs (Walsh and Reznick 2010). In this case, the selective pressures imposed by environmental factors (*i.e.* food abundance) appear to interact with those of predation, as high-predation sites are often characterized by decreased prey abundance, creating conditions of relatively high food availability to the remaining prey (Walsh 2013). Similarly, juvenile rainbow trout (*Onchorhynchus mykiss*) have demonstrated a relationship between behavioural and physiological responses to predation risk and food abundance, with food-restricted trout engaging in riskier foraging activity and displaying significantly faster heart rates than food-satiated or undisturbed fish, indicative of increased preparedness for flight (Hojesjo *et al.* 1999).

Indirect effects of predation also extend beyond physiological and reproductive consequences for prey species. Hart's rivulus introduced with wolf fish in experimental ponds altered their habitat usage patterns and demonstrated a strong preference for shallower littoral areas relative to conspecifics unexposed to wolf fish. Rivulus were also more likely to emigrate from the experimental ponds when wolf fish were present (Fraser and Gilliam 1992). Chub (*Leuciscus cephalus*) in outdoor experimental channels with pool and riffle habitat exposed to simulated avian predation were less likely to forage in the riffles and increased their time sheltering under cover in the pools at the expense of foraging activity relative to chub not exposed to simulated avian predation (Allouche and Gaudin 2001). Interestingly, when water depth was increased, chub exposed to avian predation continued to forage in the riffles, as increased water depth and turbidity may provide sufficient protection against detection and attack. Northern redbelly dace (*Phoxinus eos*) in 13 replicate Canadian shield lakes demonstrated a preference for littoral over pelagic habitat in proportion to generalized predation risk in the pelagic zones. Within littoral zones, dace also demonstrated a preference for structured versus open habitat (Dupuch *et al.* 2009). As demonstrated by the rivulus emigrating from areas of greater predation risk (Fraser and Gilliam 1992), selective habitat use in response to predation may result in the fragmentation of populations as prey avoid high-risk areas (Fraser *et al.* 1995). This is of particular importance for stream-dwelling fishes as the dendritic nature of river systems constrains migratory fish to the channel network (Grant *et al.* 2007). Avoidance of high-risk connecting channels will therefore limit both the absolute level of habitat availability and gene flow between sub-populations.

Detection of predator cues by early life history stages can also induce the development of morphological defenses against predation in certain prey taxa. The most common example of this occurs in the waterflea (*Daphnia* spp.). Exposure of daphnid eggs to the odour of planktivorous fishes (predator kairomones) results in the development of spiny structures and "helmets" which limit predation by smaller gape-limited planktivores and increase both handling time for the predator and probability of escape for the prey (Hammill and Beckerman 2010). Such morphological changes are not observed in vertebrates due to differences in ontological constraints, although predation by gape-limited piscivores may result in changes in the body morphology of prey species. Northern pike (*Esox lucius*) demonstrated a preference for shallow-bodied roach over deeper-bodied common bream of the same length, with this preference attributed to decreased handling time for shallower body morphologies (Nilsson and Bronmark 2000). With only one prey species available, predator-driven assortment may lead to increased frequency of the deep-body phenotype, which may in turn exert additional effects on the movement and behaviours of prey species.

Another indirect effect is demonstrated in Chapter 1 and Elvidge *et al.* (2013), where juvenile Atlantic salmon (*Salmo salar*) under weakly acidic conditions are deprived of an important source of chemical information on risk. As a result of chemosensory impairment, acid-impacted fish demonstrate significantly greater responses to visual threat cues than fish under neutral conditions. Similarly, juvenile salmon in a neutral stream demonstrated significantly greater responses to conspecific chemical alarm cues at night than during the day (Leduc *et al.* 2010b). Collectively, these findings demonstrate the presence of compensatory short-term behavioural mechanisms that allow prey individuals to prioritize the information conveyed by different available threat cues in inverse proportion to the number of cues available. Greater magnitudes of short-term (minutes) response to single cues and the absence of complementary responses to combinations of multiple cues in acidic waters relative to fishes in neutral waters may result in both direct deleterious effects (*e.g.*, reduced growth rate resulting from depressed foraging behaviour) and medium-term (days, seasons) differences in behaviours (*e.g.*, habitat selection, diel activity partitioning) in acid-impacted individuals and populations. Here, I describe a series of experiments designed to address whether juvenile Atlantic salmon in a subset of the neutral and acidic study streams in Elvidge *et al.* (2013) and Leduc *et al.* (2006): 1) differ in their microhabitat (foraging station) preferences, such that fish in acidic streams occupy relatively safer habitat (*e.g.*, preferring more complex substrates which provide more physical refugia from predators); 2) differ in their diel activity patterning in order to maximize use of any remaining information on predation risk by fish in acidic streams; 3) experience any differences in growth attributable to differences in trade-offs between alarm behaviours and other fitness-related activities such as foraging resulting from increased reliance and greater magnitudes of antipredator responses to visual cues in the acidic streams; and 4) experience differing levels of predation pressure and growth under fully natural, free-swimming conditions.

Materials and Methods

Study sites

Subsets of the Atlantic salmon nursery streams on the Northwest Miramichi network described in Chapter 1, as well an additional neutral stream, Otter Brook (described in Leduc *et al.* 2006), were used for this group of experiments. All sites were within 200 m of the GPS coordinates presented in Table 1.1, save Otter Brook (GPS coordinates: 46°52.749’ N, 66°2.214’ W) which is a tributary of Little Southwest whose confluence is approximately 6 km downstream (east) of Catamaran Brook (Figure 2.1).

Experiment 3a: Microhabitat selection

During July 2011, individual free-swimming 0^+ Atlantic salmon in Catamaran Brook (neutral), Lower Devil’s Brook (neutral), Upper Devil’ Brook (acidic) and Correy Creek (acidic) were located visually by an observer standing downstream and watching for 3 min in order to identify their occupied central foraging station (Steingrimsson and Grant 2008). Twenty foraging stations in each stream, as well as unoccupied control spots 1 m directly upstream of the foraging stations, were marked with flagged rocks and then examined for physical differences in order to compare microhabitats occupied by active, foraging fish in acidic and neutral streams relative to nearby available microhabitats. Physical measures consisted of pH and temperature (° C, using a portable Multiline P4 digital meter, WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany), channel width (m), distance from nearest riverbank (m), distance index (distance from bank · channel width^{-1}), depth (m), flow rate at 50% depth (m · s^{-1}, using a Marsh-McBirney Flo-Mate 2000 flow meter, Hach Company, Loveland, CO, USA), substrate complexity (described in Chapter 1 and Leduc *et al.* 2007b), canopy cover (proportion of the area sky directly overhead covered with tree canopy or other vegetation, after Leduc *et al.* 2006), and substrate composition (per cent of area within a 0.5 m radius covered with substrate < 1 cm, $1 \text{ cm} \leq x < 5$ cm, $5 \text{ cm} \leq x < 15$ cm, ≥ 15 cm) based on the grain size classes delineated in the Wolman (1954) Pebble Count. Physical measures were combined into a multivariate response and analyzed with two two-way MANOVAs against salmon preference (occupied or unoccupied sites), once for each stream class.

Experiment 3b: Diel activity partitioning

During July and August of 2008, a series of snorkeling surveys were conducted during midday (10:00 – 14:00) and midnight (22:00 – 02:00) using an underwater flashlight in two 30 m reaches in each of four streams (two neutral: Catamaran Brook, Otter Brook; two acidic: Upper Devil's Brook, Correy Creek). Otter Brook is a tributary of Little Southwest Miramichi approximately 6 km downstream from Catamaran Brook that has been included in earlier inter-stream comparisons of juvenile Atlantic salmon behaviour (Leduc *et al.* 2006). Transect lines were followed in zig-zag patterns from bank to bank such that all of the study areas within the stream reaches were examined. Every juvenile Atlantic salmon (0^+, 1^+ parr and 2^+ parr) and adult brook trout sighted during each survey was recorded and these counts were transformed into density estimates in areas defined by reach length and mean channel width ($n = 3$ measurements in each reach, at both extremes and in the middle) to control for differences in area. The terms abundance and density in this experiment do not refer to the absolute values for the populations in the study streams, but rather to the relative abundance and density of active or non-sheltering individuals at each diel period, in general accordance with previous methodology (Toobaie *et al.* 2013). Due to the low overall numbers of active, non-sheltering fish throughout the study streams during the summer of 2008, density estimates were square-root transformed to reduce the magnitude of positive skew and then examined in a series of one-way ANOVAs against diel period in each stream class (neutral or acidic). Direct comparisons of abundance/density estimates between streams and stream classes were avoided in order to focus on diel patterning within streams or classes and limit potential confounds arising from different absolute abundances or densities between populations.

Experiment 3c: Captive growth trials

Wild 0^+ Atlantic salmon were captured individually via dipnet from Catamaran Brook, weighed, measured (L_S) and placed into one of two mesocosms in either Catamaran Brook or the weakly acidic Correy Creek during July and August 2010. Fish were transported between sites using the same equipment as Experiment 2b and Jackson & Brown (2011). Mesocosms consisted of rectangular prysmic nylon nets (6 m length × 1 m width × 0.75 m height, mesh size = 3 mm) supported by aluminum conduit poles driven into the streambed and anchored to nearby trees with nylon rope. Nearby river cobbles and gravel were shoveled in to a depth of ~ 5 cm and several large rocks (> 15 cm diameter) were added to provide shelter and refugia from high water flows. These mesocosms effectively retained captive fish and delivered natural drift forage while excluding predators. Fish were placed into the enclosures at a density of $1 \cdot m^{-2}$ (*i.e.* 6 fish per enclosure) for seven days, during which one enclosure in each stream was exposed daily (11:00 or 14:00) to a 100 ml injection of salmon alarm cues to simulate a high-risk environment, or to a 100 ml injection of stream water as a mechano-chemical control. Fish were removed one day after the last injection (*i.e.* on day eight), re-weighed and measured and released at their approximate site of capture in Catamaran Brook. Five replicate trials were conducted in each stream, involving a total of 120 fish (n = 6 for each stream × treatment combination and n = 60 fish per treatment per stream). Mean changes in body measurements (standard length [mm], mass [g]) and condition (Fulton's condition index, $K = 100 \cdot \text{weight} \cdot \text{length}^{-3}$) as well rates of change in body measurements were examined against stream (neutral Catamaran Brook and acidic Correy Creek; n = 5 for each treatment in each stream) and treatment (high vs. low risk) in two-way ANOVAs. A combination of factors including suitability of substrate for the enclosures and constraints arising from travel times (a roundtrip between Catamaran Brook and Correy Creek is approximately 80 minutes of driving and was done at least once per day for the 5-6 week period over which this experiment was conducted) precluded the use of additional replicate streams.

Experiment 3d: Mark-recapture

Between June and August of 2008, individual wild 0^+ Atlantic salmon were captured via dipnet and the precise site of their capture was marked with a flagged cobble. The fish were tagged using visible implant elastomer (VIE, Northwest Marine Technologies, Shaw Island, WA, USA) in unique colour × position combinations and digitally photographed with a submersible 6MP Xacti VPC-E1 camera (Sanyo North

America Corporation, Seacaucus, NJ, USA) against a laminated sheet of millimetric graphing paper to facilitate subsequent morphometric analysis. Tagged fish were then released at their site of capture. The original plan was to tag a minimum of 100 salmon in each of the neutral Catamaran and Otter Brooks and the acidic Upper Devil's Brook and Correy Creek; however, the spring and summer of 2008 were characterized by a late spring snow melt and high levels of precipitation which resulted in a late emergence of young of the year salmon and low levels of activity, possibly due to the increased availability of drift forage and increased energetic costs associated with central-place foraging (*sensu* Nislow *et al.* 1998). Consequently, juvenile salmon were only active and foraging out of shelter for a fraction of the season and the observed densities in the streams were artificially low. During the summer of 2009, 1^+ parr densities were the highest personally observed during the course of gathering research for this thesis (2008 – 2012), indicating that the relatively high discharge and cool temperatures during 2008, while far from conducive for the activities of ecologists, nevertheless provided ideal conditions for the growth and survival of 0^+ Atlantic salmon. In any case, this attempt at a mark-recapture experiment was intended to provide estimates of inter-stream class differences in *in-situ* growth rate and body condition as well as an estimate of relative predation pressure between stream classes but was discontinued due to the considerable logistic constraints and low catch rate.

Results

Experiment 3a: Microhabitat selection

Wild 0^+ Atlantic salmon demonstrated several statistically significant differences in aspects of their habitat preferences in the neutral and acidic study streams. Habitat variables were not compared between stream classes as the neutral streams tended to be larger and deeper than the acidic streams (Figure 3.1a,b). Fish under acidic conditions occupied sites that had slower flow rates relative to control sites ($F_{1,78} = 6.442$, $P = 0.013$; Figure 3.1c). While lower flow rates may result in greater temporal persistence of chemical cues within a given area, the loss of function imposed on chemical alarm cues by acidification is unlikely to play a role in driving the establishment of this apparent preference. Lower rates of flow may also result in longer residence times within an area for drift-borne forage, as well as more abundant benthic fauna, potentially resulting in greater forage abundance and facilitating more risk-averse foraging strategies relative to high-flow conditions. Conversely, high flow sites may deliver drift forage opportunities at a greater rate than low flow sites. Fish in the neutral streams preferred habitats with greater substrate complexity ($F_{1,78} = 8.844$, $P = 0.0039$, Figure 3.1f), which likely have reduced ranges of visibility relative to sites with lower substrate complexity. There was no demonstrated difference in preference for channel position (distance from nearest bank · channel width^{-1}) within either stream class, although a general preference for near-shore habitats appears to generate lower values for channel position within the neutral streams (Figure 3.1g,h). Occupying locations closer to a shore may potentially limit the angle of attack available to foraging predatory fishes, but may also increase vulnerability to attack from terrestrial predators.

Contrary to their apparent preference for sites with greater substrate complexity as measured throughout this thesis, fish under neutral conditions demonstrated a preference for sites with greater proportions of smaller substrate particles (substrate grain size: diameter < 1 cm: $F_{1,78} = 5.849$, $P = 0.0179$, Figure 3.2b; 1 cm ≤ x < 5 cm: $F_{1,78} = 2.899$, $P = 0.093$, Figure 3.2d). Salmon in acidic streams also seem to prefer sites with lower proportions of the 1 cm ≤ x < 5 cm diameter grain size class, although this trend was not statistically significant (Figure 3.2c). Fish under neutral conditions also demonstrated a preference for sites with significantly lower proportions of the largest substrate grain size considered (diameter > 15 cm: $F_{1,78} = 9.175$, $P = 0.0033$, Figure 3.2h). Larger-grained substrate, while potentially reducing range of vision, is also likely to provide a greater abundance of physical refugia and decreased rate of flow due to turbulence. The latter potential consequence of larger grain size is demonstrated by the observation that fish under acidic conditions occupied sites characterized by greater flow rates than unoccupied sites (Figure 3.1c).

Experiment 3b: Diel activity partitioning

Overall, the three age classes of salmon recorded demonstrated trends towards greater median abundances during the day relative to at night in the acidic study streams (Figure 3.3a, b, c). Brook trout, by contrast, demonstrated qualitatively greater median abundance at night than during the day in the acidic streams. In the neutral streams, Atlantic salmon fry (0^+, Figure 3.3a) demonstrated similar median abundances during the day and night survey periods, while 1^+ (Figure 3.3b) and 2^+ parr (Figure 3.3c) and brook trout (Figure 3.3d) were all more abundant at night, although this difference only met statistical significance in the case of 1^+ salmon parr ($F_{1,22} = 3.21$, $P = 0.087$).

Atlantic salmon fry (0^+) were least abundant in the acidic Correy Creek, recorded in only 16.7% of the snorkeling surveys (*i.e.* 2 out of 12 surveys; Table 3.1). In the neutral streams, 0^+ salmon and 1^+ parr were equally abundant in both streams, but 2^+ parr were more commonly recorded in Catamaran Brook (100%) than in Otter Brook (58.3%). In order to prevent overrepresentation of estimates of salmon fry abundance from the neutral streams in the dataset, the analyses were repeated on a subset of the data consisting of one stream of each class in which salmon were most commonly recorded (Catamaran Brook and Upper Devil's Brook). In this subset of the data, 0^+ fry were significantly more abundant during the day than at night in the acidic Upper Devil's Brook ($F_{1,10} = 5.993$, $P = 0.0034$; Figure 3.4a), while the other age classes of salmon tended to be more abundant at night, as did adult brook trout ($F_{1,10} = 5.829$, $P = 0.0364$; Figure 3.4d). In the neutral Catamaran Brook, 1^+ parr were more abundant during the day than at night ($F_{1,10} = 21.20$, $P < 0.001$; Figure 3.4b) while 2^+ parr demonstrated the opposite pattern ($F_{1,10} = 57.175$, $P < 0.0001$; Figure 3.4c), as did brook trout ($P > 0.1$; Figure 3.4d). Salmon fry demonstrated a non-significant trend towards greater levels of activity during the day than at night (Figure 3.4a), in agreement with a pattern of diel activity previously identified by Toobaie *et al.* (2013).

Experiment 3c: Captive growth trials

Mean physical measurements recorded within the captive growth enclosures during each replicate are presented in Table 3.2. Captive 0^+ Atlantic salmon exposed to both the high-risk (AC) and control (SW) treatments in neutral Catamaran Brook and acidic Correy Creek demonstrated positive daily rates of growth in both standard length

(Figure 3.5a) and mass (Figure 3.5b) over the course of the experiment. While any decreases in standard length would be the result of measurement error, decreases in mass over time may have been possible. In such events, decreased mass might be attributable to reduced forage abundance within the net enclosures, resulting from mechanical exclusion of drift items or from increased competition at the experimental density. While the growth rates of subjects exposed to the different risk treatments did not differ within streams, salmon exposed to both treatments demonstrated significantly greater rates of mass increase in the acidic transplant stream, Correy Creek, than they did in their native Catamaran Brook ($F_{1,16} = 5.97$, $P = 0.0266$). Salmon exposed to three out of the four stream × treatment combinations actually demonstrated a decrease in Fulton's condition index (K), with the sole positive change occurring in the group transplanted into Correy Creek and exposed to chemical alarm cues (Table 3.3). This group of fish demonstrated rates of increase in mass similar to those fish transplanted into Correy Creek and exposed to the control (Figure 3.5b), but a smaller rate of mean increase in length (Figure 3.5a), leading to a positive change in the condition index as K is proportional to mass $\cdot$ length^{-3}.

Given the shared provenance of the salmon in this experiment and the significantly greater rates of increase in mass of the subjects in Correy Creek, it appears that the acidic transplant stream actually provided a better set of conditions for growth in the predator-excluding mesocosms than did the native stream, Catamaran Brook. Unfortunately, the experimental design incorporating only one source population from a neutral stream does not preclude the possibility that the greater mass increase of fish transplanted into the acidic stream was a result of the sudden loss of chemical alarm cue availability and less risk-averse foraging behaviour. The statistically significant trend of inter-stream differences in rates of mass increase is not evident, however, in the raw data on median values of the initial and final measurements of length (Figure 3.6a,b) and mass (Figure 3.6c,d), as the median values demonstrate similar increases between initial and final measurements in all treatment groups save the masses of fish in Catamaran Brook (Figure 3.6c).

Discussion

The four experiments presented in this chapter were intended to elucidate and quantify the indirect (non-lethal) effects of different levels of perceived predation risk mediated by sensory impairment on acid-impacted and control populations of juvenile Atlantic salmon. The fact that juvenile salmon of all the age classes considered were present every summer from 2008 to 2011 in all of the current study streams is a clear indication that acid-mediated sensory impairment does not result in either absolute mortality or behavioural avoidance of affected nursery streams by spawning adult females. This conclusion does not, however, preclude the possibility of significant inter-class (neutral or acidic) differences in interannual survival of juveniles or frequency of return of spawning adults, although detecting any such differences was beyond the logistic parameters of these experiments.

In terms of habitat selection (Experiment 3a), juvenile salmon living under neutral conditions appear to occupy sites characterized by relatively small substrate grain size and greater flow rate. Both of these physical characteristics imply a greater rate of drift forage arrival in less turbulent currents. They also demonstrated a preference for sites with greater substrate complexity, which may obstruct some portion of the field of view. This preference may reflect a behavioural trade-off between heightened ambient risk level arising from decreased visual fields and increased drift foraging opportunities mediated by the availability of chemical risk cues. By contrast, juvenile salmon under acidic conditions appear to prefer sites with lower flow rates and substrate complexity. They also prefer larger substrate grain sizes, a combination that may minimize exposure to visually foraging predators to off-set the increased direct costs of predation associated with chemosensory impairment, at the potential expense of decreased drift foraging opportunities.

Increases in both body size (L_S) and mass in the population transplant experiment (Experiment 3c), in which physical conditions were standardized and predators were excluded, indicate that weak acidification is unlikely to negatively affect growth either directly through physiological impairment or indirectly through shifts in forage abundance and/or composition. This inference is supported by the statistically significant increase in mass in fish exposed to both risk treatments in the acidic transplant relative to their counterparts in the native neutral stream, and the positive change in condition factor to the acid transplant group exposed to the high-risk treatment. The absence of treatment-derived differences between high and low risk conditions within the neutral stream supports the notion that prey fish are able to adjust their antipredator strategies in order to maintain sufficient foraging activities at the population density ($1 \cdot m^{-2}$) used in Experiment 3c. In the context of the short-term behavioural responses documented in Chapter 1 and Elvidge *et al.* (2013), energetic costs incurred from significant immediate decreases in foraging in response to discrete risky cues may be offset or replaced by increased rates of foraging following resumption of normal activities. This type of compensatory foraging pattern has recently been demonstrated in two species of wild-caught tropical fishes, the Trinidadian guppy (Elvidge *et al.* in review) and Hart's rivulus (Elvidge and Brown, in preparation), under laboratory conditions. Similar compensatory mechanisms may also occur in competitive and aggressive interactions with conspecifics and other prey guild members.

Juvenile salmon (0^+, 1^+ and 2^+ age classes) under acidic conditions demonstrated a trend towards greater levels of activity during the day than at night, while under neutral conditions, 0^+ salmon demonstrated no preference and 1^+ and 2^+ fish were more active at night (Experiment 3b). In both stream classes, predatory book trout were more active at night. These findings are mostly in agreement with an earlier study that demonstrated a preference for diurnal activity in 0^+ salmon in Catamaran Brook (Toobaie *et al.* 2013). In this experiment, the diel difference in abundance was statistically significant in the acidic Upper Devil's Brook but not in Catamaran Brook. While this observation does not reflect a direct comparison between the two streams, it suggests the possibility of more pronounced diel activity patterning in acid-impacted 0^+ salmon. Under acidic conditions, shifting to a diurnal behavioural pattern is likely to reduce the importance of chemical cues to prey survival due to the increased availability of visual cues and also reduces the likelihood of encountering a nocturnal brook trout. Temporal avoidance of fish predators under neutral conditions, by contrast, may provide smaller survival benefits given the availability of chemical cues to convey levels of risk under light-limited conditions. As demonstrated in Chapter 1 and Elvidge *et al.* (2013), the loss of chemical information due to acidification appears to elicit compensatory behavioural mechanisms which increase both the availability and value of remaining visual threat cues without limiting resource acquisition and growth potential.

Table 3.1: Percentage of snorkeling surveys in the four study streams in which adult brook trout (*Salvelinus fontinalis*) and each age class of juvenile Atlantic salmon (*Salmo salar*) were recorded ($n = 12$ surveys per stream; $n = 6$ at midday and $n = 6$ at midnight in each stream).

Stream	**Class**	**0^+ *S. salar* fry**	**1^+ *S. salar* parr**	**2^+ *S. salar* parr**	**Adult *S. fontinalis***
Catamaran Brook	Neutral	58.3%	100%	100%	25%
Otter Brook	Neutral	58.3%	100%	58.3%	83.3%
Upper Devil's Brook	Acidic	83.3%	100%	100%	91.7%
Correy Creek	Acidic	16.7%	41.7%	16.7%	75%

Table 3.2: Mean (± SD) values of physical characteristics captive 0^+ Atlantic salmon (*Salmo salar*) from Catamaran Brook were exposed to inside mesh enclosures during a cross-population transplant experiment to examine differences in acid-mediated threat-sensitive growth. *n* = 5 replicates per stream × treatment combination. AC = alarm cue (high risk), SW = stream water (control).

Stream	**Treatment**	**pH**	**Temperature (° C)**	**Depth (m)**	**Flow rate ($m \cdot s^{-1}$)**
Catamaran Brook	AC	7.158 ± 0.49	18.74 ± 1.64	0.216 ± 0.07	0.0812 ± 0.03
	SW	7.158 ± 0.49	18.74 ± 1.64	0.203 ± 0.06	0.0631 ± 0.02
Correy Creek	AC	6.846 ± 0.65	18.19 ± 1.54	0.227 ± 0.05	0.0827 ± 0.04
	SW	6.846 ± 0.65	18.19 ± 1.54	0.224 ± 0.05	0.0825 ± 0.04

Table 3.3: Mean values of fish size (standard length, mm; mass, g) and Fulton's condition index (K) of 0^+ Atlantic salmon (*Salmo salar*) from Catamaran Brook at the beginning and end of 5 replicate 6-8 day cross-population transplants. Positive differences are indicated in bold type. *n* = 6 fish per stream × treatment replicate.

Stream	**Stim**	**L_1**	**L_2**	**ΔL**	**M_1**	**M_2**	**ΔM**	**K_1**	**K_2**	***ΔK***
Catamaran Brook	AC	44.27	45.47	**1.2**	1.20	1.24	**0.04**	1.38	1.31	-0.07
	SW	44.37	45.45	**1.08**	1.16	1.18	**0.02**	1.32	1.25	-0.07
Correy Creek	AC	44.00	45.23	**1.23**	1.15	1.25	**0.1**	1.34	1.35	**0.01**
	SW	44.07	45.68	**1.61**	1.17	1.26	**0.09**	1.36	1.33	-0.03

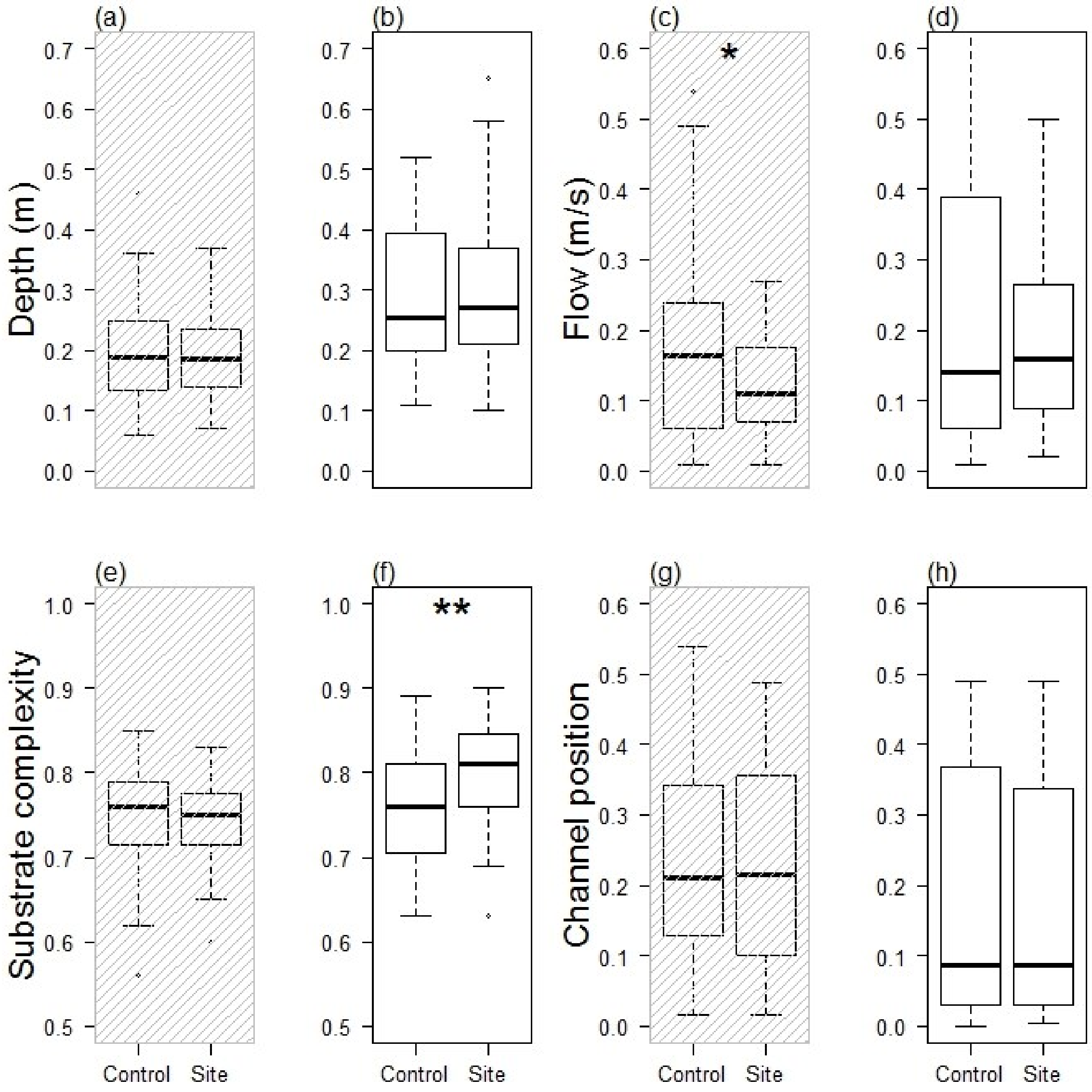

Figure 3.1: Boxplots showing the median, first and third quartiles and 95% confidence intervals of different physical habitat measurements within a 1 m radius of occupied central place foraging sites (Site) of 0^+ Atlantic salmon (*Salmo salar*) compared to unoccupied control sites (Control) 2 m directly upstream in two neutral (open plots) and two acidic (shaded plots) streams. (a,b) Depth (m); (c,d) flow rate ($m \cdot s^{-1}$); (e,f) substrate complexity (1 = perfectly flat [simple], 0 = maximum complexity); (g,h) channel position (distance from nearest bank · channel width^{-1}). *n* = 40 sites per box. Asterisks denote

significant differences between occupied and unoccupied sites as indicated by 1-way MANOVA (*: $P < 0.05$; **: $P < 0.01$).

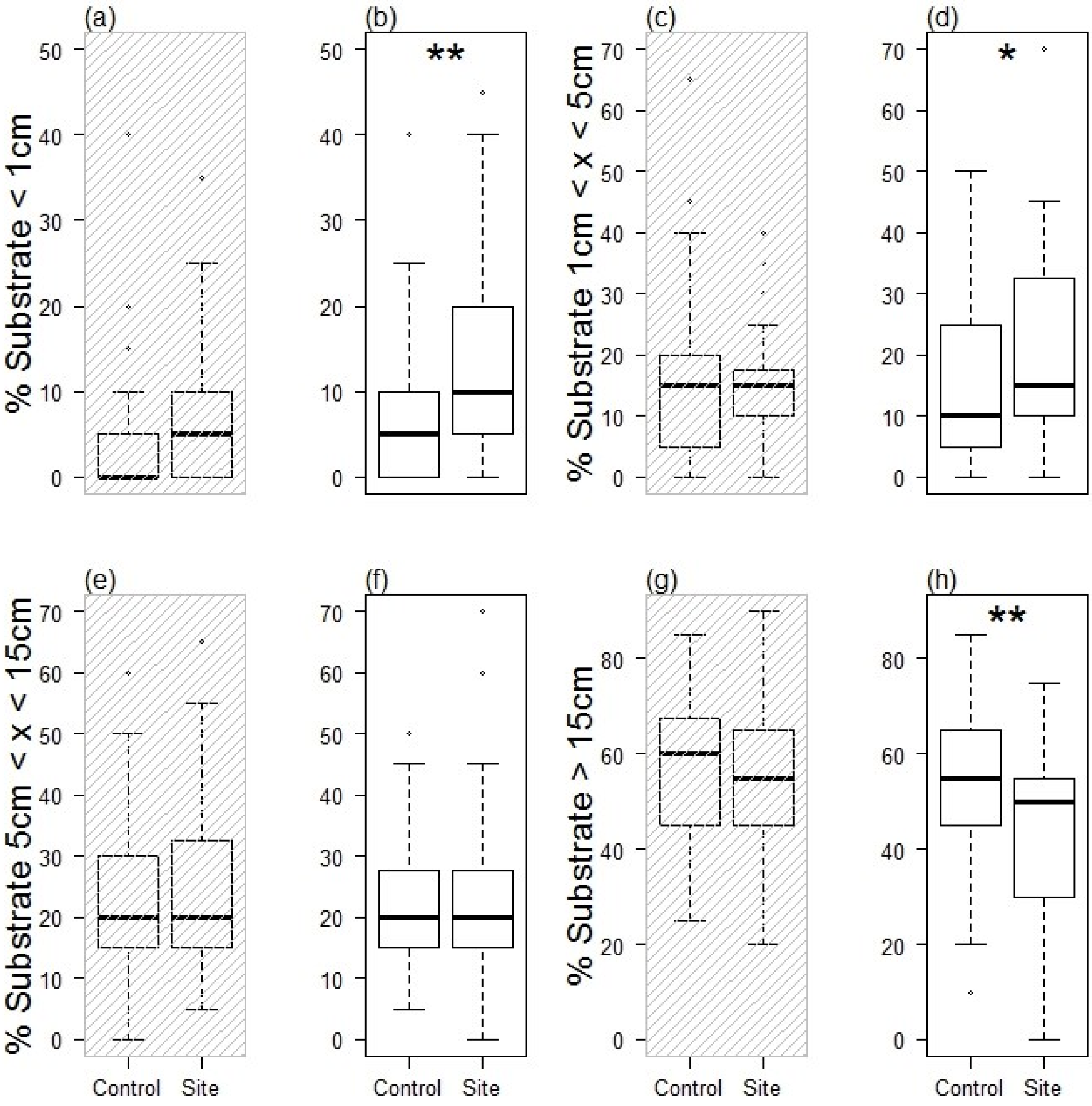

Figure 3.2: Boxplots showing the median, first and third quartiles and 95% confidence intervals of the proportion of substrate within a 1 m radius of occupied central place foraging sites (Site) of 0^+ Atlantic salmon (*Salmo salar*) compared to unoccupied control sites (Control) 2 m directly upstream in two neutral (open plots) and two acidic (shaded plots) streams. Substrate size classes correspond to the gradations specified in the Wolman (1954) Pebble Count: (a,b) < 1cm diameter; (c,d) 1 cm – 5 cm diameter; (e,f) 5 cm – 15 cm diameter; (g,h) > 15 cm diameter. *n* = 40 per bar. Asterisks denote significant

differences between occupied and unoccupied sites as indicated by 1-way MANOVA (*: $P < 0.05$; **: $P < 0.001$).

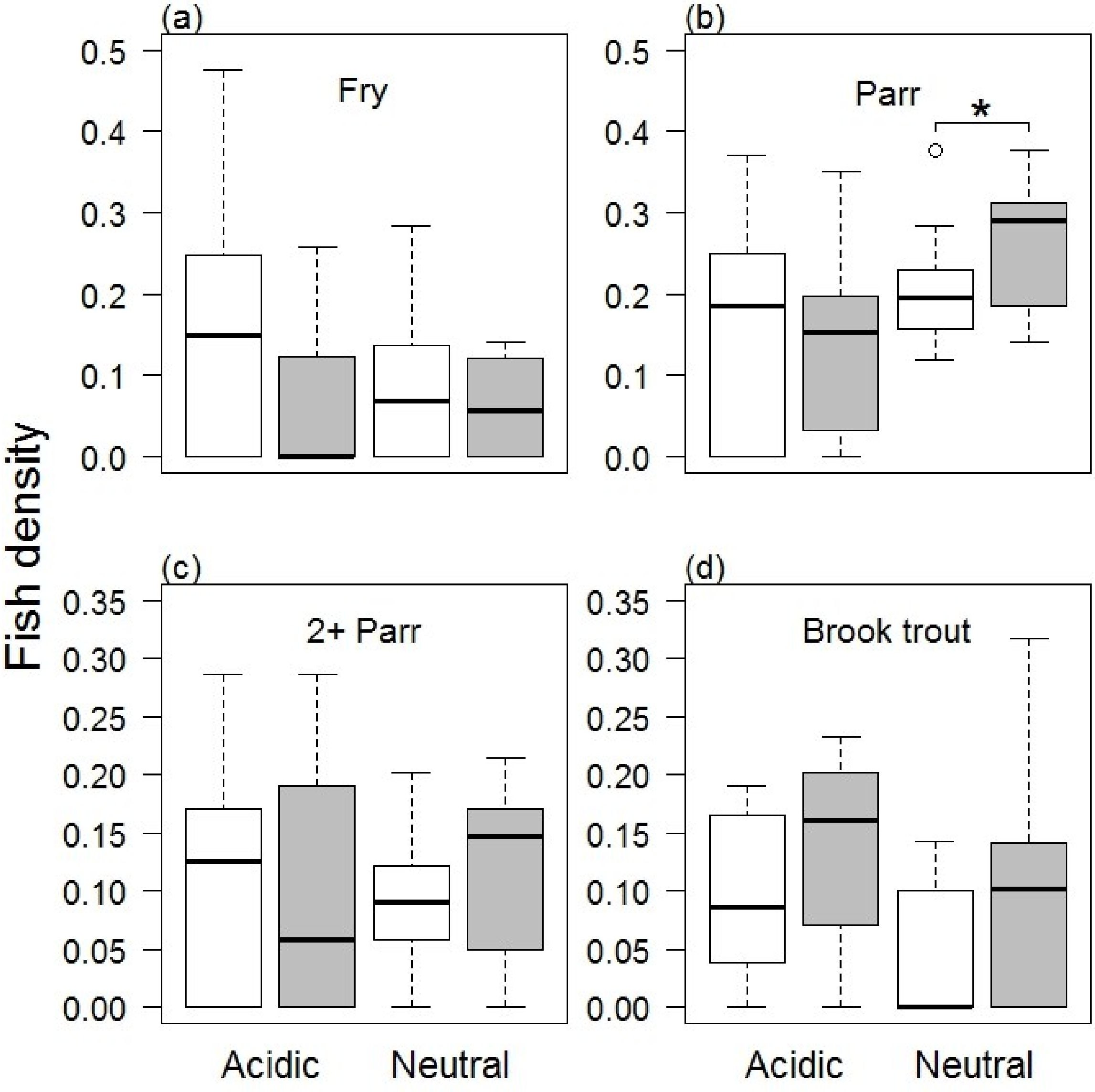

Figure 3.3: Boxplots showing the median, first and third quartiles and 95% confidence intervals of square root-transformed density estimates of three age classes of juvenile Atlantic salmon (*Salmo salar*): (a) 0^+ or fry, (b) 1^+ parr, (c) 2^+ parr as well as (d) brook trout (*Salvelinus fontinalis*) visually assessed to be large enough to consume salmon fry. Density estimates were generated via snorkeling surveys in two predefined areas in two acidic and two neutral salmon nursery streams conducted at midday (open boxes) or at

midnight (shaded boxes). Asterisks denote significant differences between diel periods within stream classes from 1-way ANOVA (*: $P < 0.1$).

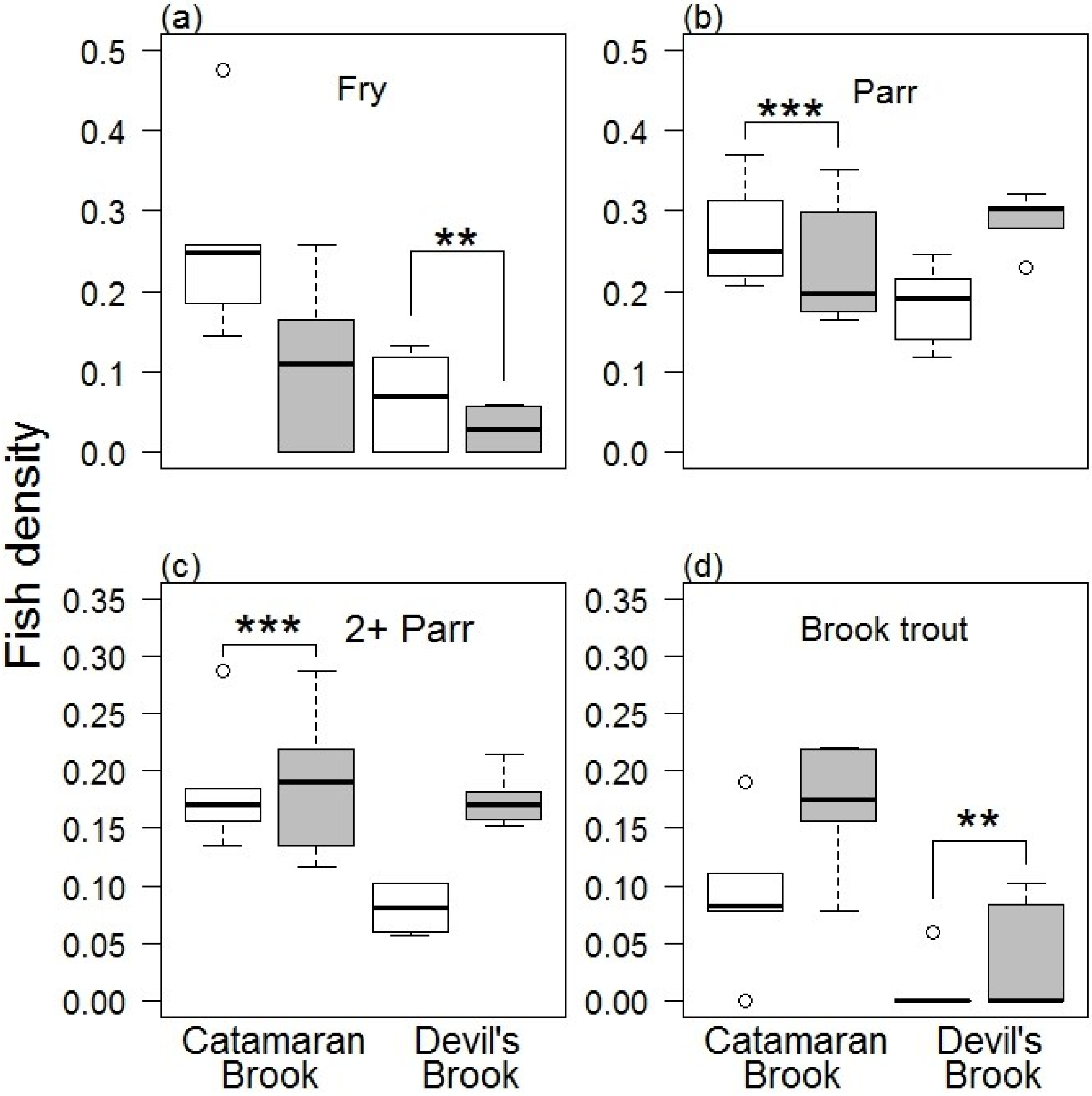

Figure 3.4: Boxplots showing the median, first and third quartiles and 95% confidence intervals of square root-transformed density estimates of three age classes of juvenile Atlantic salmon (*Salmo salar*): (a) 0^+ or fry, (b) 1^+ parr, (c) 2^+ parr as well as (d) brook trout (*Salvelinus fontinalis*) visually judged to be large enough to consume salmon fry. Density estimates were generated via snorkeling surveys in two predefined areas in Catamaran Brook (neutral) and Devil's Brook (acidic) conducted at midday (open boxes)

or at midnight (shaded boxes). Asterisks denote significant differences between diel periods within stream classes from 1-way ANOVA (**: $P < 0.05$; ***: $P < 0.001$).

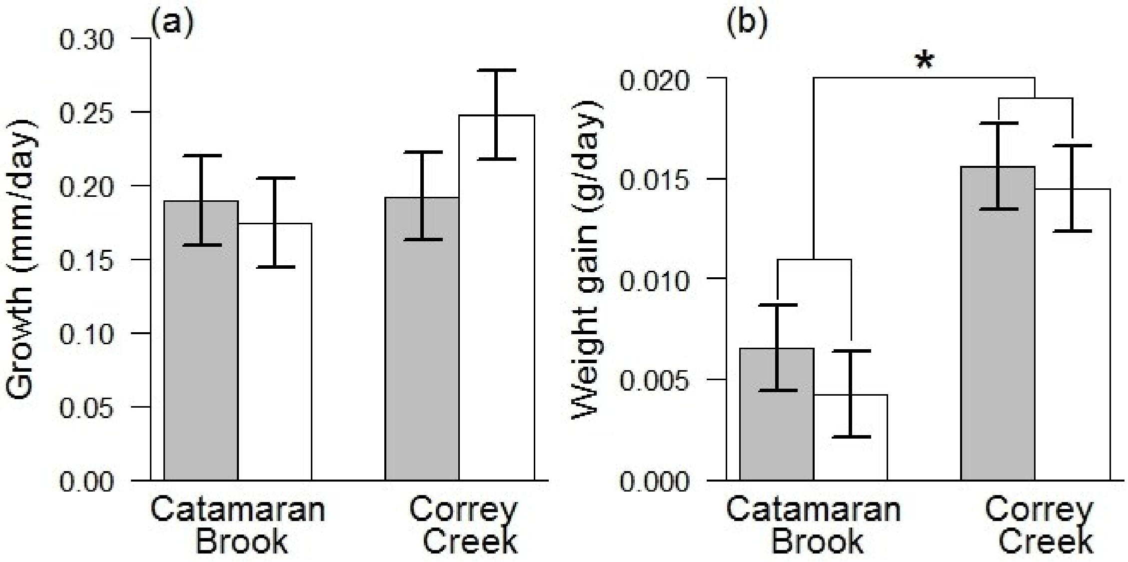

Figure 3.5: Mean (± SE) daily rates of change in (a) standard length (mm) and (b) mass (g) of wild 0^+ Atlantic salmon (*Salmo salar*) from Catamaran Brook in a cross-population transplant experiment exposed to daily injections of chemical alarm cues (shaded bars) or a stream water control (open bars). *n* = 30 measurements per bar. Asterisks denote significant differences between streams from 1-way ANOVA (*: $P < 0.05$).

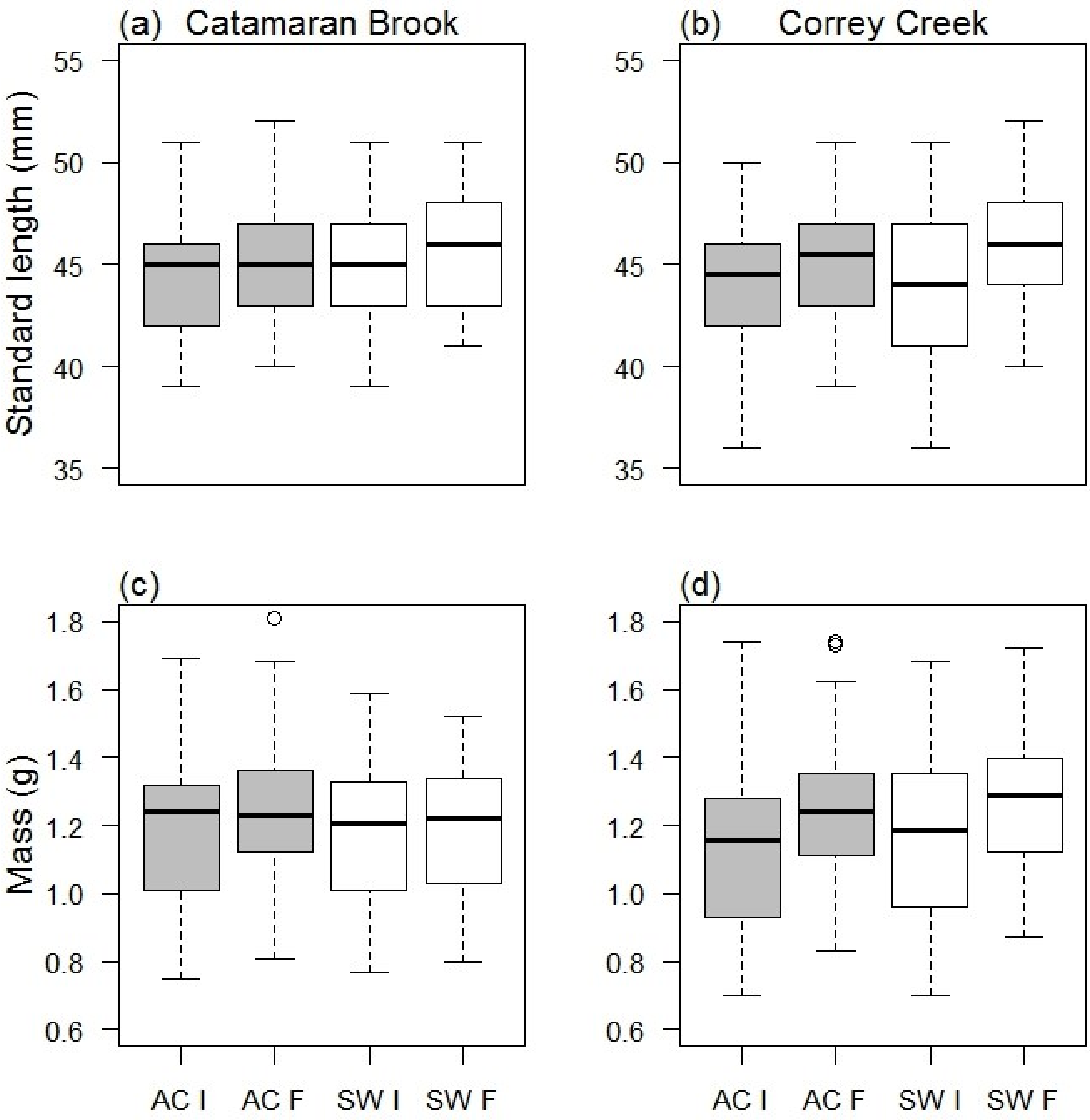

Figure 3.6: Boxplots showing the median, first and third quartiles and 95% confidence intervals of initial (I) and final (F) measurements of (a, b) standard length (mm) and (c, d) mass (g) of wild 0^+ Atlantic salmon (*Salmo salar*) exposed to daily injections of chemical alarm cues (AC, shaded bars) or a stream water control (SW, open bars) in a cross-population transplant experiment. *n* = 30 measurements per bar.

General Discussion

Amongst the various selective pressures influencing the evolution of a species, the immediate and dramatic costs imposed on prey through predation have led to a preponderance of studies on predator-prey interactions. The effects of predation are not, however, limited to the so-called evolutionary arms race (Ferrari *et al.* 2010a) driven by the direct lethal effects of predators on their prey. Indirect effects of predation, including the level of predation risk as perceived by an individual via publicly available information (Danchin *et al.* 2004), may be equally important to the evolution of prey species (Walsh 2013). Relatively high levels of predation risk have been demonstrated to exert deleterious effects on growth rate and fecundity (Fraser and Gilliam 1992), competitive ability at elevated population densities (Walsh and Reznick 2008) and can result in population fragmentation when risk level is spatially heterogeneous (Fraser *et al.* 1995). Predation on different ontogenic stages may also exert different effects on the evolution of life histories in prey species, with predation on juvenile stages typically resulting in increased growth rate and predation on adult stages typically resulting in decreased fecundity and earlier sexual maturity. In both cases, adult population density is likely to be reduced relative to predation-free or lower-risk populations.

Interestingly, perceived risk can have similar consequences. Experimental manipulation of natural stream reaches involving labeling areas as risky via daily injections of conspecific damage-released chemical alarm cues in Catamaran Brook (Northwest Miramichi River drainage, Northumberland County, NB Canada) resulted in decreased densities of juvenile Atlantic salmon relative to control sites injected daily with stream water (Kim *et al.* 2011b). Hence, information influencing the perception of spatially heterogeneous elevated levels of risk may be as important to the evolutionary ecology of prey species as physical predation risk. The apparent equality of direct risk and information on risk levels to prey responses emphasizes the importance of being able to detect and respond to public information that is both accurate and undistorted (Dall *et al.* 2005). Future and historic anthropogenic environmental changes that serve to disrupt the transfer of information on risk such as acidification interfering with damage-released chemical alarm cues (Leduc *et al.* 2006) or turbidity limiting visual cues (Gregory 1993; Domenici *et al.* 2007; Ranaker *et al.* 2012) are likely to exert greater influence over prey populations than natural or non-anthropogenic limitors of information transfer due to the differences in timescales and frequencies between natural and artificial disturbances (*sensu* Petrin *et al.* 2008). Given the widespread documented occurrence of non-lethal behavioural and physiological impacts of anthropogenic stressors on natural populations to date, impacted species may be especially vulnerable to deleterious effects arising from future disturbances. One emerging example is the interaction between increases in ocean surface temperatures and oceanic acidification associated with increased greenhouse gas (notably carbon dioxide, CO_2) emissions, whereby marine fishes may experience increased metabolic costs to offset physiological stress imposed by higher temperatures and increased partial pressure of CO_2 (Ishimatsu *et al.* 2008) as well as impaired sensory detection of ambient risk analogous to findings from acidified freshwater systems (Cripps *et al.* 2011; Ferrari *et al.* 2012).

Given similar physical habitat characteristics and restrictions on movement and dispersal via tethering, juvenile Atlantic salmon apparently experience greater predation pressure in acidic streams despite exposure to less diverse assemblages of predatory fishes (Chapter 2). Although direct estimates of mortality rates of wild salmon in the streams and stream classes examined in this thesis are not available, salmon abundance appeared quantitatively similar between inhabited stream reaches during snorkeling observations (Chapters 1 & 3). In addition, three age classes of juvenile salmon (0^+, 1^+ and 2^+) were present in all study streams between 2008 – 2011, providing anecdotal evidence that any differences in predation pressure apparently do not appear to result in differences in population persistence between neutral and acidic habitats over the study period. Free-swimming wild salmon in acidic streams appear instead to mediate their risk of predation by altering patterns of diel activity and habitat usage.

The sensory complementation model explored in Chapter 1 and Elvidge *et al.* (2013) provides an explanation for the observed differences in behavioural responses to acute risky cues. Under neutral conditions, salmon demonstrate stronger responses to combinations of multiple risky cues and weaker responses to individual cues. Under acidic conditions, salmon have been chemical alarm cue-deprived and demonstrate responses to visual cues similar in magnitude to those elicited by multiple cues in salmon under neutral conditions. As a result of past experiences pairing complementary visual and chemical information on predation risk (*i.e.* seeing a predator attack a conspecific or prey guild member and then detecting chemical alarm cues), salmon in neutral streams are better able to adopt graded, threat-sensitive responses to risky cues than in acidic streams, which instead demonstrate hypersensitive responses to any remaining cues. Brown *et al.* (2009) found an analogous graded versus non-graded response pattern between two populations of Trinidadian guppies exposed to high and low levels of predation, respectively. While guppies have demonstrated rapid evolution of their antipredator behaviours in response to different predation levels in transplant experiments (O'Steen *et al.* 2002), laboratory rearing of guppies bred from wild-caught fish resulted in reduced magnitudes of response relative to parental guppies from high predation sites (Kelley and Magurran 2003).

The similarities between responses to different combinations of risky cues in salmon of both neutral and acidic provenance in a cross-population transplant experiment under neutral conditions (Chapter 1) reinforce the notion that experience, independent of any habitat or population-derived differences such as decreased innate response or physiological impairment, plays a significant role in shaping the antipredator responses of prey. Direct comparison of 0^+ salmon in Catamaran Brook (neutral) and Upper Devil's Brook (acidic) reveals that under acidic conditions, salmon are significantly more active during the day than at night (Figure 3.4a). Under neutral conditions in Catamaran Brook, 0^+ salmon are equally abundant during the day and night (Figure 3.4a) and demonstrate significantly greater responses to chemical alarm cues at night than during the day (Leduc *et al.* 2010b). These salmon are able to adjust their antipredator response patterns from graded and threat-sensitive during the day when both chemical and visual cues are available to hypersensitive in response to chemical cues at night. This behavioural plasticity is likely to be the result of an interaction between personal experience and the temporal predictability of the presence or absence of visual cues throughout the photoperiod. While salmon from acidic streams transplanted into neutral streams demonstrated graded responses to risky visual and chemical cue combinations following seven day acclimation periods, it remains unknown whether they also adopt diel behavioural plasticity similar to that demonstrated by resident salmon and over what timescale such a change occurs. Pulses of anthropogenic increases in turbidity, such as those which occur following deforestation and other land use changes in riparian ecosystems, are likely to interfere with both visual predator recognition and paired learning mechanisms enabling acclimation to novel predators (Ferrari *et al.* 2010c) in the same way anthropogenic acidification has been demonstrated to preclude the learning and recognition of novel chemical cues (Leduc *et al.* 2007a; Brown *et al.* 2012).

Acid-impacted juvenile salmon also demonstrate significant differences in their habitat preferences relative to conspecifics in neutral streams by preferentially occupying foraging stations characterized by larger substrate grain size (Wolman 1954). While larger substrate particles may provide more abundant physical refugia from predators and shelter from strong currents (Dolinsek *et al.* 2007), they are also likely to limit the distance over which salmon can visually detect predators (Cronin 1997). Salmon in neutral streams, by contrast, prefer sites with smaller substrate grain sizes. Such foraging stations may provide increased abundance of drift foraging opportunities due to less flow turbulence at the potential cost of increased predation risk due to increased visibility to predators and fewer physical refugia. Given equal abundances of juvenile salmon, stronger habitat preferences are likely to result in increased densities within suitable stream habitats in acidic streams compared to neutral streams. Under conditions of equal forage abundance, relative increases in density are likely to result in increases in intraspecific competition for foraging opportunities resulting in increased aggression and potential reductions in both territory size (Wood *et al.* 2012) and number of foraging stations used (Kim *et al.* 2011a). A multi-year dataset collected from Catamaran Brook demonstrated a negative relationship between body size (fork length L_F) and population density of 0^+ salmon at the end of the growth season in the fall, although this trend was mostly driven by increased growth rates during low density years resulting in a curve of best fit described by a negative power function (Imre *et al.* 2005). At higher densities, salmon may experience increased mortality or avoid this potential cost by emigration, although these possibilities remain unexamined in natural populations due largely to logistic constraints. High density rearing conditions have also been demonstrated to have a negative effect on the development of behavioural life skills such as exploratory foraging and recognizing novel foraging opportunities in another salmonid species (Brockmark *et al.* 2010). Upon their release after the tethering experiments (Chapter 2), surviving hatchery-reared salmon typically returned to the sites they had been occupying prior to retrieval consistent with site preferences resulting from enforced residency. Wild juvenile salmon may similarly avoid emigrating from familiar optimal foraging sites when faced with increased levels of competition and risk of predation. Salmon population enhancement and restocking efforts using hatchery-reared fish could potentially benefit from similar methods involving temporary predator exclusion and induced site preferences to overcome initial high mortality upon introduction to natural stream habitats (Mirza and Chivers 2000; Brown and Laland 2001). Differences in habitat preferences resulting in different densities of juvenile salmon between stream classes could also elicit positive or negative effects on heterospecific predators and prey through behaviourally mediated indirect interactions (BMII: Dill *et al.* 2003). BMIIs in this case could potentially benefit heterospecific competitors by providing more habitat space that is unoccupied by salmon, or by reducing the level of predation pressure experienced by other prey guild members should salmon density be sufficiently high to drive local recruitment of predators.

In the captive growth experiment (Chapter 3), no differences were detected in initial and final mean values of body size (standard length L_S, mass), although salmon transplanted into the acidic Correy Creek demonstrated significantly greater rates of increase in body mass relative to salmon held in the neutral Catamaran Brook. The protocol involved stocking the captive fish at a constant density of 1 salmon $\cdot$ m^{-2}, precluding a comparison of any differences in natural density between stream classes arising from acid-derived variation in selective usage of different microhabitat types. An additional possible effect of relative increases in prey density is increased predator recruitment to areas with more forage opportunities. As mentioned above, increases in prey density in habitats in acidic streams characterized by greater abundances of physical refugia to prey and decreased line-of-sight distances to both predators and prey are unlikely to confer foraging advantages to predators sufficient to overcome increases in shelter to prey. Habitat selection in both neutral and acidic streams appears to be determined by trade-offs between forage opportunities and predation risk, with the relative importance of each factor determined by the availability of information on ambient risk level.

Acidification of both freshwater (Brown *et al.* 2002; Leduc *et al.* 2004a) and marine (Ferrari *et al.* 2012) ecosystems results in the loss of information on risk level conveyed by damage-released chemical alarm cues. Impacted fishes mitigate the costs of this loss of information through the adoption of acute (minutes) and chronic (days, weeks) antipredator behaviours which serve to maximize fitness-related activities while minimizing the risk of predation experienced by an individual. Density-mediated effects stemming from chronic alterations in antipredator strategies including enhanced diel activity patterning and habitat selectivity, while potentially deleterious to acid-impacted individuals, are apparently of secondary importance to maximizing fitness benefits accrued from the trade-off between survival and foraging. Interannual persistence of populations affected by weak levels of acidification (pH 6 – pH 6.6) provides additional support to the notion that plasticity of behavioural strategies over different timescales informed by past experience through paired associative learning mechanisms is sufficient to offset the loss of information on risk resulting from environmental interference with chemical alarm cues. While acidified waterbodies may or may not return to historic chemical profiles, remediation efforts and/or decreased acid deposition are likely to result in increased pH levels. The results of this thesis suggest that above some pH threshold (*i.e.* pH 6), remediation and reduction efforts may begin to achieve diminishing returns. Consequently, efforts to reduce the acid levels in impacted freshwater systems would best be focused on waterbodies acidified to below pH 6. From an evolutionary perspective, weak acidification appears unlikely to influence selection for particular phenotypes in Atlantic salmon due to the observed patterns of avoidance of more heavily acidified streams by spawning adults (Kitamura and Ikuta 2000; Kitamura and Ikuta 2001) in combination with the behavioural plasticity observed in the cross-population transplant experiment and interannual comparison presented in this thesis (Chapter 1).

General Conclusion

Environmentally-mediated sensory impairment results in a suite of alterations to the antipredator behavioural strategies of impacted prey fish species including the culturally and commercially valuable Atlantic salmon (*Salmo salar*). These behavioural changes appear to compensate for the increased uncertainty of ambient risk level by reducing the exposure of an individual to predation threats. While these patterns were detected in juvenile salmon in nursery streams under the influence of anthropogenic acidification, they are also predicted to apply to prey species at large that are affected by interference with the transfer of different forms of information on risk level. Weak levels of acidification (pH 6 – pH 6.6) are unlikely to negatively impact affected fish species, and as such remediation efforts should focus on more heavily acidified waterbodies. Costly restocking and population enhancement projects may benefit from continued evaluations of the effectiveness of enforcing site preferences in hatchery-reared fish upon release in natural streams at enhancing growth and survival, while stream remediation and improvement programs should focus on providing sufficiently complex substrate in order to promote higher population densities while limiting negative density effects at the individual and population levels by increasing the availability of preferred habitats.

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