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Interspecific competition among juvenile salmonids: social behaviour and HORMONE LEVELS OF ATLANTIC SALMON AND TWO NON-NATIVE TROUT SPECIES

Jessica Arden Van Zwol

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INTERSPECIFIC COMPETITION AMONG JUVENILE SALMONIDS: SOCIAL BEHAVIOUR AND HORMONE LEVELS OF ATLANTIC SALMON AND TWO NON-NATIVE TROUT SPECIES

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Jessica A. Van Zwol

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The University of Western Ontario
London, Ontario, Canada

THE UNIVERSITY OF WESTERN ONTARIO
School of Graduate and Postdoctoral Studies

CERTIFICATE OF EXAMINATION

Supervisor	Examiners
Dr. Bryan Neff	Dr. Jim Staples
Supervisory Committee	Dr. Brock Fenton
Dr. Jack Millar	Dr. Jack Millar
Dr. Beth MacDougall-Shackleton	

The thesis by

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Abstract

Competition with ecologically similar non-native salmonids may hinder efforts to restore Atlantic salmon (*Salmo salar*) in Lake Ontario. I examined the competitive effects of juvenile brown trout (*S. trutta*) and rainbow trout (*Oncorhynchus mykiss*), two non-native competitors, on aggression, dominance, growth, and hormone concentrations of three candidate strains of juvenile Atlantic salmon selected for reintroduction into Lake Ontario. Interspecific competition in semi-natural streams reduced aggression, dominance, and growth of Atlantic salmon, coincident with increasing concentrations of cortisol, a hormone that functions in part in the stress response. An aggression-associated hormone, 11-ketotestosterone, was largely unaffected. Interestingly, the most ecologically similar competitor, rainbow trout, had less impact on Atlantic salmon behaviour and growth, relative to brown trout. Atlantic salmon from Lac Saint-Jean were least affected, implicating genetic differences among strains and specific management recommendations. This study highlights the necessity of competition experiments to understand how competition may influence restoration of extirpated populations.

Keywords: Behaviour, Atlantic salmon, non-native species, restoration, cortisol, 11-ketotestosterone, aggression, dominance, growth, brown trout, rainbow trout, Lake Ontario

Co-authorship

Chapter 2

Jessica Van Zwol: Observed juvenile fish behaviours, analyzed data and drafted the manuscript.

Bryan Neff: Provided input into experimental design and the manuscript.

Chris Wilson: Provided the fish and facilities where the experiments were conducted; as well as offered suggestions for experimental design.

Chapter 3

Jessica Van Zwol: Observed juvenile fish behaviours, analyzed data and drafted the manuscript.

Bryan Neff: Provided input into experimental design and the manuscript.

Shawn Garner: Performed enzyme immunoassays.

Chris Wilson: Provided the fish and facilities where experiments were conducted.

Chapter 4

Jessica Van Zwol: Observed juvenile fish behaviours, performed enzyme immunoassays, analyzed data and drafted the manuscript.

Bryan Neff: Provided input into experimental design and the manuscript.

Chris Wilson: Provided the fish and facilities where experiments were conducted.

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Chapter 1. General Introduction

Behaviour and Conservation

Ecosystems worldwide are currently undergoing critical losses of biodiversity. Conservation efforts endeavour to counteract the decline through the preservation and restoration of species (Dobson et al. 1997). One of the leading causes of losses of biodiversity is the presence of non-native species (IUCN 2000), which alter the behaviour of native species (Gamradt et al. 1997), introduce diseases (Ruesink et al. 2005), reduce food availability (Nystrom et al. 1999) and disrupt social hierarchies (Blanchet et al. 2007) across a wide variety of taxa. Behavioural ecology is a discipline that will contribute to the restoration of native species (Caughley 1994; Curio 1996). Understanding the role of interspecific variation in behaviour can assist in determininng (i) an organism's response to interactions with non-native species (Caro 1999) and (ii) the feasibility of reintroducing extirpated native species to restored habitat will enhance conservation efforts (Curio 1996). Knowledge derived from behavioural studies can provide the necessary tools to establish effective management strategies for the preservation of biodiversity.

Non-native species' influence on native species

The presence of non-native species may adversely affect native species by influencing population dynamics, abundance and persistence through competition, predation, or parasitism (e.g., Race 1982; Hamilton et al. 1999; Blanchet et al. 2007). Additionally, non-native species may also have impacts at the scale of entire communities or ecosystems by altering productivity and nutrient cycling (Vitousek 1990; D'Antonio and Vitousek 1992), disrupting existing interspecies interactions (e.g., Vázquez and Simberloff 2003), and interrupting resource flow across ecosystems (e.g., Baxter et al. 2004). When non-native species successfully invade an ecosystem, declines in biota abundance and diversity within the ecosystem often follow (D'Antonio and Vitousek 1992; Olden et al. 2004). For example, the recent introduction of the highly competitive zebra mussels (*Dreissena polymorpha*) in the Hudson River (New York, USA) led to an 85% decline in phytoplankton biomass as a result of feeding by the mussels (Caraco et al.

1997). The reduction in phytoplankton was linked to the corresponding decline of less competitive native mussel populations (Strayer et al. 1998). Other examples of introduced species include that have had a negative impact include Eurasian water milfoil (*Myriophyllum spicatum* L.), Nile perch (*Lates niloticus*), and several trout species (Madsen et al. 1991; Kaufman 1992; Knapp and Matthews 2000). Non-native species can overwhelm ecosystems causing native species to either adapt or decline in population size and face extinction.

Amongst social species such as territorial fish, a rapid population increase of a non-native species can disrupt social hierarchies through interspecific resource and habitat competition leading to decreases in dominance, activity, and growth rate of native species (e.g., Blanchet et al. 2007). Similarly, Roberge et al. (2008) found that hierarchal disruption not only affects native species behaviour but also brain gene transcription patterns: dominant and subordinate native individuals that differed in the absence of a non-native competitor became more similar when held with the non-native competitor. Therefore, the presence of non-native species may have long-standing implications by disrupting behaviour patterns in social hierarchies.

Stable social hierarchies

In many species, the establishment of stable social hierarchies benefits both dominant and subordinate individuals by decreasing the frequency of detrimental intraspecific interactions (Smith and Smith 2003). Individuals assume a rank position based on dominance and submissiveness through a series of interactions including fights, bluffs and threats, the outcome of which depends on the relative fighting ability of the opponents (Johnsson and Åkerman 1998). Organization of competitive individuals into hierarchies stabilizes competitive relationships allowing energy to be allocated to other pursuits such as growth (Johnsson and Åkerman 1998; Smith and Smith 2003). Although there is stability among individuals, it is generally thought resource partitioning occurs for favourable shelters and sites (Figler et al. 1999), food (Ryer and Olla 1996), and mates (Choe 1994) to which dominant individuals have greater access (Johnsson and Åkerman 1998). The benefits of remaining atop the hierarchy are assumed to include enhanced growth, survival, and/or reproductive output (e.g., Huntingford et al. 1990;

Frank et al. 1995). For example, over their lifetime, dominant female red deer (*Cervus elaphus*) produce more offspring that survive to adulthood than subordinate conspecifics (Clutton-Brock et al. 1984). Yet, there are conflicting results about the long-term benefits of social dominance on breeding success. For example, in some species that use alternative mating tactics like cuckoldry (Gross 1982), dominant individuals do not necessarily achieve higher reproductive success in instances where the high cost of maintaining dominance comes at a cost to possible reproductive investment (Packer et al. 1995). While stable social hierarchies diminish agonistic interactions that arise through competition, resource distribution remains unequal as dominant individuals secure greater benefits.

The establishment of dominance within a hierarchy is associated with aggressive behaviours typically exhibited by dominant individuals, while subordinate individuals tend to exhibit less aggressive behaviours (e.g., Fisher and Matthews 2001). Characteristically, subordinate individuals show reduced aggression, activity, and movement compared to dominant individuals (e.g., Abbott et al. 1985; Winberg et al. 1991; Øverli et al. 1998). Additionally, subordinate individuals seek shelter and avoid interacting with dominants (Blanchard et al. 2001). However, the behaviour of subordinates is plastic. For example, Rowell (1974) found that subordinate behaviour is altered by formation of hierarchies and is correlated with physiological changes. Captive studies support this concept; after the removal of a dominant individual, subordinates returned to normal aggression levels after a period ranging from 24 h to 14 days (Frey and Miller 1972; Francis 1983).

After hierarchies form, long term behavioural changes can be observed in subordinate individuals. For example, even in the event of surplus food, the feeding rates of Arctic charr (*Salvelinus alpinus*) subordinates remain low in the presence of dominant individuals (Jobling and Wandsvik 1983). The suppression of feeding may be due to exclusion by dominants that monopolize the best feeding areas in a stream or simply that dominants out-compete subordinate individuals at foraging (Yamagishi et al. 1974; Höjesjö et al. 2005). In addition, Metcalfe (1986) found that growth rates of subordinate rainbow trout (*Oncorhynchus mykiss*) were negatively correlated with food intake, possibly as a result of being driven to more energetically-costly stream sites by dominant individuals. Cunjak and Green (1984) found that in interspecies dominance hierarchies including rainbow trout and brook trout (*Salvelinus fontinalis*), subordinates lost mass. Abbott et al. (1985) found that size-matched rainbow trout juveniles classed as subordinates early in trials were unable to become dominant even when fed supplemental rations to surpass the weight of the established dominant individuals. Finally, subordinate individuals have elevated concentrations of cortisol, a glucocorticoid stress-associated hormone, which has been attributed to the social interactions within hierarchies (Sloman et al. 2001). Sparks et al. (1972) found that measuring the cortisol responses of bluegill sunfish (*Lepomis macrochirus*) to toxic chemicals allowed them to establish that dominant bluegill are under less stress and able to tolerate increased external stress than subordinates. Thus, subordinate individuals are less aggressive, obtain less food items and grow less, and are more stressed in comparison to their dominant counterparts, all of which can have a major long-term impact on the fitness of subordinate individuals.

The relationship between social behaviour and hormones

The ability to cope with stress is determined by physiology and behaviour (Øverli et al. 2004). When a fish experiences a stressful situation such as competition for resources (e.g., Øverli et al. 1999), or social stress (Sloman et al. 2001), the typical physiological response is an increase in the secretion of glucocorticoids such as cortisol (Iwama et al. 2004). Cortisol is the primary circulating glucocorticoid hormone in teleost fish (> 80%; Donaldson 1981). In response to a stressor, the primary response of teleosts involves the release of catecholamines from the chromaffin tissue in the head kidney and the signalling of the hypothalamic-pituitary-interrenal (HPI) axis (similar to the HPA-axis in mammals). This activation stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland thereby initiating the release of cortisol from the interrenal tissue of the anterior head kidney (Iwama et al. 2004).

Salmonids are socially aggressive and territorial and form social hierarchies (e.g., Yamagishi 1962) strongly linked to cortisol concentrations (Øverli et al. 1999). Socially subordinate salmon have chronically elevated cortisol (Øverli et al. 1999). In addition to increased cortisol levels, subordinate fish alter their behaviour in response to stressors by exhibiting reduced feeding (Gregory and Wood 1999), slowed growth (Gregory and

Wood 1999), reduced aggression towards other fish (Øverli et al. 2004), and altered locomotion (Winberg et al. 1993). Thus, social interactions strongly influence subordinate fish physiology and behaviour.

While subordinate fish exhibit submissive behaviours and elevated cortisol, dominant individuals of a social hierarchy most often exhibit aggressive behaviour, which is thought to be largely controlled by androgen hormones (for a review, see Nelson 2005). Androgens and aggression are believed to be linked as castration reduces aggression, while the administration of testosterone restores normal aggression levels (Nelson 2005). The association between androgens and aggression is complex and bidirectional in that increases in that changes in one can influence the other (Nelson 2005). 11-Ketotestosterone (11-KT), a commonly studied key fish androgen, has been linked to aggression and social status, with subordinate fishes exhibiting lowered 11-KT and aggression (Oliveira et al. 1996). Taves et al. (2009) found that newly dominant male cichlids (*Neolamprologus pulcher*) exhibited elevated levels of 11-KT compared to newly subordinate males, findings which supported similar research by Desjardins et al. (2008) on cichlids (*N. pulcher*). Interestingly, a relationship between cortisol and 11-KT levels have been found in common carp (*Cyprinus carpio* L.), where males with elevated cortisol were found to have lower levels of 11-KT (Consten et al. 2002). Further, Young et al. (1996) found that elevated 11-KT suppressed cortisol production in the interrenal cells of rainbow trout. Thus, differences between socially-ranked fish go beyond agonistic behaviours and may be characterized by hormone composition.

Lake Ontario Atlantic salmon

Understanding the link between an organism's physiological state and its behaviour could influence plans to reintroduce Atlantic salmon to areas from which they have been extirpated, where its native habitat is now occupied by introduced and naturally-reproducing salmonids. Investigating this link may reveal how Atlantic salmon will respond to the presence of these introduced species.

Lake Ontario Atlantic salmon were once a prevalent top predator, playing a key ecological role in their ecosystem (MacCrimmon 1977). Although the topic of debate, the Lake Ontario population of Atlantic salmon was thought to spend its entire life cycle in fresh water (Blair 1938; Gage 1963). Adult Atlantic salmon would remain in the lake for the majority of their adult life (one to two years) feeding and growing before returning to their natal streams to spawn (COSEWIC 2006). Unlike Pacific salmonids, Atlantic salmon are iteroparous, meaning they can spawn repeatedly throughout their lifetime. Once emerged from their stream nests, juvenile Atlantic salmon would spend one to three years in stream riffles defending territories and feeding on aquatic insect larvae, before migrating down stream to the lake (COSEWIC 2006).

The Lake Ontario salmon were so abundant it appeared that one could walk across the backs of the salmon during upstream migration (MacCrimmon 1977). In addition, salmon in Wilmot Creek (Newcastle, Ontario) were so common, a special report from 1869 claimed:

> [Atlantic salmon] were so plentiful forty years ago, that men killed them with clubs and pitchforks – women seined them with flannel petticoats – and settlers bought and paid for farms and built houses from the sale of salmon. Later they were taken with nets and spears, over one thousand being often caught in the course of one night. (Whitcher and Venning 1869, Appendix 9)

This "king of fish" was valued by aboriginals and early settlers alike, providing a valuable food source (COSEWIC 2006). By 1869, the US Department of Fisheries began to realize that the overfishing of Atlantic salmon was unavoidable in Wilmot Creek and similar streams. Despite the creation of some of Canada's first hatcheries in efforts to stave off the decline of the once-predominant Atlantic salmon (Knight 2007), this renowned fish disappeared from Lake Ontario by the end of the 19th century from overfishing and habitat destruction caused by the constructions of dams for electricity, deforestation, and farming practices (Crawford 2001; Knight 2007).

Throughout the past century, numerous attempts have been made to restore this species to its native waters, yet a self-sustaining population has not been achieved (MacCrimmon 1977; DFO 2009). Despite stocking nearly a million juvenile Atlantic salmon in Lake Ontario tributaries in the 1990s, returns have been virtually non-existent (Hoyle and Shaner 2002). The most recent of these restoration efforts is the creation of the Lake Ontario Atlantic Salmon Restoration Program (LOASRP), a collaborative effort of public and private sectors focusing on research, raising awareness and involving the public to bring back the salmon (OFAH 2011). The efforts to date include removing many of the decaying hydroelectric dams on Lake Ontario tributaries and addressing the issues of siltation and deforestation that led to the extirpation (Clément 2000).

Restoring a self-sustaining population of Atlantic salmon in Lake Ontario could prove to be beneficial for a number of reasons. First, restoring a native top predator to the lake's ecosystem would be a substantial step in the recovery of lost biodiversity in the Great Lakes by reinstating a native predator fish to the food web (OMNR 2006). Currently, most populations of top predators are not self-sustaining in the Great Lakes and are supported by stocking efforts (EPA 2005). A viable population would help stabilize an ecosystem dominated for the last half century by non-natives and curb the extirpation rate in the Great Lakes by reducing populations of alewife (*Alosa pseudoharengus*), that prey on the eggs of native fish including yellow perch (*Perca flavescens*) and lake trout (*Salvelinus namaycush*) (NYSDC 2009). Second, as a top predator, Atlantic salmon are an important component of the food web as top predators provide food for human and wildlife consumers as well as maintain healthy fish communities (EPA 2005). Being at the top of the food chain, these predators also represent valuable bio-indicators of ecosystem health in one of Canada's most important freshwater ecosystems (OMNR 2006). Therefore, a sustainable population of Atlantic salmon would provide stability to the Lake Ontario food web. In addition to the biological benefits of establishing a sustainable population, the restoration of Atlantic salmon would create socio-economic benefits including employment opportunities through enhanced recreational fisheries which bring increased tourism around the lake. The Atlantic salmon angling industry in Atlantic Canada and Quebec produces revenue of roughly $200 million CAD annually with most revenue remaining in rural areas for small businesses including outfitters, lodges, and restaurants (ASF 2008). Thus, re-establishing a population of Atlantic salmon would prove beneficial at many levels.

Community structure within an ecosystem can be organized by bottom-up or top-down controls. Certain species within the community may exert a strong influence on the observed community structure, such as predatory starfish in the rocky intertidal zone (Smith and Smith 2003). Bottom-up control occurs when populations at any given trophic level are controlled by the level below them within a food chain, whereas top-down control can occur when predator populations control the prey species below them. In lakes and oceans, fish can exert top-down controls on the food web and strongly influence the fluxes of nitrogen and phosphorus (e.g., Vanni et al. 1997) and prey populations (Worm and Myers 2003). In the stream environment, habitat complexity (e.g., Basquill and Grant 1998; Höjesjö et al. 2004), density (e.g., Imre et al. 2005), and prey availability (e.g., Fuller and Keith 1975) are among the factors that interact to control populations of salmonids and determining their relative contributions to bottom-up or top-down controls is complex. Nonetheless, by controlling for these factors, conclusions may be drawn concerning the relative strengths of competition among individuals.

Current competitive environment

Since the extirpation of Atlantic salmon in Lake Ontario, a number of non-native salmonids have been routinely stocked in tributaries draining into the lake in support of recreational fisheries and have, in some cases, established naturally-reproducing populations; they include brown trout (*S. trutta*), rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), and sockeye salmon (*O. nerka)* (Crawford 2001). Recreational fisheries for these non-native salmonids have provided a strong economic base for this sector (NYSG 2009) but these salmonids may also be hindering efforts to restore Atlantic salmon as some of the introduced salmonids exhibit similar niche requirements (Grieg et al. 2003).

In the stream environment as juveniles, brown trout and rainbow trout are two of the introduced salmonids that exhibit the greatest temporal and spatial niche overlap with Atlantic salmon (Gibson 1981; Armstrong et al. 2003), which may lead to heightened aggression and competition for food and shelter (e.g., Gibson 1981; Hearn and Kynard 1986). Rainbow trout were introduced in the late 1800s as native Atlantic salmon populations were declining (Crawford 2001), and naturalised by the 1940s (Kerr and Lasenby 2000). This species was strongly stocked in the 1960s to exert biological control on populations of non-native planktivorous rainbow smelt (*Osmerus mordax*) and alewife. Smelt were purposely introduced as a prey fish population for dwindling populations of Atlantic salmon (Crawford 2001) while alewife, accidentally introduced in the Great Lakes, were considered an uncontrolled aesthetic, economic and ecological annoyance (Stewart et al. 1981). Juvenile rainbow trout inhabit a similar stream environment as Atlantic salmon and are considered to have niche overlap inhabiting riffles, and pools to a lesser extent, of streams (Gibson 1981; Hearn and Kynard 1986). Stanfield and Jones (2003) suggest that naturalised populations of rainbow trout will interfere with survival of juvenile Atlantic salmon stocks in historic Lake Ontario tributaries through competition for resources. Similarly, stocked brown trout were first introduced into Lake Ontario in 1913 as an angling challenge (Crossman 1984) and this species exhibits considerable niche overlap with Atlantic salmon in sympatric populations, although brown trout may prefer pool habitats and Atlantic salmon can inhabit faster reaches of a stream (Armstrong et al. 2003; Stradmeyer et al. 2008). Brown trout have been shown to displace rainbow trout, a species with similar food habits and habitat preferences (e.g., Kaeding and Kaya 1978; Barwick et al. 2004). Although brown trout and Atlantic salmon populations commonly live in sympatry in Europe (Harwood et al. 2002; Armstrong et al. 2003), their ranges did not overlap in North America, which may have consequences for reintroduction efforts of Canadian strains of Atlantic salmon. The presence of these two particular non-native salmonids may hinder efforts to restore Atlantic salmon as past research has shown that individually, brown trout and rainbow trout are more dominant and aggressive than Atlantic salmon (e.g., Volpe et al. 2001; Stradmeyer et al. 2008). It is likely that streams with the highest habitat quality for Atlantic salmon to successfully reproduce are currently being used by rainbow trout and brown trout, which may possibly inhibit Atlantic salmon establishment through stream competition.

The aforementioned studies have examined the interactions of Atlantic salmon and one other salmonid species but, in Lake Ontario tributaries, rarely will an Atlantic salmon encounter only one non-native salmonid species, but rather a combination of non-native salmonids. The addition of more than one species may compound the effects of each species (Ritchie and Olff 1999), as observed with three common species of dry acidic grasslands on a single plant *Hieracium pilosella* (Weigelt et al. 2007). In communities made up of different combinations of multiple grassland species, the biomass of *H.*

pilosella was reduced compared to the individual effects of each species (Weigelt et al. 2007). Brown trout and rainbow trout present together may increase competition and compound aggression and dominance towards Atlantic salmon if all three species are together in similar stream environments of Lake Ontario tributaries. This combination of competition sources may have negative consequences if only by decreasing the carrying capacity for Atlantic salmon through increased total fish density (Jonsson et al. 1998; Crawford 2001). The effect of a multiple species environment in Lake Ontario tributaries may also impact Atlantic salmon juveniles indirectly through the decline of benthic invertebrate abundance due to trout predation; competition with the trouts may force hiding and decrease foraging attempts of Atlantic salmon inhibiting growth and survival rates; displacement resulting from more overtly aggressive and competitive trout; or a combination of these indirect effects (McDowall 2006). For example, galaxiid species in Australia have been removed from optimal foraging sites because of competition from introduced brown trout and rainbow trout (McDowall 2006). Thus, the presence of two non-native salmonids in Lake Ontario tributaries may compound the individual effects on Atlantic salmon establishment.

Candidate strains of Atlantic salmon

Among populations (individuals within a species that live within the same geographical area) and strains (variants that are maintained by culture) of a species, there are likely differences in both physiology (e.g., hormones) and behaviour (e.g., Pickering and Pottinger 1989; Bell and Stamps 2004). As such, there are potential differences in competitive ability (Weber and Fausch 2003). Population level differences in response to stress may enable some individuals to perform better under conditions of intra- and interspecific competition (Øverli et al. 2004; Schjolden and Winberg 2007). The original Lake Ontario strain of Atlantic salmon is thought to be extinct (COSEWIC 2006). Thus, current restoration efforts are investigating the potential benefits and comparative ability of three candidate strains (or populations) of Atlantic salmon for stocking purposes to enhance restoration efforts from (i) LaHave River, Nova Scotia, (ii) Rivière aux Saumons (Lac Saint-Jean), Quebec, and (iii) Sebago Lake, Maine (Figure 1.1). If there is a strain of Atlantic salmon which has above average competitive ability towards the non-native trout species and a lower stress response, it may have a competitive advantage relative to other strains in Lake Ontario streams and may be advantageous to stock.

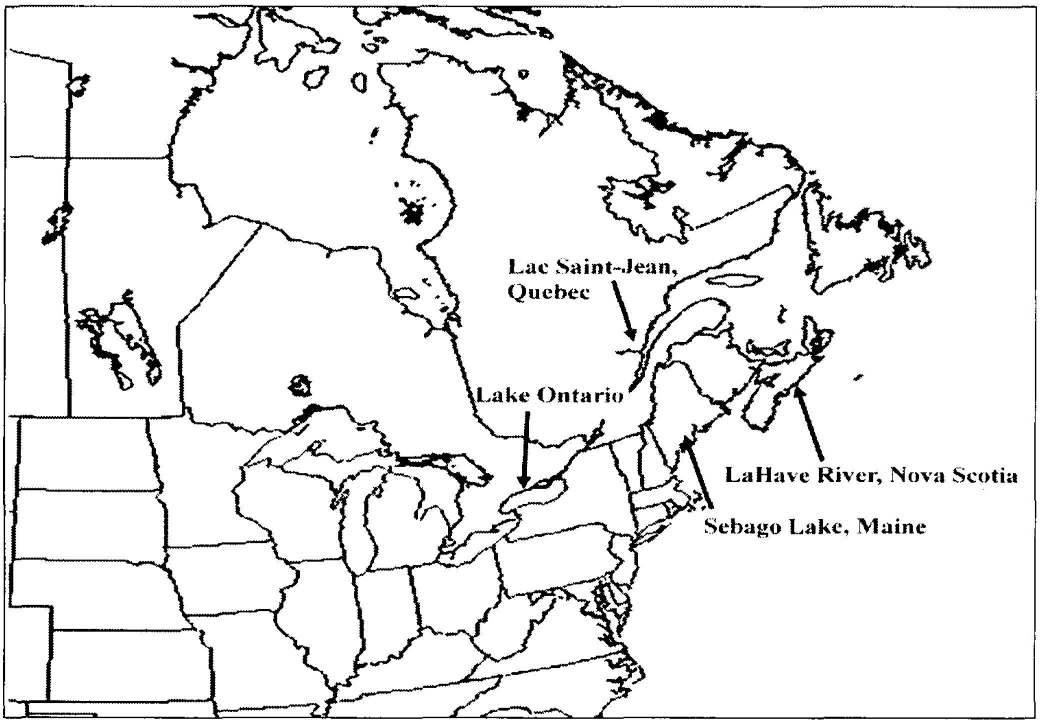

Figure 1.1: Geographical location of origin of the three Atlantic salmon (*Salmo salar*) strains and Lake Ontario, the historical native habitat of a population of Atlantic salmon.

Restoration efforts in the past used many strains of Atlantic salmon for stocking, although most recently, efforts have focused on stocking one strain of Atlantic salmon that originated in the LaHave River, Nova Scotia (e.g., Stanfield and Jones 2003) largely because of the strain's availability as broodstock (Kerr 2006). However, this anadromous (i.e., lives in the ocean, but spawns in freshwater streams) strain of Atlantic salmon does not coexist with brown trout or rainbow trout in its native environment and has been raised for a number of generations in hatchery settings (C. Wilson, Ontario Ministry of Natural Resources, Peterborough, Ontario, personal communication, 2008). Physiologically, this strain may or may not be different from the original in that original was believed to retain the capacity for anadromy thus, would have maintained ion channels necessary for osmoregulation in the ocean (see Willmer et al. 2005). The

LaHave strain continues to be stocked in Lake Ontario tributaries (e.g., Stanfield and Jones 2003) although a self-sustaining population has not yet been produced.

The other two candidate strains were chosen by the province of Ontario for their genetic or ecological traits that appear to be similar to the original Lake Ontario strain or their past performance (Grieg et al. 2003; Dimond and Smitka 2005). The Lac Saint-Jean strain lives in fresh water (Carter 1974) and is potamodromous (i.e., migration occurs within freshwater lakes and streams) rather than anadromous, although no physical barriers prevent the strain from returning to the ocean (Grieg et al. 2003). Although this strain does not coexist with brown trout and rainbow trout, the strain was chosen because of its genetic similarity, geographic proximity, and possible ancestral link to the original strain (Tessier and Bernatchez 2000; Dimond and Smitka 2005). Using genetically similar strains may be advantageous in the event of local adaptations (Conover 1998; Joshi et al. 2001) in that these similar strains may have a fitness advantage for the Lake Ontario environment. Indeed, Montalvo and Ellstrand (2000) found that the long-term success of restored populations hinged on the genetic similarity of transplants and not necessarily geographic distance for different varieties of a southern Californian coastal sage scrub (*Lotus scoparius*). They found that varieties that shared local adaptations with original population fared the best (Montalvo and Ellstrand 2000). The Lac Saint-Jean strain may do well in Lake Ontario tributaries compared to the LaHave strain because of the possible genetic similarity to the extinct Lake Ontario population.

Finally, the other strain under consideration is the freshwater Sebago Lake strain which was physiologically and is now physically landlocked (Watts 1999; Boucher 2004). Individuals of the Sebago Lake strain have been transplanted into Lake Champlain and appear to have successfully established (Dimond and Smitka 2005), despite competition with both brown and rainbow trout in tributaries of the lake. Such establishment would indicate that the Sebago Lake salmon may also fare better than LaHave individuals in Lake Ontario tributaries. Another important difference among the strains is that the Lac Saint-Jean and Sebago Lake individuals available for stocking in Lake Ontario are from wild parentage. The research outlined below will discuss agonistic behaviours and hormone concentrations of each of these strains when exposed to brown trout and rainbow trout, in efforts to provide recommendations concerning viable stocking options in Lake Ontario tributaries.

Objectives

The objective of my thesis is to examine agonistic behaviours and hormone concentrations of juvenile Atlantic salmon in the presence of non-native salmonids, specifically noting the relative performance of individual Atlantic salmon strains, and to use this information to provide recommendations to management or restoration programs focused on restoring Atlantic salmon in Lake Ontario. To accomplish these tasks, I use behavioural trials to identify agonistic and feeding behaviours of all three Atlantic salmon strains in the presence of one or both non-native salmonids, i.e., brown trout and rainbow trout. I further explore the impacts of these non-native salmonids by performing immunoassays to evaluate cortisol and 11-KT hormone concentrations of the Atlantic salmon strains. If brown trout and rainbow trout are stronger competitors than Atlantic salmon, then agonistic and feeding behaviours and cortisol and 11-KT levels of Atlantic salmon will decline when in competition with these species. Additionally, if competitive ability varies among the Atlantic salmon strains, then individuals of the Sebago Lake strain may be the strongest competitor towards the trout species by exhibiting more agonistic and feeding behaviours, lower cortisol levels and higher 11-KT levels than the other strains. Finally, I examine these behaviours and cortisol in both brown trout and rainbow trout to determine whether exposure to Atlantic salmon influences their behaviour and hormone concentrations. If competition does arise among Atlantic salmon and the trout species, than aggressive and feeding behaviours and cortisol levels of the trout species will also decline.

My research endeavoured to answer the following key scientific questions surrounding competition among juvenile Atlantic salmon, brown trout, and rainbow trout: (i) Do non-native salmonids impact Atlantic salmon behaviours and hormone concentrations, (ii) do non-native salmonids differ in their effect on Atlantic salmon behaviours, (iii) does the number of non-native salmonids present effect Atlantic salmon behaviours and hormone concentrations, (iv) do Atlantic salmon strains differ in their competitive performance and hormone concentrations, (v) do stress-associated hormones, specifically cortisol, influence aggressive behaviour in Atlantic salmon as has been noted in other fish species (Øverli et al. 2004), (vi) are androgen hormones, specifically 11-KT, positively associated with aggression as this relationship is largely unexplored in juvenile fish, and (vii) do Atlantic salmon have any impact on non-native behaviour and stress-associated hormone concentrations? I discuss the results of the experiments in light of restoration efforts of Atlantic salmon in Lake Ontario and provide recommendations to organizations given the task to restore this native species.

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Chapter 2. The effect of non-native salmonids on social dominance and growth of juvenile Atlantic salmon (*Salmo salar*)*

2.1 Introduction

The introduction of non-native species can have wide-ranging negative effects from the individual level (Hamilton et al. 1999) through to entire community or ecosystem levels (D'Antonio and Vitousek 1992). Biodiversity and abundance of biota reductions within the ecosystem typically follow their introduction (D'Antonio and Vitousek 1992; Olden et al. 2004) as non-native species often overwhelm ecosystems leaving native species to cope or risk extirpation (Ricciardi et al. 1998).

Among fishes, salmonids are among the most widely introduced species around the world (Crawford and Muir 2008). Indeed, Edge et al. (1993) and Dewald and Wilzbach (1992) showed that native fishes fed less in the presence of brown trout, while Nakano et al. (1998) found that introduced brook charr (*Salvelinus fontinalis*) decreased foraging frequency and distances of native bull charr (*S. confluentus*) and restrained microhabitat use. Similarly, Kitano (2004) found that rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and brook trout have reduced native populations of white-spotted charr (*Salvelinus leucomaenis*), Dolly Varden charr (*Salvelinus malma*), masu salmon (*O. masou*), and Sakhalin taimen (*Hucho perryi*) either directly by predation, or indirectly by competition for resources. In fact, rainbow trout and brown trout have had such widespread negative effects on native ecosystems they have been listed as among the top 100 of the world's worst invasive alien species (Lowe et al. 2000).

In the Great Lakes, non-native salmonids, such as brown trout and rainbow trout may also be impacting restoration efforts of native fishes (Crawford 2001; Grieg et al. 2003). Specifically, extirpated Atlantic salmon (*Salmo salar*) in Lake Ontario have been unable to produce a self-sustaining population despite attempts to restore the species

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(MacCrimmon 1977; Stanfield and Jones 2003). Scott et al. (2005) examined brief interactions (< 1 d) between some of these non-native salmonids found in Lake Ontario and Atlantic salmon and noted adverse impacts on social behaviour of juvenile Atlantic salmon (also see Scott et al. 2003). Other research revealed that interactions with rainbow trout may heighten aggression, territoriality, and competition for resources in stream because of niche overlap with Atlantic salmon (Gibson 1981; Hearn and Kynard 1986). In their native range in Europe, brown trout coexist with Atlantic salmon (Armstrong et al. 2003), but they are more aggressive and socially dominant of the two species (Stradmeyer et al. 2008). In sympatric populations, the dominance of brown trout appears to shift resource use in Atlantic salmon both temporally (Harwood et al. 2001) and/or spatially (Kennedy and Strange 1986). It is currently unknown whether coexistence of brown trout and Atlantic salmon is achievable outside the native range of brown trout, here in North America. Aggression of the brown trout may hinder Atlantic salmon restoration efforts. Additionally, dominant individuals typically have preferential access to resources, which can lead to increased growth and survivorship (e.g., Ens and Goss-Custard 1984). In addition, in Lake Ontario, the presence of rainbow trout, brown trout, and other non-native salmonids increases species richness in the streams and lake which can increase competition for the same resources and likely alters the carrying capacity for Atlantic salmon simply through density effects (Crawford 2001).

Understanding variation in behaviour within a species is crucial for determining the role phenotypic differences play in restoration efforts and for understanding the impact of non-native species (Curio 1996; Caro 1999). As such, I examined the potential effect that non-native and ecologically similar salmonids have on Atlantic salmon during the juvenile life stage. Using semi-natural stream environments, I observed agonistic (the suite of aggressive and submissive social behaviours related to competition) and feeding behaviours of juvenile Atlantic salmon in the presence of juvenile brown trout and rainbow trout to determine if their presence hindered aggression, food consumption, or growth of Atlantic salmon. I predicted that competition with brown trout and rainbow trout, two species known for their aggression, would reduce initiated aggression, food consumption and growth of juvenile Atlantic salmon. I examined the comparative performance of three different strains of Atlantic salmon, (LaHave River, Rivière aux

Saumons [Lac Saint-Jean], and Sebago Lake) that are being used as part of a large-scale effort to re-establish Atlantic salmon in Lake Ontario (Grieg et al. 2003). I predicted that Atlantic salmon from Sebago Lake, a strain that has been successfully established in other watersheds with brown trout and rainbow trout, would show the least reductions in aggression, food consumption, and growth compared to the other strains. Thus, I was able to examine population-specific differences in Atlantic salmon behaviour and performance when in competition with the non-native salmonids, and assess the potential importance of performance differences within and among strains for re-establishing this formerly native species in Lake Ontario.

2.2 Methods

Study animals

In this study, brood stocks were used from three Atlantic salmon populations: the LaHave River, Nova Scotia; Lac Saint-Jean, Quebec; and Sebago Lake, Maine (see Chapter 1 for detailed ecology of each Atlantic salmon strain). As juvenile mortality among salmonids is high (Elliott 1990; Good et al. 2001), restoration efforts in Lake Ontario stock various age groups of Atlantic salmon from recently-hatched fry to sub-adult fish (Stanfield and Jones 2003; Kerr 2006). My behavioural trials involved 1.5-year-old (yearling) Atlantic salmon ($N = 504$), brown trout ($N = 180$), and rainbow trout ($N = 180$). All fish were reared from brood stocks established by the Ontario Ministry of Natural Resources (OMNR) in support of the effort to re-establish Atlantic salmon in Lake Ontario (Grieg et al. 2003). LaHave Atlantic salmon ($N = 168$) and brown trout were obtained from the OMNR Harwood Fish Culture Station (Harwood, Ontario, Canada), while Lac Saint-Jean ($N = 168$) and Sebago Lake ($N = 168$) Atlantic salmon and rainbow trout came from the OMNR Normandale Fish Culture Station (Normandale, Ontario, Canada). Fish were of the same age and culture history as those routinely stocked in streams feeding Lake Ontario. As such, the yearlings of the three species differed in size (see below) as they do under local natural conditions. Prior to the start of the experiment, fish were held for one month at the OMNR Codrington Fisheries Research Facility (Codrington, Ontario, Canada) in flow-through tanks with an average density of 0.6 fish/L, exposed to a natural light cycle, and fed trout chow (Corey Aquafeeds, Fredericton, NB).

Experimental set-up

The experiments performed in this study conformed to animal care guidelines as outlined by the Canadian Council on Animal Care. Semi-natural streams were used to perform six behavioural trials in blocks between May and July 2009 at the Codrington hatchery. The streams were designed to provide substrate and flow conditions similar to those used by Atlantic salmon and trout found in southern Ontario streams as evidence suggests habitat use overlaps among the species (Gibson 1973; Hearn and Kynard 1986; Armstrong et al. 2003). Although both pools and riffles are inhabited by the three species, brown trout may prefer slower moving waters, while Atlantic salmon and rainbow trout may inhabit faster reaches of a stream (Gibson 1973; Hearn and Kynard 1986; Armstrong et al. 2003). Each stream channel had an overall length of 2.4 m with a riffle and pool section (Figure 2.1). The upstream riffle section was 1.6 m long, 0.4 m deep, and 0.5 m wide with a water depth of approximately 0.2 m and flow velocity of 0.18 ± 0.05 m/s. Substrate in the riffle consisted of 7-10 cm river rock and two 15-18 cm rocks to provide potential cover. The riffle section was followed by a pool section measuring 0.8 m long, 0.8 m deep, and 0.5 m wide. The pool water depth was 0.6 m with a surface current of 0.027 ± 0.025 cm/s. Pool substrate consisted of river rock ranging in size from 2-10 cm.

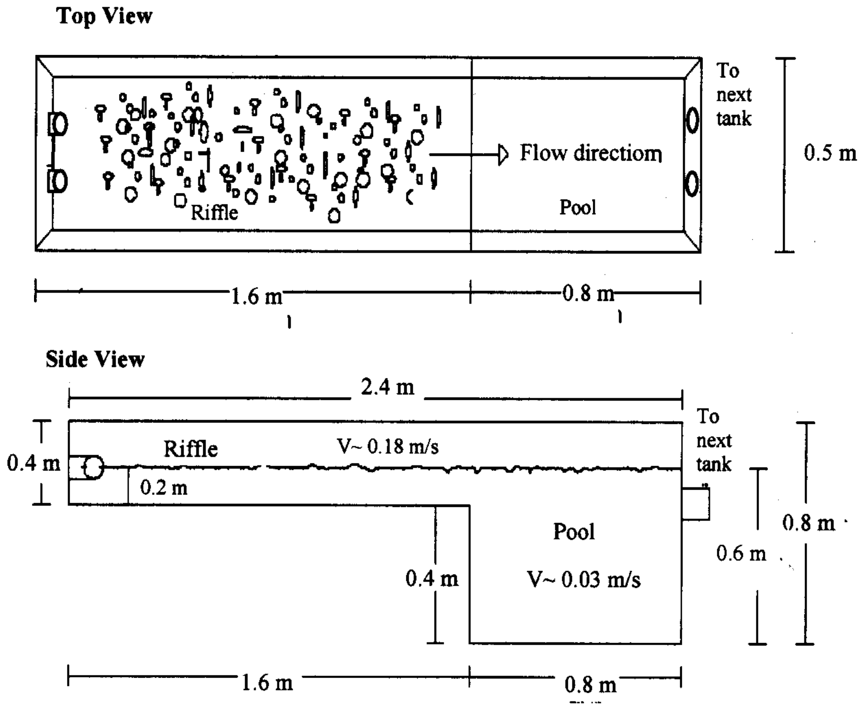


Figure 2.1: Schematic diagram of a semi-natural stream channel.

Water from the hatchery's surface-water head pond (gravity-fed system) was piped to the stream channels through a headbox inside the hatchery, which ensured equal flow to all stream channels. Water temperature was 9.8 ± 1.4 °C (± S.D.). Stream channels were set up in two parallel series of six channels each. Water flowed from the headbox through the first two channels and then into subsequent channels in both series. Channels were connected using two 10 cm PVC pipes, which were covered with wire mesh on one end to prevent the movement of fish between channels.

Each trial block was comprised of 12 treatments, with 12 fish per treatment. Each Atlantic salmon strain underwent four treatments: Atlantic salmon alone (12 fish); Atlantic salmon with brown trout (+BT; 6 salmon, 6 trout); Atlantic salmon with rainbow trout (+RT; 6 salmon, 6 trout); and Atlantic salmon with both brown trout and rainbow trout (+BTRT; 4 salmon, 4 of each trout species). Density in the stream channels was 10 fish/m^2, which is the upper end of densities found in the wild (Fransen et al. 1993). This design allowed me to effectively determine the relative strengths of intraspecific and interspecific competition among ecologically similar species (e.g., Fausch 1998). The three Atlantic salmon strains were considered separately in all trials in order to independently evaluate their comparative performance. There were seven trial start dates (one trial block had a pair of dates, due to logistical constraints at the onset of the experiment, with the commencement of four treatments followed by eight treatments).

At the beginning of each trial, using similar catch effort to obtain fish, individuals were randomly selected from stock tanks and anaesthetized with MS-222. Once sedated, the initial mass and total length of each fish were recorded. In order to observe and record individual behaviour and feeding, each fish was tagged with a coloured 2 cm vinyl anchor tag (Floy Tag & Mfg., Inc., Seattle, Washington). Tags were applied using a fine fabric gun (Avery Mark II Fine Fabric Pistol Grip) with a maximum insertion depth of 0.95 cm. Tags were applied to either the left or right side of the fish just below the dorsal fin to ensure all fish within each channel could be uniquely identified. Between fish, the needle was disinfected with hydrogen peroxide and rinsed with water. Fish were released into a flow-through holding tank to recuperate before being placed in the appropriate stream channel. A random number generator was used to determine the placement of each treatment in the 12 channels for each trial block.

Behavioural observations began the day after the fish were tagged (Day 1), and continued for 7 days. Behaviours were monitored each day in both a morning and afternoon session using an assembled rig of three high definition camcorders set up above a stream channel (Sony HDR-XR200V): one camera above the pool and two equally spaced out above the riffle section. The camcorder rig could easily be moved from channel to channel and was situated approximately 1 m above the water. Two rigs were constructed (six cameras total), which enabled two stream channels to be simultaneously recorded before moving the rigs to the next pair of channels. Fish were given 15 minutes to acclimate to the presence of the camcorder rig before recording began. Aggressive and feeding behaviours were then recorded for 30 minutes.

In the morning session (0800-1230 h), each day for seven days, fish were fed trout chow (Corey Aquafeeds, Fredericton, NB) and frozen bloodworms (Chironomidae; Hikari®, Japan). Specifically, every minute for the first 10 minutes of a recording session, either 50-100 bloodworms or 1 g of trout chow were alternately released at the top and middle of the stream channel, with the current carrying food items through the channel to simulate natural invertebrate drift (~2% of biomass in each stream channel). Care was taken to avoid being seen by the fish. The afternoon recordings (1400-1830 h) did not involve food. The order that channels were filmed was randomized using a random number generator for each day.

On Day 8 of each trial, fish were collected from the stream channels for final mass and length measurements. Collection of fish began at the channels farthest from the headbox to prevent disturbance. Netted fish were sedated with MS-222 before final masses and lengths were recorded. The initial and final mass measurements were used to calculate standard growth rate (%/day) using (Bernier et al. 2004): SGR = 100 × [ln (final mass) - ln (initial mass)]/days fed.

Video analysis

Analysis of the videos focused on aggressive and feeding behaviours. Aggressive behaviours monitored comprised chasing, charging, and nipping (see Keenleyside and Yamamoto 1962 for definitions of behaviours). Feeding observations included number of items consumed. Behaviours of each fish were summed across Days 1, 3, 5, and 7. Agonistic behaviours and food consumed were converted to a rate by dividing by the total number of hours observed. Approximately 864 hours of video were observed in real time and paused every time an action occurred, with actor, act, and recipient recorded. In some instances (rarely), either the actor or recipient could not be determined definitively, yet the act was still recorded.

Statistical analysis

Dominance was calculated using David's score, which creates an index for individuals within a social hierarchy based on an individual's initiated and received aggressive acts, while accounting for repeated interactions among group members (David 1988, see Gammell et al. 2003). David's score was calculated using:

$$DS_i = \sum_i P_{ij} + \sum_i(P_{ij} \times \sum_j P_{jk}) - \sum_i L_{ij} - \sum_i(L_{ij} \times \sum_j L_{jk});$$

where P_{ij} is the number of initiated aggressive acts for individual i towards individual j divided by the total number of aggressive acts between i and j, and L_{ij} is the number of aggressive acts received by i from j divided by the total number of aggressive acts between i and j. P_{jk} and L_{jk} are analogous to P_{ij} and L_{ij}. The summations are across all fish that individual i interacted with. David's scores avoid disproportionately weighting minor deviations from the main dominance direction within a pair of fish because win/loss asymmetries are taken into account by using the proportions (David 1988, Gammell et al. 2003). For example, a fish that did not participate in any agonistic interactions was not given a dominance index higher than that of a fish that was on the receiving end of many aggressive acts as a result of actively initiating aggression towards other fish. Higher David's scores correspond to a more dominant individual.

Differences in initial mass and total length of the Atlantic salmon strains were analysed using one-way analysis of variance (ANOVA) models. Student's *t*-tests were used post-hoc to determine differences between pairs of strains. Data of initiated aggression, received aggression, and food consumption were normalised using $\log_{10}(x + 1)$ transformation. Next, I conducted linear mixed models to test the effects of strain and treatment on initiated and received aggression, David's scores, food consumption, and standard growth rate. The interaction between strain and treatment was included while initial mass was entered as a covariate in the models. Trial block and channel number were entered as random effects. I used a variance components covariance structure and denominator degrees of freedom were calculated using a Satterthwaite approximation (Satterthwaite 1946). This approximation can lead to non-integer values (SPSS Inc. 2005). When main effects were significant or a significant interaction existed between strain and treatment, Student's *t*-tests were used post-hoc to determine differences in variables. To test the effect of dominance on growth parameters, linear regression analysis was used to compare David's score and food consumption or standard growth rate.

To examine the effect of non-native trout species on each Atlantic salmon strain in multivariate space, I used direct discriminant function analysis (DFA). The DFA examined the variation in the five aggression and growth variables (initiated and received aggression, David's score, food consumption, and standard growth rate) to assess how the three Atlantic salmon strains clustered when alone or with non-native trout species by grouping each strain by the presence or absence of non-native trout species (e.g., LaHave individuals alone or LaHave individuals with non-natives). All non-native treatments were grouped together for this analysis. All five dependent variables were included in the analysis and the pooled within-group structure matrix was analyzed to determine which variables most strongly correlated with the discriminant functions. A two-way ANOVA was then used to examine the effects of treatment (alone versus non-native) and strain on the first two DFA axes. All statistics were performed using JMP 4 (version 4.0.2, SAS Institute Inc., 2000), SPSS 16.0 (SPSS Inc., 2007), or Microsoft Office Excel 2003 (Microsoft Corporation, USA). Presented P-values are for two-tailed probabilities ($\alpha < 0.05$).

2.3 Results

The strains of Atlantic salmon differed significantly from one another in initial mass and total length (mass: $F_{2,297} = 71.5$, $P < 0.001$; total length: $F_{2,297} = 37.8$, $P < 0.001$, Table 2.1). Atlantic salmon from the Sebago Lake strain were the largest, followed by Lac Saint-Jean fish, while those from the LaHave strain were the smallest. Overall, the average mass of Atlantic salmon was 40 ± 16 g (S.D.), while the average length was 164 ± 22 mm. Brown trout had an average mass of 39 ± 14 g and length of 151 ± 18 mm, while rainbow trout were on average 21 ± 10 g and 126 ± 19 mm in length. Both mass and length differed among the three species (initial mass: $F_{2,861} = 130.7$, $P < 0.001$; length: $F_{2,861} = 212.5$, $P < 0.001$), with Atlantic salmon being longer but not heavier than brown trout (length: post-hoc t-test, $t_{401} = 7.85$ $P < 0.001$, mass: $t_{365} = 0.72$, $P = 0.47$), while both species were longer and heavier than rainbow trout (Atlantic salmon length:

post-hoc *t*-test, t_{370} = 21.9, Atlantic salmon mass: t_{520} = 19.5, brown trout length: t_{358} = 13.0, brown trout mass: t_{322} = 15.1; $P < 0.001$ for all comparisons).

Table 2.1: Summary of phenotypic and behavioural characteristics of juvenile Atlantic salmon (*Salmo salar*). Data presented are means (± one standard deviation) of each strain (LaHave, Lac Saint-Jean, and Sebago Lake) across treatments (alone, with rainbow trout, with brown trout, and with both brown trout and rainbow trout) in semi-natural stream channels.

	Atlantic salmon strains		
Characteristic	LaHave	Lac Saint-Jean	Sebago Lake
Initial mass (g)	30.9 ± 11.7^{a}	36.6 ± 9.7^{b}	53.2 ± 16.1^{c}
Total length (mm)	150 ± 22^{a}	162 ± 15^{b}	181 ± 18^{c}
Initiated aggression/hour	2.8 ± 4.3^{a}	3.6 ± 4.4^{b}	1.3 ± 2.8^{c}
Received aggression/hour	6.8 ± 6.2^{a}	6.6 ± 5.6^{b}	3.1 ± 3.2^{c}
David's score	-4.7 ± 13.5	-3.7 ± 14.8	-1.5 ± 7.8
Food consumed/hour	21.4 ± 21.0^{a}	17.6 ± 14.1^{ab}	14.7 ± 14.6^{b}
Standard growth rate (%/day)	-0.12 ± 0.99	-0.06 ± 0.85	-0.15 ± 0.64

Note: N = 168 in each strain. Different lowercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate a significant difference among strains ($P < 0.05$).

Agonistic interactions and David's score

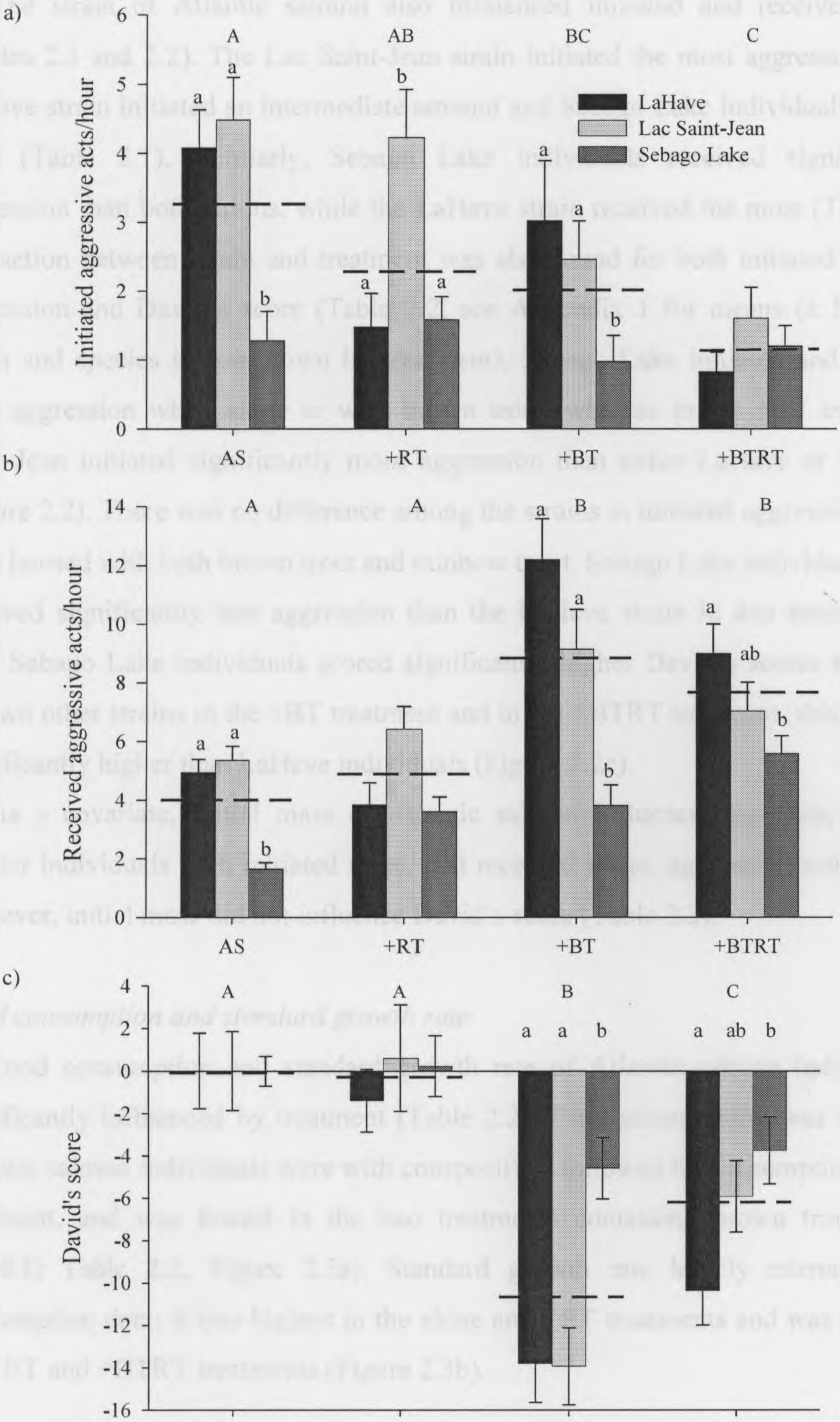
Treatment significantly influenced initiated and received aggression/hour and David's score of Atlantic salmon (Table 2.2). Across all strains, Atlantic salmon juveniles initiated more aggression when alone than in either +BT and +BTRT treatments and were significantly more aggressive in the +RT treatment than in the +BTRT treatment (Figure 2.2a). Initiated aggression by Atlantic salmon varied between the +BT and +BTRT treatment with aggression observed to be higher in the +BT treatment (Figure 2.2a). Atlantic salmon also received much less aggression when alone or in the +RT treatment compared to either +BT or +BTRT treatments (Figure 2.2b). When Atlantic salmon were alone or in the +RT treatment, they scored higher David's scores than in the +BT and +BTRT treatments; David's scores in the +BT treatment were lower than the +BTRT treatment (Table 2.2, Figure 2.2c).

Table 2.2: Summary of linear mixed model results for the behavioural analyses in juvenile Atlantic salmon (*Salmo salar*). The analyses examine the influence of non-native species on agonistic, foraging, and growth characteristics of Atlantic salmon in semi-natural stream behavioural trials.

Dependent variable	Independent	Degrees of freedom	F statistic	*P* value
Initiated aggression/hour	Treatment	3, 380.0	8.60	< 0.001
	Strain	2, 355.1	28.1	< 0.001
	Initial mass	1, 482.7	7.41	0.007
	Strain × Treatment	6, 168.4	6.89	< 0.001
Received aggression/hour	Treatment	3, 474.3	27.6	< 0.001
	Strain	2, 462.9	26.0	< 0.001
	Initial mass	1, 488.8	9.90	0.002
	Strain × Treatment	6, 366.9	6.67	< 0.001
David's score	Treatment	3, 491.0	26.0	< 0.001
	Strain	2, 491.0	2.61	0.07
	Initial mass	1, 491.0	0.63	0.43
	Strain × Treatment	6, 491.0	2.22	0.04
Food consumption/hour	Treatment	3, 466.7	13.29	< 0.001
	Strain	2, 451.7	6.76	0.001
	Initial mass	1, 489.8	0.61	0.43
	Strain × Treatment	6, 334.7	3.64	0.002
Standard growth rate (%/day)	Treatment	3, 472.7	5.16	0.002
	Strain	2, 472.8	0.21	0.81
	Initial mass	1, 486.6	18.6	< 0.001
	Strain × Treatment	6, 368.4	1.94	0.07

Note: Strain (LaHave, Lac Saint-Jean, and Sebago Lake) and treatment (alone, with rainbow trout, with brown trout, and with both brown trout and rainbow trout) were coded as main factors; while initial mass was included as a covariate. Initiated and received aggression and food consumption were $\log_{10}(x + 1)$ transformed. Significance achieved when $P < 0.05$. $N = 168$ for each strain and denominator degrees of freedom calculated using Satterthwaite approximation.

Figure 2.2: Agonistic interactions and dominance of juvenile Atlantic salmon (*Salmo salar*) in treatments with and without non-native trout species. (a) The number of initiated aggressive acts per hour, (b) the number of received aggressive acts per hour and (c) David's score of three strains of Atlantic salmon (LaHave, Lac Saint-Jean, and Sebago Lake). The four experimental treatments include Atlantic salmon alone (AS, $N = 72$ for each strain), Atlantic salmon with rainbow trout (*Oncorhynchus mykiss,* +RT, $N = 36$ for each strain), Atlantic salmon with brown trout (*S. trutta,* +BT, $N = 36$ for each strain), and Atlantic salmon with both brown trout and rainbow trout (+BTRT, $N = 24$ for each strain). Bars denote mean ± S.E. for each of the three strains, while dashed lines depict the mean of the three strains for each treatment. Different uppercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate significant differences between treatments ($P < 0.05$), while different lowercase letters also denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate significant differences between Atlantic salmon strains within a specific treatment ($P < 0.05$).


a)
Initiated aggressive acts/hour
0
1
2
3
4
5
6
A
AB
BC
C
a
a
b
a
b
a
a
a
b
AS
+RT
+BT
+BTRT
LaHave
Lac Saint-Jean
Sebago Lake
b)
Received aggressive acts/hour
0
2
4
6
8
10
12
14
A
A
B
B
a
a
b
a
a
b
a
ab
b
AS
+RT
+BT
+BTRT
c)
David's score
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
A
A
B
C
a
a
b
a
ab
b
AS
+RT
+BT
+BTRT
Treatment

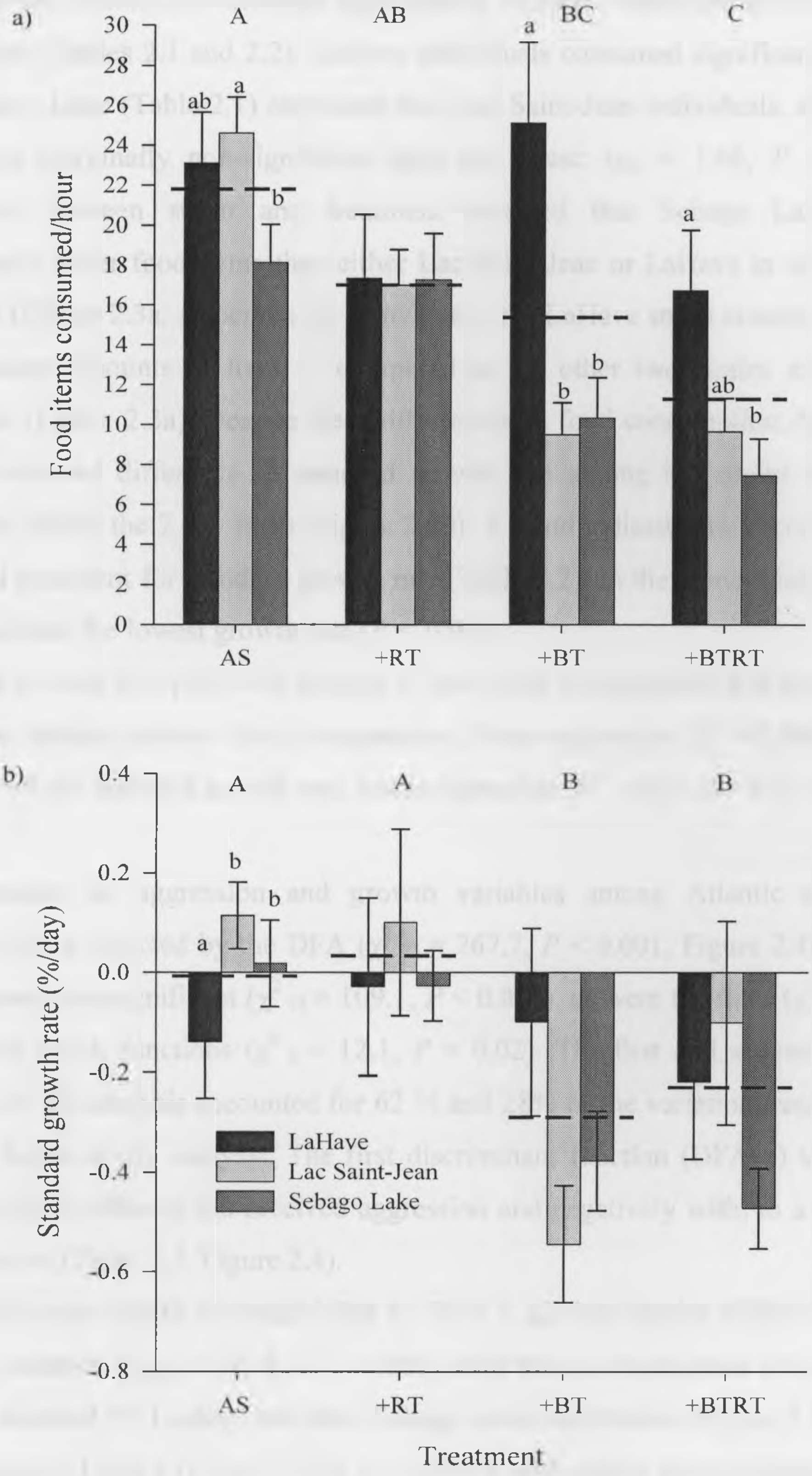
The strain of Atlantic salmon also influenced initiated and received aggression (Tables 2.1 and 2.2). The Lac Saint-Jean strain initiated the most aggression, while the LaHave strain initiated an intermediate amount and Sebago Lake individuals initiated the least (Table 2.1). Similarly, Sebago Lake individuals received significantly less aggression than both strains, while the LaHave strain received the most (Table 2.1). An interaction between strain and treatment was also found for both initiated and received aggression and David's score (Table 2.2, see Appendix 1 for means (± S.D.) of each strain and species broken down by treatment). Sebago Lake initiated and received the least aggression when alone or with brown trout, whereas in the +RT treatment, Lac Saint-Jean initiated significantly more aggression than either LaHave or Sebago Lake (Figure 2.2). There was no difference among the strains in initiated aggression when they were housed with both brown trout and rainbow trout. Sebago Lake individuals, however, received significantly less aggression than the LaHave strain in this treatment (Figure 2.2). Sebago Lake individuals scored significantly higher David's scores than either of the two other strains in the +BT treatment and in the +BTRT treatment, this strain scored significantly higher than LaHave individuals (Figure 2.2c).

As a covariate, initial mass of Atlantic salmon influenced agonistic interactions: heavier individuals both initiated more, and received fewer, aggressive acts (Table 2.2). However, initial mass did not influence David's score (Table 2.2).

Food consumption and standard growth rate

Food consumption and standard growth rate of Atlantic salmon individuals were significantly influenced by treatment (Table 2.2). Food consumption was highest when Atlantic salmon individuals were with conspecifics, followed by consumption in the +RT treatment, and was lowest in the two treatments containing brown trout (+BT and +BTRT; Table 2.2, Figure 2.3a). Standard growth rate largely mirrored the food consumption data: it was highest in the alone and +RT treatments and was the lowest in the +BT and +BTRT treatments (Figure 2.3b).

Figure 2.3: Feeding behaviours and growth of .juvenile Atlantic salmon (*Salmo salar*) in treatments with and without non-native trout species. (a) The number of food items consumed per hour, and (b) the standard growth rate of three strains of Atlantic salmon (LaHave, Lac Saint-Jean, and Sebago Lake). The four experimental treatments include Atlantic salmon alone (AS, $N = 72$ for each strain), Atlantic salmon with rainbow trout (*Oncorhynchus mykiss,* +RT, $N = 36$ for each strain), Atlantic salmon with brown trout (*S. trutta,* +BT, $N = 36$ for each strain), and Atlantic salmon with both brown trout and rainbow trout (+BTRT, $N = 24$ for each strain). Bars denote mean ± S.E. for each of the three strains while dashed lines depict the mean of the three strains for each treatment. Different uppercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate significant differences between treatments ($P < 0.05$), while different lowercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate significant differences among strains within a specific treatment ($P < 0.05$).


a)
Food items consumed/hour
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
A
AB
BC
C
ab
a
b
a
b
b
a
ab
b
AS
+RT
+BT
+BTRT
b)
Standard growth rate (%/day)
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
A
A
B
B
a
b
b
LaHave
Lac Saint-Jean
Sebago Lake
AS
+RT
+BT
+BTRT
Treatment

The three strains also differed significantly in food consumption but not standard growth rate (Tables 2.1 and 2.2). LaHave individuals consumed significantly more food than Sebago Lake (Table 2.1) and more than Lac Saint-Jean individuals, albeit the latter effect was marginally non-significant (post-hoc *t*-test: $t_{292} = 1.96$, $P = 0.052$). An interaction between strain and treatment revealed that Sebago Lake consumed significantly fewer food items than either Lac Saint-Jean or LaHave in all but the +RT treatment (Figure 2.3a, Appendix 1). Conversely, the LaHave strain consumed more food or equivalent amounts of food as compared to the other two strains across the four treatments (Figure 2.3a). Despite these differences in food consumption, however, there was no observed difference in standard growth rate among the strains in any of the treatments during the 7 day trials (Figure 2.3b). A trend indicated an interaction between strain and treatment for standard growth rate (Table 2.2). In the alone treatment, LaHave individuals had the lowest growth rate ($P < 0.05$).

David's score was positively related to both food consumption and standard growth rate of the Atlantic salmon (food consumption, linear regression: $R^2 = 0.008$, $\beta = 0.09$, $N = 504$, $P = 0.05$; standard growth rate, linear regression: $R^2 = 0.01$, $\beta = 0.11$, $N = 504$, $P = 0.01$).

Differences in aggression and growth variables among Atlantic salmon strain groupings were detected by the DFA ($\chi^2_{25} = 267.7$, $P < 0.001$, Figure 2.4). The second function was also significant ($\chi^2_{16} = 109.1$, $P < 0.001$), as were the third ($\chi^2_{9} = 30.1$, $P < 0.001$) and fourth functions ($\chi^2_{4} = 12.1$, $P = 0.02$). The first and second discriminant functions of the analysis accounted for 62 % and 28% of the variation, respectively, and were the focus of my analysis. The first discriminant function (DFA 1) was positively correlated with initiated and received aggression and negatively with, to a lesser extent, David's score (Table 2.3, Figure 2.4).

The two-way ANOVA revealed that for DFA 1, all three strains differed significantly from one another ($F_{2,498} = 82.8$, $P < 0.001$) with the Lac Saint-Jean strain scoring the highest, followed by LaHave and then Sebago Lake individuals (Figure 2.4). Treatment also influenced DFA 1 ($F_{1,498} = 7.30$, $P = 0.007$), with higher scores generally observed in the non-native treatments. There was also, however, an interaction between strain and presence of non-natives ($F_{2,498} = 11.6$, $P < 0.001$): Sebago Lake and Lac Saint-Jean, but not LaHave individuals, had higher DFA 1 values in the non-native versus alone treatments (Figure 2.4).

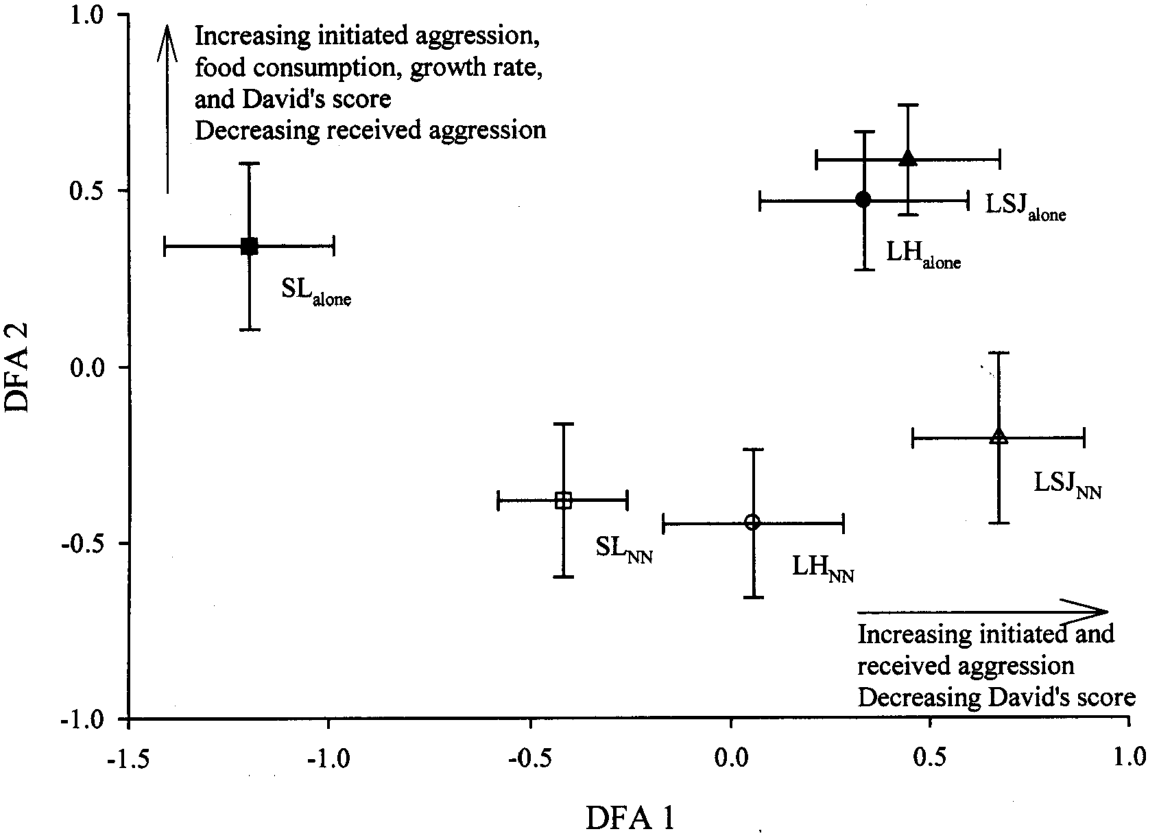


Figure 2.4: Canonical plot of the first two functions of the discriminant function analysis examining the variation of aggression and growth measurements among juvenile Atlantic salmon (*Salmo salar*). The data are grouped by strain comprising LaHave (LH), Lac Saint-Jean (LSJ), and Sebago Lake (SL) and are divided based on alone and non-native (NN) treatments. The symbols represent strain centroids (with 95% confidence intervals).

Table 2.3: Summary of discriminant function analysis (DFA) of agonistic and growth measurements in Atlantic salmon (*Salmo salar*). The DFA was performed on five agonistic and growth measurements in three strains comprising LaHave, Lac Saint-Jean, and Sebago Lake individuals in alone and non-native semi-natural streams, with pooled within-group correlations of canonical roots and standardized canonical discriminant function coefficients.

	Correlation of variables with discriminant functions				Standardized canonical discriminant function coefficients			
Variable	DFA 1	DFA 2	DFA 3	DFA 4	DFA 1	DFA 2	DFA 3	DFA 4
Initiated aggression/hour	0.641	0.632	-0.05	0.395	1.26	0.706	-0.460	-0.679
David's score	-0.161	0.564	-0.463	0.587	-0.797	-0.81	-0.169	1.26
Received aggression/hour	0.640	-0.397	0.462	0.461	0.157	-0.792	0.344	0.962
Food consumption/ hour	0.067	0.580	0.786	0.198	-0.493	0.565	0.788	0.271
Standard growth rate (%/day)	-0.022	0.263	0.490	-0.137	0.16	-0.164	0.247	-0.220

The second discriminant function (DFA 2) was positively correlated with initiated aggression, food consumption, growth rate, and David's score and negatively with received aggression (Table 2.3). For this function, a two-way ANOVA found that while the strains did not vary ($F_{2,498} = 2.08$, $P = 0.12$), the presence of non-native trout species significantly influenced canonical scores ($F_{1,498} = 80.6$, $P < 0.001$), with strains initiating less aggression, consuming less food, growing less and having lower dominance scores, but receiving more aggression in the presence of the non-native trout species. I observed no significant interaction between strain and the presence of non-natives ($F_{1,498} = 0.39$, $P = 0.68$).

2.4 Discussion

This study showed that the presence of non-native salmonids affects the aggressive and foraging behaviour of juvenile Atlantic salmon. When juvenile Atlantic salmon were with conspecifics only, the level of aggression received by individual fish was lowest and the level of food consumption was highest. Additionally, Atlantic salmon were most aggressive in the conspecific treatment as the presence of non-native trouts suppressed the amount of aggression Atlantic salmon initiated. Specifically, brown trout exerted a stronger influence on Atlantic salmon than rainbow trout. These data mirror those Stradmeyer et al. (2008), who found that juvenile brown trout were always dominant to Atlantic salmon. Using both stream channels and field surveys, Hearn and Kynard (1986) found that wild rainbow trout and juvenile Atlantic salmon compete and Blanchet et al. (2009) found that food consumption of juvenile Atlantic salmon was lowered in the presence of rainbow trout. Collective evidence now suggests that non-native salmonids, particularly brown trout, can have strong behavioural effects on Atlantic salmon.

Body size is an important factor in determining the outcome of contests among conspecifics. Many studies have shown that dominance in fish is directly linked to larger body size (e.g., Abbott et al. 1985; Beaugrand et al. 1996). However, this relationship between body size and dominance did not exist for Atlantic salmon in my study. Consistent with my data, Huntingford et al. (1990) examined dominance competitions between pairs of juvenile Atlantic salmon in spring and summer (when I conducted my trials) and found no evidence that dominance tests were won by larger fish, regardless of the size difference between a pair (neither in small differences: 0.1-5% nor large differences: >5%). Interestingly, when the experiment was conducted in September, the relationship did exist with 72% of the dominance tests being won by the larger fish of a pair (in both small and large body size differences: >0.1%; Huntingford et al. 1990). The study found, however, that aggression levels strongly influenced the social dominance of an individual. Aggressive individuals that became dominant in the spring and summer regardless of size may have obtained more profitable positions in stream over the summer that were less energetically-demanding and had greater food abundance, resulting in greater growth. Although I was examining Atlantic salmon behaviour in the context of multiple individuals where fish were of all different sizes, these data suggest that dominance is a function of behaviour and that large body size may be a consequence, not a cause, of dominance, at least in some salmonids.

It is well known that subordinate fish exhibit less growth as a result of the behaviours of dominant fish. This pattern has been shown in a number of salmonid species (e.g., Atlantic salmon and brook trout, Gibson 1973). Consistent with these studies, I found that the presence of brown trout suppressed the growth rate of Atlantic salmon, which were typically subordinate to the brown trout. I also found that in the brown trout and Atlantic salmon treatment, subordinate fish grew at rates much lower than dominant fish, and food consumption of Atlantic salmon significantly declined as compared to when Atlantic salmon were alone. While dominant brown trout are known to monopolise feeding areas, reducing feeding opportunities of subordinates (Höjesjö et al. 2005), Metcalfe (1986) postulated that regardless of the actions of the dominant fish, it is better for subordinate fish to minimize energetic costs, rather than maximizing food intake. Although I did not directly quantify the behavioural tactics used to acquire food, my results are consistent with those of Metcalfe (1986). For example, Sebago Lake salmon appeared to choose a growth strategy that minimized energy expenditure, opting out of the competition and consequently consuming the least amount of food and losing the most mass of the three strains in the treatments with non-native trouts. However, anecdotal evidence suggests that all three species preferred the slower-moving pool over the riffle for both feeding and initiating aggressive interactions, possibly in an attempt to minimize energy expenditure. Regardless of the actual feeding tactics used by Atlantic salmon, my data clearly show that Atlantic salmon feed less in the presence of dominant brown trout and consequently display reduced growth.

Community ecology studies have long shown that competition among ecologically similar species can lead to spatial separation or shifts in resource use if the species continue to live in sympatry (e.g., Werner and Hall 1977; Langeland et al. 1991). Brown trout and Atlantic salmon have historically coexisted in rivers in Europe (Höjesjö et al. 2005), but tend to spatially separate in streams, largely driven by the aggressive behaviour of brown trout (Armstrong et al. 2003). My study confirmed the dominance of brown trout over Atlantic salmon as has been shown by Stradmeyer et al. (2008). Additionally, I found that food consumption and growth of Atlantic salmon declined in the presence of brown trout. Rainbow trout and Atlantic salmon, however, have not historically coexisted, yet studies have shown there is a degree of niche overlap (Gibson

1981; Hearn and Kynard 1986), which I expected would influence the agonistic interactions and growth of Atlantic salmon in my study. Similar to that found by Blanchet et al. (2008), the presence of rainbow trout did not affect food consumption or growth rate of Atlantic salmon. Atlantic salmon received no more aggression in the presence of rainbow trout than they did in the conspecifics treatment. These data support Volpe et al. (2001) who found that although rainbow trout were much more aggressive than Atlantic salmon, agonistic interactions were largely between rainbow trout conspecifics and not Atlantic salmon. Hence, it is conceivable that although there is niche overlap between these two species, the agonistic interactions I observed suggest rainbow trout compete most heavily with conspecifics and largely ignore Atlantic salmon. Thus, density issues aside, these data suggest that brown trout, more than rainbow trout influence Atlantic salmon agonistic and feeding behaviours and unless spatial separation is possible for brown trout and Atlantic salmon, competition between these two species poses a threat to Atlantic salmon establishment in Lake Ontario streams.

Behavioural differences among populations or strains within a species have been observed across many taxa (e.g., Jones 1977; Rex et al. 1996; Moretz et al. 2007) and comparing these differences can provide an understanding of phenotypic attributes that will strengthen efforts of native species reintroduction (Curio 1996). One important attribute for successful establishment and persistence is aggression (Holway and Suarez 1999). Specifically, I have shown differences among Atlantic salmon strains in both aggressive and feeding behaviours. Indeed, the Lac Saint-Jean strain initiated significantly more aggression than individuals from the Sebago Lake and LaHave strains and LaHave individuals received the most aggression. However, the LaHave strain consumed more food than the Sebago Lake strain. Patterns suggest that the Lac Saint-Jean strain lost the least mass of the three strains, and consumed more food than Sebago Lake individuals. These data together may suggest that the Lac Saint-Jean fish are better competitors against brown trout and rainbow trout, two non-native species, prevalent in Lake Ontario tributaries. The discriminant function analysis confirmed these strain differences by showing that the presence of non-native trout species influenced the LaHave and Sebago Lake strains the most, but had less of an impact on the Lac Saint-Jean strain. Differences observed here suggest that stocking the Lac Saint-Jean strain, the strain believed to be the closest geographically and genetically of the three strains to the original Lake Ontario population (Dimond and Smitka 2005), will achieve greater restoration success as they are better competitors against brown trout and rainbow trout. Indeed, the fact that LaHave strain was affected by the presence of the non-native trouts may explain the previous failed attempts of restoring Atlantic salmon with this strain.

High species richness can lead to competition for resources, resulting in declines in growth rates of the competing species. This effect has been shown in, for example, sunfish (Centrarchidae, Mittelbach 1988), *Daphnia* spp. (Bengtsson 1993), and desert annuals, where competition among the plants leads to decreases in growth, biomass, and fecundity (Inouye et al. 1980). I found that the presence of multiple salmonid species led to increases in received aggression for Atlantic salmon in addition to reductions in food consumption and growth. Such interactions often lead to partitioning of habitat and resources among the competing species allowing the individuals to coexist (e.g., Robertson and Gaines 1986; Young 2001). Because of my experimental setup, I could not easily assess potential habitat or resource partitioning. Nevertheless, I found no evidence that Atlantic salmon shifted habitat use across the pool and riffle sections when alone versus with either or both of the non-native species. Regardless, my data suggest that high salmonid species richness could be detrimental for Atlantic salmon during the stream stage of life. Assessing the species community of targeted streams and rivers for Atlantic salmon restoration may also help to alleviate competition for Atlantic salmon.

In conclusion, my data point to some considerations that may help to direct restoration of Atlantic salmon in Lake Ontario tributaries. First, the three strains are predicted to have differential post-stocking ecological success in tributary environments, with Atlantic salmon originating from Lac Saint-Jean outperforming the LaHave and Sebago Lake strains. Whether these differences would similarly extend to variable fitness in Lake Ontario in terms of growth, survival, and adult returns still needs to be determined. Second, the successful establishment of juvenile Atlantic salmon may be greatly impeded by the presence of brown trout. Rainbow trout appear to have less of an influence on Atlantic salmon, albeit high species richness did impede the performance of Atlantic salmon. As such, I recommend avoiding stocking juvenile Atlantic salmon in Lake Ontario streams with high densities of brown trout and also in streams with multiple established salmonid species. Nevertheless, juvenile survivorship in streams is currently the major hurdle impeding re-introduction of this important native species in the Great Lakes.

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Chapter 3. The influence of non-native salmonids on circulating levels of cortisol and 11-ketotestosterone in juvenile Atlantic salmon*

3.1 Introduction

The relationship between hormones and behaviour has been studied to understand the proximate mechanisms involved in how organisms respond to their environment. One important hormone studied is cortisol (or corticosterone, the homolog in reptiles and birds), which is a glucocorticoid that mediates the hormonal response to stress (Barton and Iwama 1991; Wendelaar Bonga 1997). Exposure to a stressor can disrupt homeostatic equilibrium of an organism and elicit compensatory or adaptive physiological and behavioural responses to mobilize energy reserves and overcome the stress (for a review, see Wendelaar Bonga 1997). These responses are initiated by the activation of the hypothalamic-pituitary-interrenal (HPI) axis in fish, or the hypothalamic-pituitary-adrenal axis in birds and mammals, which stimulates a cascade effect of several pituitary hormones in the kidney, signalling the release of cortisol or corticosterone (Mommsen et al. 1999; Barton 2002; Iwama et al. 2004; Nelson 2005). On the other hand, chronic stressors, which include stress from social confrontations (Fuchs and Schumacher 1990), can cause the stress response to become maladaptive and dysfunctional when cortisol levels are chronically elevated (Pickering and Pottinger 1989; DiBattista et al. 2005). When migration to an area where the stressor is absent is not an option, coping involves readjusting biological activities, which may include reducing aggression or food consumption (Kelsey et al. 2002; Pankhurst et al. 2008), which in turn can lead to lowered social status and reduced growth (McCormick et al. 1998; Iwama et al. 2004).

While lowered social status and aggression has been associated with elevated cortisol levels, elevated androgen levels have been associated with elevations of these

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characteristics (Dzieweczynski et al. 2006; Parikh et al. 2006). For example, aggression has been shown to decline in experiments involving the removal of androgens, whereas supplementation of the hormone increases agonistic interactions (Arnold 1975; Hume and Wynne-Edwards 2005). Furthermore, the Challenge Hypothesis suggests that androgen levels fluctuate according to social interactions in that social challenges such as male-male competition for mates can increase androgen levels (Wingfield et al. 1990). Testosterone is the main circulating androgen linked to aggression in mammals and birds (Nelson 2005), whereas in teleost fishes, 11-ketotestoterone (11-KT) is the predominant androgen (Borg 1994; Oliveira et al. 2009). The relationship of androgens and aggressive behaviour is complex and bidirectional, although it is thought that winning agonistic interactions contributes to elevated androgen level through positive feedback mechanisms (Nelson 2005). Winning stimulates the release of luteinizing hormone from the hypothalamus, which initiates the gonads and adrenal glands to increase secretion of androgens (Nelson 2005). Even observing agonistic interactions has been found to increase androgen concentrations in fish not directly involved in the agonistic act (Oliveira et al. 2001). Among conspecifics, dominance in social hierarchies has been linked to heightened aggression and androgen levels (Bouissou 1983; Schoech et al. 1991; Desjardins et al. 2008), but a corresponding link in heterospecific interactions is not as well established. Furthermore, most research on androgens and aggression has been on adult individuals (Oliveira et al. 2009); the evidence that exists concerning juveniles suggests androgens are similarly linked to aggression (e.g., Anestis 2006; Kent et al. 2009). However, while it is known that 11-KT levels in juvenile fish are low (Antonopoulou et al. 1995; Berglund et al. 1995; Shrimpton and McCormick 2002), the link between aggression and 11-KT in this taxon remains largely unexplored. Thus, measuring 11-KT concentrations and observing aggressive behaviour in juvenile fish allows us to contribute to a void in hormone and behaviour research.

Androgens and aggression are typically associated with male individuals; however, females may also exhibit elevated androgens and be highly aggressive (see Nelson 2005). Indeed, both males and females of bi-parental monogamous cichlids (*Neolamprologus pulcher*) actively defend territory against conspecific and heterospecific individuals (Desjardins et al. 2006). Circulating levels of 11-KT increased in both males and females in response to a simulated territory intruder, but elevations in testosterone were only noted in the female. Research has shown that females across taxa can be as, or more, aggressive than males (see Nelson 2005). von Engelhard et al. (2000) found that while androgen levels in female ring-tailed lemurs (*Lemur catta*) did not exceed those of males, a two-fold increase in both androgen levels and conflict behaviour was observed during the mating season. Meanwhile, testosterone has been implicated in the hormone-dependent aggression in female rats (Albert et al. 1989). Thus, in juvenile individuals who actively defend territory, such as Atlantic salmon, androgen levels may not differ greatly between the sexes.

One important application of the study of hormones and behaviour is to the effect that non-native species have on native species assemblages (Wingfield et al. 1997; Mooney and Cleland 2001). Competition with non-native species may be considered a chronic stressor for many native populations (Hamilton et al. 1999; Ruiz et al. 1999; Roberge et al. 2008). For example, native young-of-the-year burbot (*Lota lota*) have increased cortisol levels when in the presence of non-native spinycheek crayfish (*Orconectes limosus*) and alter their behaviour to avoid the non-native species (Hirsch and Fischer 2008).

I examined the effect that non-native salmonids have on Atlantic salmon hormone concentrations and the subsequent relationship with behaviour. Restoration efforts have been unable to produce a self-sustaining population of Atlantic salmon following its extirpation from Lake Ontario (MacCrimmon 1977; Stanfield and Jones 2003; Bowlby et al. 2007). I hypothesized that these efforts may have been unsuccessful in part because of historical introductions and ongoing stocking of highly aggressive and competitive non-native salmonids, specifically brown trout (*S. trutta*) and rainbow trout (*Oncorhynchus mykiss*) (Volpe et al. 2001; Stradmeyer et al. 2008), that have been introduced and inhabit historic Atlantic salmon habitat (Crawford 2001; Stewart and Schaner 2002). Competition with these two non-native salmonids may be altering both behaviour and hormonal levels of Atlantic salmon, and may be contributing to the lack of success of restoration efforts.

In this study I evaluated the cortisol and 11-KT response of juvenile Atlantic salmon in the presence and absence of juvenile brown trout and rainbow trout in a semi-natural stream environment. I predicted that if brown trout and rainbow trout are stronger competitors than Atlantic salmon, then cortisol concentrations of Atlantic salmon will be greater and 11-KT concentrations lower in treatments where the non-native species are present compared to concentrations of individuals exposed only to conspecifics as cortisol concentrations would increase in response to stress and 11-KT concentrations would decrease in response to losing agonistic interactions. Using three strains of Atlantic salmon (LaHave, Lac Saint-Jean, and Sebago Lake), which are currently being used in a large-scale Lake Ontario restoration efforts (Grieg et al. 2003), I compared their hormonal responses and investigate the relationship between the hormones and agonistic interactions, feeding behaviour and growth. I predicted that the Sebago Lake strain, which has successfully been stocked in other watersheds, would prove to be the least affected by the competition with the non-native trout species and exhibit cortisol concentrations similar to unstressed salmonids and have the highest 11-KT concentrations in order to compete successfully against the non-natives.

3.2 Methods

Study animals

For the current study, 1.5-year-old juvenile Atlantic salmon (*N* = 564 total), brown trout (*N* = 180), and rainbow trout (*N* = 180) were obtained from Ontario Ministry of Natural Resources (OMNR). The three candidate Atlantic salmon strains examined in this study were the LaHave, Lac Saint-Jean, and Sebago Lake strains (*N* = 188 for each strain; see Chapter 1 for detailed ecology of each Atlantic salmon strain). Fish were obtained as detailed in Chapter 2. All fish species in the study were representative (same age and culture history) of those currently stocked in Lake Ontario streams (see Table 3.1 for mass and length). Fish were held at the Codrington Fisheries Research Facility (Codrington, Ontario) as detailed in Chapter 2. I was unable to determine the sex of Atlantic salmon as post-mortem visual inspection of juveniles is inconclusive and molecular markers are unknown for Atlantic salmon (Pat O'Reilly, Department of Fisheries and Oceans, Dartmouth, Nova Scotia, personal communication, 2010). However, there is no evidence that the Atlantic salmon strains depart from a 1:1 sex ratio (Chris Wilson, Ontario Ministry of Natural Resources, personal communication, 2011).

Experimental set-up

Semi-natural stream channels were set up at the Codrington facility to perform six behavioural trial blocks, each 8 days in length, between May and July 2009. Each channel simulated natural substrate and flow conditions of Lake Ontario streams used by salmonids (Gibson 1973; Hearn and Kynard 1986; Kemp et al. 2003). Details concerning the stream channel construction and set up are outlined in Chapter 2.

Each trial block was made up of 9 treatments, with 12 fish in each treatment. Each Atlantic salmon strain underwent three treatments: (i) Atlantic salmon with conspecifics of the same strain ("Alone" treatment, 12 salmon), (ii) Atlantic salmon housed with one non-native trout species: either with brown trout or rainbow trout ("+1NN" treatment, 6 salmon, 6 trout of one species), and (iii) Atlantic salmon with two non-native trout species: both brown trout and rainbow trout ("+2NN" treatment, 4 salmon, and 4 of each trout species). Hormone data of Atlantic salmon in treatments with either brown trout or rainbow trout did not differ and thus, were combined (see below). Fish were selected, tagged, and placed in stream channels as detailed in Chapter 2. The provision of food items and monitoring and analysis of behavioural observations of each fish are also detailed in Chapter 2. Using initial (pre-trial) and final mass, standard growth rate (%/day) was calculated as: SGR = 100 × [ln (final mass) – ln (initial mass)]/days fed (Bernier et al. 2004).

Blood samples

On Day 8 of each trial, Atlantic salmon were collected from the stream channels for final mass and length measurements, and terminal blood sampling. Collection of fish began at the channels farthest from the headbox. Netting, anaesthetizing, and sampling blood of all 12 fish in a stream channel took an average of 30 minutes. Care was taken to ensure that remaining fish were minimally disturbed during the collection of individual fish within the stream channels (Collection order did not influence hormone concentrations; see below). Netted fish were sacrificed by submersion in a MS-222 solution until operculum movement ceased, followed by a cranial blow. To obtain blood samples, the caudal fin was severed posterior to the adipose fin at the peduncle using a scalpel. Blood was collected in Microvette CB 500 Li Heparin tubes (Sarstedt, Quebec).

Each fish provided approximately 0.25-0.50 ml blood. Plasma was separated by centrifugation (1500 g for 5 minutes) and stored in 0.5 ml Eppendorf® tubes and frozen at -20°C for later analysis of cortisol and 11-ketotestosterone (11-KT) concentrations.

To assess basal hormone concentrations, 20 fish from each strain were netted from the source stock tanks in the middle of the experimental period (July 1, 2009) and sacrificed as outlined above. Mass and total length were recorded for these fish and blood samples were collected and processed as above. Basal blood samples from Atlantic salmon not participating in the stream channels serve as a means of comparison with treatment-exposed Atlantic salmon as past research has shown that when salmonids are kept at high densities, such as the density of the stock tanks in this study, (0.6 fish/L, as compared to 0.03 fish/L in stream channels), hierarchies typically do not form, reducing the number of social interactions that may increase stress (e.g., Kjartansson et al. 1988; Brown et al. 1992).

Enzyme immunoassays

Prior to the enzyme immunoassay for 11-KT, 20 μL plasma samples were extracted three times with an equal volume of diethylether (Van Der Kraak et al. 1989) in order to reduce intra-well variation and increase the detection capacity of the assay kit. The diethylether was evaporated in a fume hood overnight. Samples were reconstituted with assay buffer prior to the assay. Plasma concentrations of cortisol and 11-KT were determined by conducting assays according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, Michigan). The limit of detection for the cortisol and 11-KT kits was 12 pg/ml and 1.3 pg/ml, respectively. Each sample was run in triplicate, with 50 μL (1/80 or 1/40 dilution for cortisol and 11-KT, respectively) loaded into each well. The absorbance of individual wells was read at 412 nm. For the cortisol analysis, a subset of the 564 Atlantic salmon juveniles used in the behavioural trials were selected (N = 100 for each strain, or 300 total samples) to represent treatments across trials; four individuals per stream channel were randomly generated (i.e., 20 samples of each strain for basal, alone, and +2NN treatments, while there were 40 samples of each strain in the +1NN treatment; 20 from each of the Atlantic salmon with rainbow trout and Atlantic salmon with brown trout treatments). Of those 300 samples, 184 were randomly selected for 11-KT analysis (LaHave, N = 59; Sebago Lake N = 60; Lac Saint-Jean, N = 65) representing all treatments across trials as well as the basal condition. Additional details of the sample sizes can be found in Appendix 2. Precision was analysed by determining the intra-assay coefficient of variation (CV) and was found to be 20% (N = 11) for cortisol and 17% for 11-KT (N =13). An internal standard was included in the assays in order to determine the inter-assay CV, which was 20% (N = 8) for cortisol (standard curve average: r^2 = 0.98, Appendix 3) and 14% (N = 4) for 11-KT (standard curve average: r^2 = 0.98, Appendix 4).

Statistical analysis

Differences among strains in initial mass and total length were analysed using analyses of variance (ANOVA). Student's post-hoc *t*-tests were conducted between pairs of strains if significance was obtained in the ANOVA. Data of aggression initiated and received, and food consumption were normalised using $\log_{10}(x + 1)$ transformation. The hormonal data were also skewed, so were $\log_{10}(x + 1)$ transformed for statistical analyses.

To examine hormonal differences among three strains of Atlantic salmon across treatments, I used linear mixed models with strain (LaHave, Lac Saint-Jean, and Sebago Lake), treatment (basal, alone, +1NN, or +2NN), and the interaction of these two factors. Initial mass was included as a covariate, while trial block and channel number were entered as random effects. Random effects that were not significant were removed from the final model. Linear mixed models used a variance components covariance structure and a Satterthwaite approximation calculated denominator degrees of freedom (Satterthwaite 1946). When significance was achieved for strain, treatment or the interaction, one-way ANOVAs and post-hoc Student's *t*-tests were used to determine pair-wise differences in hormone concentrations. I also used one-way ANOVAs to explore trends when marginally non-significant results were obtained (P < 0.10) in the main model.

Linear mixed models were also used to examine the relationships of the hormones with the behaviours and growth measurements of juvenile Atlantic salmon. Basal hormone levels were excluded from these models as I did not measure agonistic and feeding behaviours of the fish from which I obtained basal hormone levels. Plasma cortisol or 11-KT concentrations were treated as covariates, while strain and treatment were treated as fixed factors. The interactions of each hormone with strain and treatment were also entered in the model, and initial mass was included as a covariate. Trial block and channel number were again entered as random effects and I used the same covariance structure and approximation of degrees of freedom in the model as noted above. Initiated and received aggression, David's score, food consumed, and standard growth rate were dependent variables in these models. The individual influence of strain and treatment effects on behaviour and growth has been discussed in detail in Chapter 2; here, I focus my discussion on the relationships between the hormones and the behavioural results. When significance was detected for a hormone, linear regression analysis was performed. When an interaction was found between strain or treatment and plasma cortisol or 11-KT, linear regression analysis was used to determine which strain had a significant relationship between the hormone and the dependent variable in a specific treatment. Finally, a Pearson's correlation was used to determine the relationship between cortisol and 11-KT. All statistics were performed using JMP 4 (version 4.0.2, SAS Institute Inc., 2000), SPSS 16.0 (SPSS Inc., 2007), or Microsoft Office Excel 2003 (Microsoft Corporation, USA) and P-values presented are for two-tailed probabilities ($\alpha \leq 0.05$).

3.3 Results

Mass and length comparisons

The Atlantic salmon strains differed in both their initial mass and total length (mass: $F_{2,297} = 71.5$, $P < 0.001$; total length: $F_{2,297} = 37.8$, $P < 0.001$; Table 3.1). Fish from the Sebago Lake strain were the heaviest and longest, followed by Lac Saint-Jean fish, while individuals from the LaHave strain were the smallest (Table 3.1). Among the three species, both mass and length differed (initial mass: $F_{4,859} = 161.5$, $P < 0.001$; length: $F_{4,859} = 191.0$, $P < 0.001$). Rainbow trout were the smallest and shortest of all the fish, while Sebago Lake Atlantic salmon were both heavier and longer than brown trout and rainbow trout (Table 3.1). Brown trout, however, were heavier than both the LaHave and Lac Saint-Jean salmon, but were shorter than both strains (Table 3.1). Means (± SD) of each strain and species broken down by treatment are presented in Appendix 2.

Table 3.1: Summary of physical characteristics of juvenile Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), and rainbow trout (*Oncorhynchus mykiss*).

	Atlantic salmon strains				
Variable	LaHave	Lac Saint-Jean	Sebago Lake	**Brown trout**	**Rainbow trout**
Initial mass (g)	31.9 ± 12.3^a	37.9 ± 11.2^b	53.2 ± 15.2^c	39.4 ± 13.6^d	20.6 ± 9.6^e
Total length (mm)	154 ± 22^a	167 ± 15^b	177 ± 18^c	151 ± 17^a	126 ± 19^d

Note: Data presented are means (± 1 standard deviation) across treatments. Strain data obtained from basal, alone, +1 and +2 non-native treatments. Trout data obtained from +1 non-native and +2 non-natives treatments. $N = 188$ for each strain (including 20 individuals for basal samples), $N = 180$ for each trout species. Different lowercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate a significant difference among strains or species ($P < 0.05$).

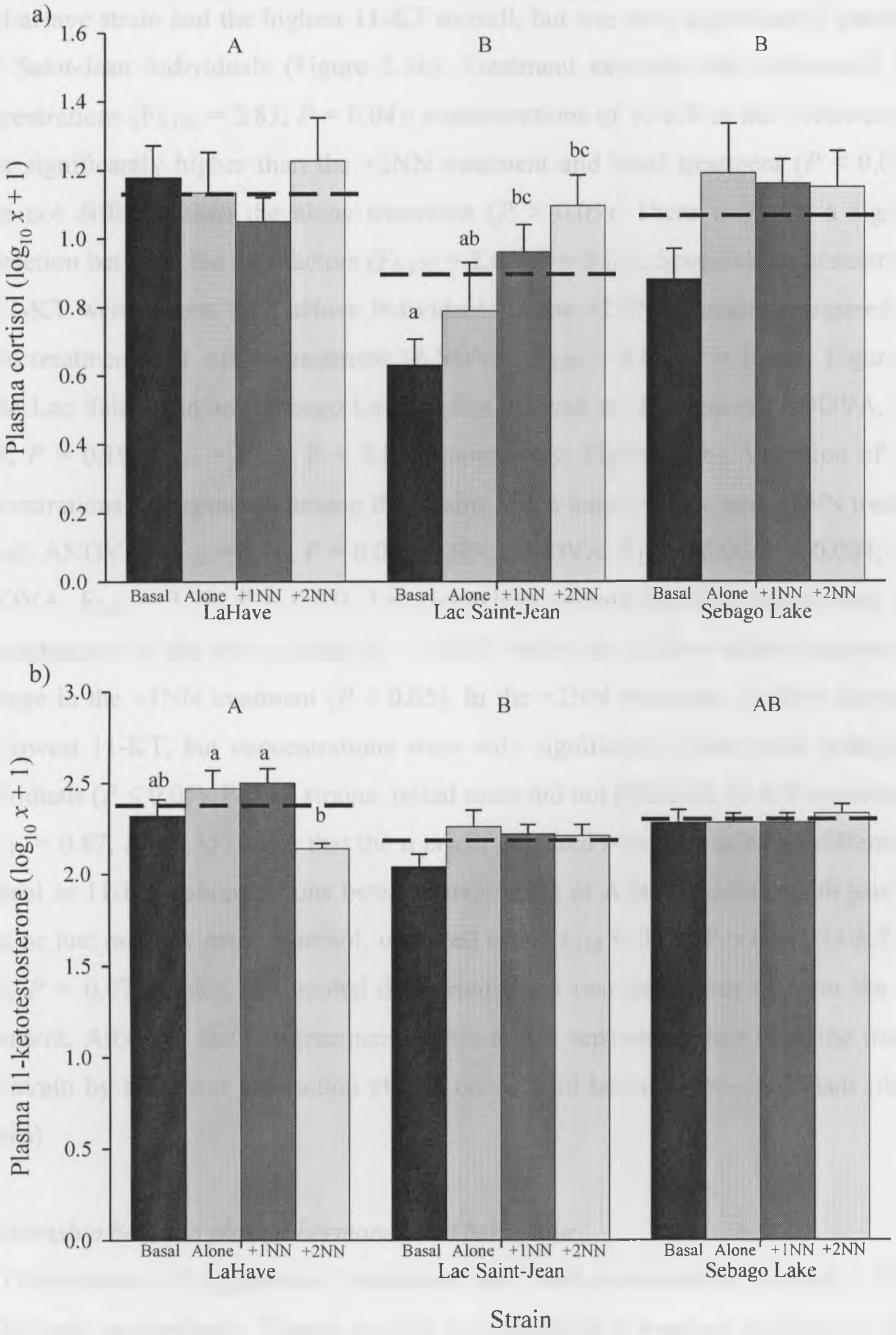
Influence of strain and treatment on plasma hormones

Collection order did not influence either cortisol or 11-KT concentrations (cortisol, Pearson's correlation: $r = 0.10$, $N = 284$, $P = 0.10$; 11-KT, Pearson's correlation: $r = -0.08$, $N = 181$, $P = 0.31$; note cortisol collection order for 16 Atlantic salmon and 11-KT collection order for 3 Atlantic salmon were accidentally not recorded).

Strains of Atlantic salmon differed in plasma cortisol concentrations across all treatments ($F_{2,287} = 12.3$, $P < 0.001$). Lac Saint-Jean salmon exhibited the lowest cortisol concentrations, Sebago Lake individuals were intermediate but not significantly different from Lac Saint-Jean fish, whereas LaHave salmon had the highest concentrations (Figure 3.1a). Experimental treatment also significantly influenced plasma cortisol ($F_{3,287} = 2.82$, $P = 0.04$), with cortisol concentrations lowest in the basal treatment, which were significantly lower than the two non-native treatments ($P < 0.05$; Figure 3.1a). This pattern appeared to be driven primarily by the Lac Saint-Jean strain as there was a marginally non-significant interaction between strain and treatment ($F_{6,287} = 1.96$, $P = 0.07$, Figure 3.1a). Specifically, cortisol concentrations varied significantly across treatments for Lac Saint-Jean individuals (ANOVA, $F_{3,95} = 3.19$, $P = 0.03$); basal cortisol concentrations were the lowest, followed by concentrations in the alone, +1NN, and

+2NN treatments (Figure 3.1a). Cortisol concentrations did not vary across treatments for LaHave and Sebago Lake salmon (LaHave: ANOVA, $F_{3,95} = 1.10$, $P = 0.35$; Sebago Lake: $F_{3,95} = 2.18$, $P = 0.10$; Figure 3.1a). Cortisol variation among strains was apparent only in the basal treatment (basal: ANOVA, $F_{2,56} = 10.9$, $P < 0.001$): LaHave basal cortisol was significantly higher than the other two strains ($P < 0.05$; Figure 3.1a). For all strains, initial mass was positively correlated with plasma cortisol concentrations ($F_{1,287} = 25.2$, $P < 0.001$).

Figure 3.1: Circulating plasma hormone concentrations for juvenile Atlantic salmon (*Salmo salar*). Data presented are of (a) cortisol and (b) 11-ketotestosterone (11-KT) concentrations for three Atlantic salmon strains, LaHave, Lac Saint-Jean, and Sebago Lake, obtained from basal, alone, +1 non-native (+1NN), and +2 non-natives (+2NN) treatments. All data were $\log_{10}(x + 1)$ transformed (for treatment means and standard deviations of each variable for each strain, see Appendix 2). Untransformed cortisol concentration data was measured in ng/ml and 11-KT in pg/ml. black dashed lines denote the mean concentration of either cortisol or 11-KT of for each strain across all treatments. Different uppercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate significant differences among strains ($P < 0.05$), while different lowercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate differences within strains (cortisol: $N = 100$ for each strain; 11-KT: LaHave, $N = 59$; Lac Saint-Jean, $N = 65$; and Sebago Lake, $N = 60$).


a)
Plasma cortisol (log$_{10}$ x + 1)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A
B
B
a
ab
bc
bc
Basal Alone +1NN +2NN
LaHave
Basal Alone +1NN +2NN
Lac Saint-Jean
Basal Alone +1NN +2NN
Sebago Lake
b)
Plasma 11-ketotestosterone (log$_{10}$ x + 1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A
B
AB
ab
a
a
b
Basal Alone +1NN +2NN
LaHave
Basal Alone +1NN +2NN
Lac Saint-Jean
Basal Alone +1NN +2NN
Sebago Lake
Strain

Atlantic salmon strains differed in 11-KT concentrations ($F_{2,171} = 6.70$, $P = 0.002$); the LaHave strain had the highest 11-KT overall, but was only significantly greater than Lac Saint-Jean individuals (Figure 3.1b). Treatment exposure also influenced 11-KT concentrations ($F_{3,171} = 2.83$, $P = 0.04$): concentrations of 11-KT in the +1NN treatment were significantly higher than the +2NN treatment and basal treatment ($P < 0.05$), but were not different than the alone treatment ($P > 0.05$). There was also a significant interaction between the two factors ($F_{6,171} = 2.65$, $P = 0.02$). Specifically, concentrations of 11-KT were lowest for LaHave individuals in the +2NN treatment compared to the alone treatment and +1NN treatment (ANOVA, $F_{3,55} = 4.88$, $P = 0.005$; Figure 3.1b) while Lac Saint-Jean and Sebago Lake strains showed no differences (ANOVA, $F_{3,61} = 1.64$, $P = 0.19$; $F_{3,56} = 0.22$, $P = 0.88$, respectively; Figure 3.1b). Variation of 11-KT concentrations was apparent among the strains in the basal, +1NN, and +2NN treatments (basal: ANOVA, $F_{2,30} = 3.96$, $P = 0.03$; +1NN: ANOVA, $F_{2,64} = 6.00$, $P = 0.004$; +2NN: ANOVA, $F_{2,41} = 3.79$, $P = 0.03$). Lac Saint-Jean salmon had the lowest basal 11-KT concentrations of the three strains ($P < 0.05$), while the LaHave salmon highest strain average in the +1NN treatment ($P < 0.05$). In the +2NN treatment, LaHave salmon had the lowest 11-KT, but concentrations were only significantly lower than Sebago Lake individuals ($P < 0.05$). For all strains, initial mass did not influence 11-KT concentrations ($F_{1,171} = 0.87$, $P = 0.35$). Note that the *a priori* unpaired *t*-tests revealed no differences in cortisol or 11-KT concentrations between treatments of Atlantic salmon with just brown trout or just rainbow trout (cortisol, unpaired t-test: $t_{118} = 0.29$, $P = 0.77$; 11-KT: $t_{60} = 0.42$, $P = 0.67$), hence, we pooled data from these two treatments to form the +1NN treatment. Albeit, if the two treatments were coded separately, then both the treatment and strain by treatment interaction effects on cortisol become non-significant (data not shown).

Relationship between plasma hormones and behaviour

Observations of aggressive behaviours and food consumption totalled 5,154 and 18,049 acts, respectively. Plasma cortisol was unrelated to received aggression, David's score, or standard growth rate (Table 3.2). However, cortisol concentrations were negatively associated with initiated aggression, such that as cortisol concentrations increased, initiated aggression decreased (Table 3.2, Figure 3.2a). A trend indicated a negative relationship between plasma cortisol and food consumption and plasma cortisol and strain interacted to influence food consumption (Table 3.2; Figure 3.3). Specifically, Atlantic salmon from Lac Saint-Jean and Sebago Lake with elevated plasma cortisol exhibited depressed food consumption (Lac Saint-Jean, Linear regression, $R^2 = 0.09$, $F_{1,78} = 7.66$, $N = 80$, $P = 0.01$; Sebago Lake, $R^2 = 0.10$, $F_{1,78} = 8.89$, $N = 80$, $P = 0.004$), but the pattern was not significant for the LaHave salmon (Linear regression, $R^2 = 0.01$, $F_{1,78} = 1.12$, $N = 80$, $P = 0.29$: Figure 3.3). There were also direct effects of strain, treatment, and initial mass on several of the behaviours (Table 3.2; see Chapter 2 for a full discussion of those effects).

Table 3.2: Summary of linear mixed model results for the plasma cortisol analyses in juvenile Atlantic salmon (*Salmo salar*). The analyses examine the relationship of cortisol with agonistic, foraging, and growth characteristics of Atlantic salmon in semi-natural stream behavioural trials.

Dependent variable	Independent	d. f.	F	*P*
Initiated aggression/hour	Cortisol	1, 224.7	4.25	0.04
	Cortisol × Strain	2, 225.5	1.31	0.27
	Cortisol × Treatment	2, 218.9	0.34	0.71
	Strain	2, 226.2	9.04	< 0.001
	Treatment	2, 221.9	2.12	0.12
	Initial mass	1, 226.8	0.05	0.82
Received aggression/hour	Cortisol	1, 225.2	0.05	0.83
	Cortisol × Strain	2, 225.9	1.85	0.16
	Cortisol × Treatment	2, 220.4	0.14	0.87
	Strain	2, 225.4	8.24	< 0.001
	Treatment	2, 222.9	1.33	0.27
	Initial mass	1, 227.0	3.45	0.07
David's score	Cortisol	1, 225.0	0.99	0.32
	Cortisol × Strain	2, 223.4	1.24	0.29
	Cortisol × Treatment	2, 219.3	0.35	0.70
	Strain	2, 226.8	0.21	0.81
	Treatment	2, 221.9	3.81	0.02
	Initial mass	1, 220.9	0.14	0.71
Food consumed/hour	Cortisol	1, 225.8	3.22	0.07
	Cortisol × Strain	2, 226.2	4.71	0.01
	Cortisol × Treatment	2, 222.4	0.22	0.80
	Strain	2, 220.6	1.96	0.14
	Treatment	2, 224.4	0.69	0.50
	Initial mass	1, 228.0	1.63	0.20
Standard growth rate (%/day)	Cortisol	1, 221.4	0.57	0.45
	Cortisol × Strain	2, 222.7	0.59	0.55
	Cortisol × Treatment	2, 217.8	0.41	0.67
	Strain	2, 226.5	0.72	0.49
	Treatment	2, 219.7	1.58	0.21
	Initial mass	1, 224.1	9.84	0.002

Note: Strain (LaHave, Lac Saint-Jean, and Sebago Lake) and treatment (alone, +1 non-native, and +2 non-natives) were coded as main factors; while cortisol and initial mass were included as covariates. Initiated and received aggression and food consumption were $\log_{10}(x + 1)$ transformed. d.f. = degrees of freedom; significance achieved when $P < 0.05$. $N = 300$ and fractional denominator degrees of freedom calculated using Satterthwaite approximation.

Figure 3.2: The relationship of plasma hormone concentrations and initiated aggressive acts for juvenile Atlantic salmon (*Salmo salar*). Data presented are of (a) cortisol and (b) 11-ketotestosterone (11-KT) concentrations for three Atlantic salmon strains, LaHave, Lac Saint-Jean, and Sebago Lake, obtained from alone, +1 non-native, and +2 non-natives treatments. All data were $\log_{10}(x + 1)$ transformed (for treatment means and standard deviations of each variable for each strain, see Appendix 2). Untransformed cortisol concentration data was measured in ng/ml and 11-KT in pg/ml. The solid line denotes a significant linear regression between initiated aggressive acts/hour and plasma cortisol: $\log_{10}$ (initiated aggressive acts/hour + 1) = -0.13·$\log_{10}$ ([plasma cortisol] + 1) + 0.48; $P = 0.002$) while the dashed line denotes a non-significant relationship between initiated aggression and 11-KT: $\log_{10}$ (initiated aggressive acts/hour + 1) = 0.002·$\log_{10}$ ([11-KT] + 1) + 0.37; $P = 0.99$) (cortisol, $N = 80$ for each strain; 11-KT, $N = 48$ for LaHave, $N = 54$ for Lac Saint-Jean, $N = 49$ for Sebago Lake).

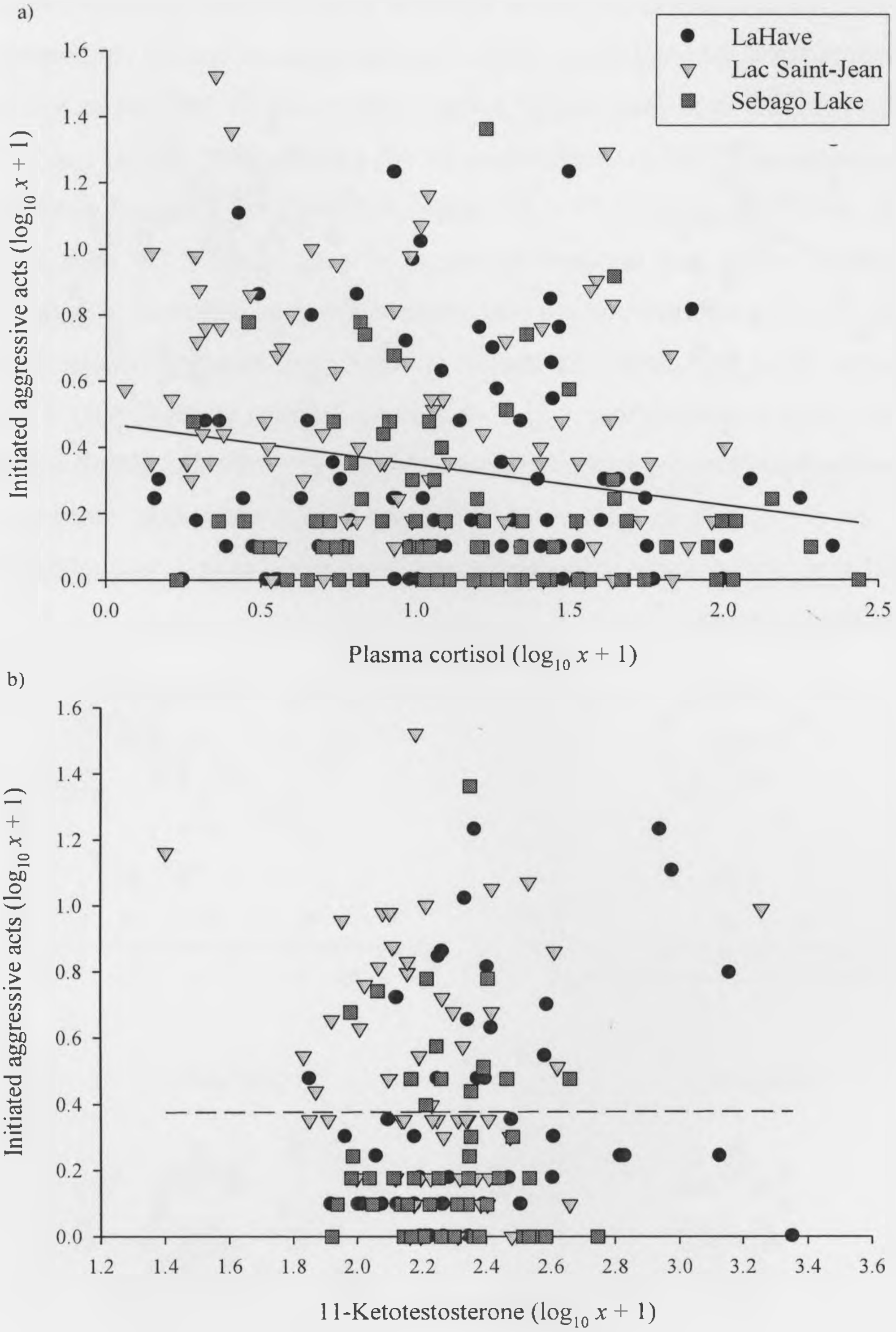
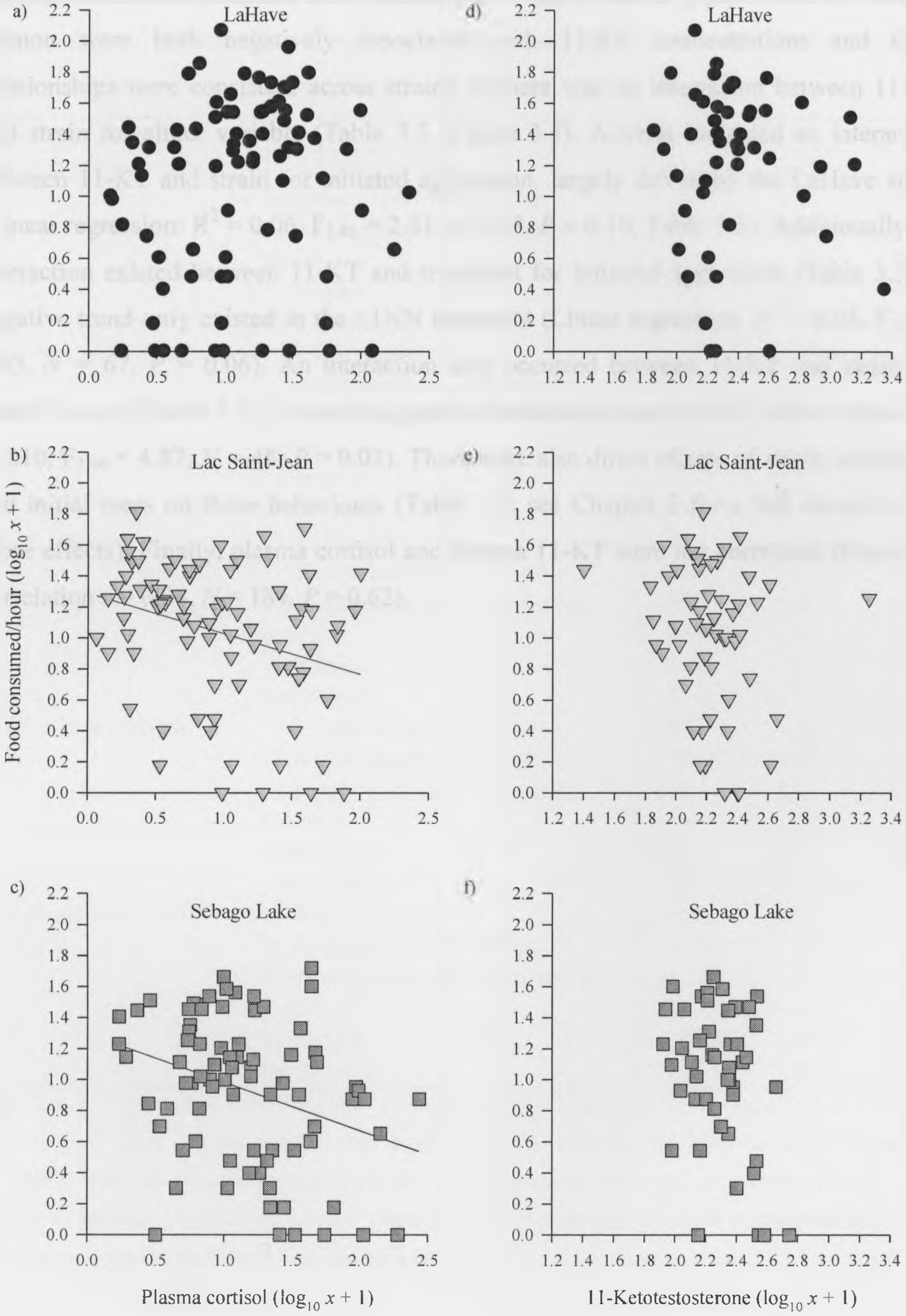

a)
LaHave
Lac Saint-Jean
Sebago Lake
Initiated aggressive acts ($\log_{10} x + 1$)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.5
1.0
1.5
2.0
2.5
Plasma cortisol ($\log_{10} x + 1$)
b)
Initiated aggressive acts ($\log_{10} x + 1$)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
11-Ketotestosterone ($\log_{10} x + 1$)

Figure 3.3: The relationship of plasma hormone concentrations and food consumption for juvenile Atlantic salmon (*Salmo salar*). Data presented are cortisol concentrations for (a) LaHave, (b) Lac Saint-Jean, and (c) Sebago Lake strains (N = 80 for each strain) and 11-ketotestosterone (11-KT) concentrations for (d) LaHave (N = 48), (e) Lac Saint-Jean (N = 54), and (f) Sebago Lake (N = 49) strains. All data were $\log_{10}(x + 1)$ transformed (for treatment means and standard deviations of each variable for each strain, see Appendix 2). Untransformed cortisol concentration data was measured in ng/ml and 11-KT in pg/ml. Solid lines denote the results of significant linear regressions (Lac Saint-Jean: $\log_{10}$ (food consumed/hour + 1) = $-0.26 \cdot \log_{10}$ ([plasma cortisol] + 1) + 1.29, P = 0.007; and Sebago Lake: $\log_{10}$ (food consumed/hour + 1) = $-0.31 \cdot \log_{10}$ ([plasma cortisol] + 1) + 1.29, P = 0.003). In the case of 11-KT and food consumption, regressions were non-significant for each strain, although the combined relationship across all three strains was significant (see text).


a)
LaHave
b)
Lac Saint-Jean
c)
Sebago Lake
d)
LaHave
e)
Lac Saint-Jean
f)
Sebago Lake
Food consumed/hour ($\log_{10} x + 1$)
Plasma cortisol ($\log_{10} x + 1$)
11-Ketotestosterone ($\log_{10} x + 1$)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
0.0
0.5
1.0
1.5
2.0
2.5
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4

Linear mixed models examining the relationships of 11-KT with aggressive and feeding behaviours revealed food consumption and standard growth rate of Atlantic salmon were both negatively associated with 11-KT concentrations and these relationships were consistent across strains as there was no interaction between 11-KT and strain for either variable (Table 3.3, Figure 3.3). A trend indicated an interaction between 11-KT and strain for initiated aggression, largely driven by the LaHave strain (Linear regression: $R^2 = 0.06$, $F_{1,46} = 2.81$, $N = 48$, $P = 0.10$, Table 3.3). Additionally, an interaction existed between 11-KT and treatment for initiated aggression (Table 3.3): a negative trend only existed in the +1NN treatment (Linear regression, $R^2 = 0.05$, $F_{1,65} = 3.63$, $N = 67$, $P = 0.06$). An interaction also occurred between 11-KT and strain for David's score (Table 3.3), driven by a positive relationship only in the LaHave strain ($R^2 = 0.10$, $F_{1,46} = 4.87$, $N = 48$, $P = 0.03$). There were also direct effects of strain, treatment, and initial mass on these behaviours (Table 3.3; see Chapter 2 for a full discussion of those effects). Finally, plasma cortisol and plasma 11-KT were not correlated (Pearson's correlation $r = 0.04$, $N = 184$, $P = 0.62$).

Table 3.3: Summary of linear mixed model results for the 11-ketotestosterone (11-KT) analyses in juvenile Atlantic salmon (*Salmo salar*). The analyses examined the relationship of plasma 11-KT with agonistic, foraging, and growth characteristics of Atlantic salmon in semi-natural stream behavioural trials.

Dependent variable	Independent	d.f.	F	*P*
Initiated aggression/hour	11-Ketotestosterone	1, 136.6	0.37	0.54
	11-Ketotestosterone × Strain	2, 134.3	2.71	0.07
	11-Ketotestosterone × Treatment	2, 133.8	4.85	0.01
	Strain	2, 132.8	3.03	0.05
	Treatment	2, 134.1	4.85	0.04
	Initial mass	1, 139.6	0.03	0.87
Received aggression/hour	11-Ketotestosterone	1, 138.2	0.15	0.70
	11-Ketotestosterone × Strain	2, 137.5	0.95	0.39
	11-Ketotestosterone × Treatment	2, 136.7	2.58	0.08
	Strain	2, 137.2	2.17	0.12
	Treatment	2, 136.6	3.63	0.03
	Initial mass	1, 139.6	0.63	0.43
David's Score	11-Ketotestosterone	1, 136.4	0.96	0.33
	11-Ketotestosterone × Strain	2, 135.8	2.81	0.02
	11-Ketotestosterone × Treatment	2, 135.3	1.86	0.16
	Strain	2, 136.9	4.83	0.01
	Treatment	2, 135.6	1.42	0.25
	Initial mass	1, 137.0	0.30	0.58
Food consumed/hour	11-Ketotestosterone	1, 137.7	4.26	0.04
	11-Ketotestosterone × Strain	2, 135.6	0.55	0.58
	11-Ketotestosterone × Treatment	2, 135.3	2.35	0.10
	Strain	2, 134.4	0.23	0.80
	Treatment	2, 135.6	2.55	0.08
	Initial mass	1, 139.9	1.31	0.26
Standard growth rate (%/day)	11-Ketotestosterone	1, 135.1	8.20	0.005
	11-Ketotestosterone × Strain	2, 134.4	0.22	0.80
	11-Ketotestosterone × Treatment	2, 132.8	0.86	0.43
	Strain	2, 133.5	0.16	0.85
	Treatment	2, 133.1	0.69	0.50
	Initial mass	1, 138.2	5.40	0.02

Note: Strain (LaHave, Lac Saint-Jean, and Sebago Lake) and treatment (alone, +1 non-native, and +2 non-natives) were coded as main factors; while plasma 11-ketotestosterone and initial mass were treated as covariates. Initiated and received aggression and food consumption were $\log_{10}(x + 1)$ transformed. d.f. = degrees of freedom, significance achieved when $P < 0.05$. $N = 151$ and fractional denominator degrees of freedom calculated using Satterthwaite approximation.

3.4 Discussion

Basal circulating hormone concentrations and the sensitivity of these hormone concentrations to social interactions and stressors may vary among populations. I found that basal cortisol concentrations of individuals from the LaHave strain (population) were the highest among the three Atlantic salmon strains, while basal concentrations of Sebago Lake and Lac Saint-Jean individuals were up to three-fold lower. Social interactions with in the treatments with both one and two non-native species present were a cause of cortisol elevation for the Atlantic salmon as concentrations in both treatments were higher than basal concentrations and those of individuals in the conspecific treatments. Additionally, Lac Saint-Jean fish showed a greater response to the presence of non-native salmonids, while cortisol concentrations varied little among the treatments for the other two strains. Concentrations of 11-KT were also lowest in the Lac Saint-Jean strain, but it was the LaHave strain that showed the greatest variation in 11-KT response to the presence of non-native salmonids, with concentrations lowest in the presence of both non-natives. Similar to my results, Pickering and Pottinger (1989) found significant variation in basal cortisol concentrations of five strains of rainbow trout and three strains of brown trout. Furthermore, those authors found that the response to a stressor varied among the strains (also see Pottinger and Moran 1993). Pottinger and Carrick (2001) went on to show that the variation in basal cortisol concentrations across strains was genetic because cortisol concentrations were heritable. Similar results have also been found in other taxa including mammals (Kemp and Drinkwater 1989; Malisch et al. 2007). As my strains were each bred using similar protocols and raised in a common hatchery environment, it is likely that the differences in both basal hormone concentration and stress response among the strains has a genetic basis. Collectively, these data suggest that population differences in basal hormone concentrations and response to stressors such as the presence of non-native competitors, is at least in part explained by genetic differences among populations.

Patterns of circulating cortisol concentrations associated with chronic stress may differ among populations. A number of studies of salmonids have shown that chronic stress can be associated with circulating concentrations around 10 ng/ml, whereas unstressed concentrations are associated with concentrations of less than 5 ng/ml (Maule et al. 1987; Pickering and Pottinger 1989). In my study, LaHave salmon had cortisol concentrations almost twice the chronic amount even when being held in large groups (i.e., the stock tanks) where hierarchies and other social interactions are not expected to occur (Kjartansson et al. 1988; Brown et al. 1992). On the other hand, individuals from Lac Saint-Jean and to a lesser extent, Sebago Lake, showed more expected patterns of stress response, with cortisol concentrations being higher in treatments with one or both non-native species present as compared to the basal condition. Concentrations of these two strains in the non-native treatments were much higher than those observed in unstressed juvenile salmonids (approximately 0-5 ng/ml; e.g., Pickering and Pottinger 1989). To better understand the high cortisol concentrations in the LaHave strain, it would be fruitful to measure cortisol concentrations of fish in the wild to determine if the hatchery environment is the source of the apparent stress. Alternatively, LaHave individuals may be less sensitive to circulating cortisol (see Carragher et al. 1989; Pickering and Pottinger 1989) or have glucocorticoid receptors with a low affinity for the hormone (Maule and Schreck 1991; Maule et al. 1993) or have fewer glucocorticoid receptors (see Levine 2005). In such cases, the observed elevated cortisol levels may not actually indicate chronic stress. Nevertheless, the differences among the three strains of salmon that I observed suggest quantitative differences in the functioning of the pituitary-interrenal axis in response to environmental stressors (also see Pottinger and Moran 1993).

Elevated plasma cortisol concentrations have been associated with reductions in food consumption in a number of species (Gregory and Wood 1999; Crockett et al. 2000). For example, Gregory and Wood (1999) examined the influence of cortisol on the feeding behaviour of juvenile rainbow trout and found that chronically elevating plasma cortisol concentrations through implants were associated with reduced individual appetite and food consumption. In pigtailed macaques (*Macaca nemestrina*), Crockett et al. (2000) found that, in a laboratory setting, elevated cortisol was associated with appetite suppression. I also found a strong negative relationship between cortisol and food consumption in two of the three strains of Atlantic salmon. Other studies have linked the reduced food consumption to reduced searching and capturing of food items (Beitinger 1990). I did not differentiate between these two behaviours in my study. Regardless, the patterns uncovered in my study implicate long-term obstacles for chronically stressed Atlantic salmon as reduced food consumption can significantly impact performance and survival.

Research has shown clear patterns between aggression and both glucocorticoid and androgen levels across taxa (Cavigelli and Pereira 2000; Øverli et al. 2004; Parikh et al. 2006). Although well established in adults, the patterns are not as well defined in juveniles. Agonistic interactions can be a source of stress, but the effect of that stress differs among individuals, typically with aggressive individuals having low cortisol levels and less aggressive individuals having high levels (Ejike and Schreck 1980; Hannes et al. 1984; Elofsson et al. 2000). My data confirmed this pattern in juvenile Atlantic salmon: individuals that initiated more aggression had low concentrations of cortisol, whereas individuals that initiated less aggression had high levels. It is also well known that elevated androgen levels are associated with increased aggression in adult individuals (Dzieweczynski et al. 2006; Parikh et al. 2006), yet, to my knowledge, this relationship in juvenile fish is largely unexplored. My results suggest that a direct relationship between 11-KT and initiated aggression does not exist, at least in juvenile Atlantic salmon and in the context of my study. The levels of 11-KT that I observed in juvenile Atlantic salmon were similar to levels found in juvenile coho salmon (*O. kisutch*) and rainbow trout (Patiño and Schreck 1986; Hou et al. 1999) of similar age and size, but substantially lower than levels in adult salmonids (Sato et al. 1997; Olsén et al. 1998). Additionally, competition with the non-native salmonids did not appear to influence 11-KT concentrations of either Lac Saint-Jean or Sebago Lake fish, while 11-KT concentrations were lowest for the LaHave strain when in the presence of both trout species. Interestingly, a pattern revealed that this strain also received more aggression in this treatment than the other two strains (see Chapter 2). Although 11-KT is considered to be the primary androgen in fish (see Borg 1994; Oliveira et al. 2009) and has been linked to aggression in adults (Taves et al. 2009), it may be that other androgens such as testosterone, play a larger role in aggression in juveniles.

Androgens are also believed to positively aid juvenile development (e.g., Schwabl 1996), although the benefits associated with heightened levels of the hormones may not always outweigh the costs. Faster growth, commonly associated with androgens, may only be a benefit in some instances, and recent studies postulate that in the long-term it may be costly (e.g., Metcalfe and Monaghan 2001). Evidence of the costs associated with elevated androgen levels has been shown in the development of offspring of a number of organisms with androgen-supplemented individuals exhibiting reduced or delayed growth and other traits (McGivern et al. 1996; Henry and Burke 1999; Sockman and Schwabl 2000). Similarly, Gannam and Lovell (1991) found that while feeding low doses of 11-KT to channel catfish (*Ictalurus punctatus*) stimulated growth, with higher doses having the opposite effect. The pattern has been observed in adult male song sparrows implanted with testosterone early on in the breeding season; Wingfield (1984) noted that individuals exhibited lower body mass and less fat. By the end of the season, however, both controls and testosterone-implanted individuals were lean (Wingfield 1984). I found that elevated 11-KT concentrations in all three Atlantic salmon strains negatively correlated with both food consumption and standard growth rate. Heightened metabolic costs and increased energy expenditure associated with elevated androgen levels may limit energetic resources available for growth (Marler et al. 1995; Buchanan et al. 2001), possibly explaining the negative relationship. Thus, elevated androgen levels may benefit juvenile development in some instances, but may come at the cost of growth.

In conclusion, my research generally supports previous literature showing that circulating levels of glucocorticoid hormones may mediate aggressive and feeding behaviour. My research also suggests that although particular androgens such as 11-KT are associated with feeding behaviours in juvenile fish, they are not linked to aggression. Moreover, my data have important implications for restoration of Atlantic salmon in the Great Lakes. The documented differences among the three Atlantic salmon strains in basal cortisol concentrations and stress responses to the presence of non-native salmonids may well affect the post-stocking ecological success of the strains in natural stream environments. Whether these extend to Lake Ontario streams has yet to be determined. Nevertheless, the differences suggest that the Lac Saint-Jean strain may fare better in natural streams environments where stocked Atlantic salmon juveniles have to contend and compete with non-native brown trout or rainbow trout.

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Chapter 4. Social dominance and hormone concentrations of juvenile non-native trout species in the presence of juvenile Atlantic salmon

4.1 Introduction

Ecosystem services are human benefits derived from natural resources and processes provided by the environment, including clean drinking water, waste decomposition, and spiritual and recreational benefits (Holmlund and Hammer 1999; Millennium Ecosystem Assessment 2005). It has been argued that the sustainability of such ecosystem services critically depends on the preservation and restoration of native biodiversity and the associated natural ecosystem processes (Chichilnisky and Heal 1998; Duarte 2000; Worm et al. 2006).

Invasive species, and more generally the presence of non-native species, however, can threaten ecosystem processes and ultimately the associated services. For instance, the introduction of non-native grasses can impact productivity and nutrient cycling (Vitousek 1990; D'Antonio and Vitousek 1992). Non-native species that exhibit niche overlap with ecologically-similar native species can consequently drive competition for food and shelter (Hearn and Kynard 1986) and unless the species diverge in resource use, the introduction may eventually alter biodiversity and ecological processes as a result of displacement or extirpation of the native species (Morita et al. 2004). Indeed, controlling invasive species in the Great Lakes are estimated to cost Canada and the US close to $1 billion a year (CESD 2001; Lovell and Stone 2005). Sustainably managing natural resources and their services thus requires the preservation of native biodiversity or limiting competitive interactions of native and non-native species.

Alternatively, non-native species can contribute to ecosystem processes and services (Ewel and Putz 2004). Blanket criticism of non-native species is not always productive, for when their presence does not threaten ecosystem health, such species can aid in ecosystem maintenance or development and even improve the restoration process of native species (see Ewel and Putz 2004). For example, while the introduction of zebra mussels (*Dreissena polymorpha*) in inland waters of North America has typically been associated with a number of negative biotic and abiotic changes, the species enhances both water clarity and quality, increasing light transmittance and enabling greater growth of benthic plants and filtering a wide range of particles from the water column (for a review, see MacIsaac 1996). Ecosystem services provided by non-native species can also provide extensive monetary and cultural contributions through, for instance, recreational fisheries (see Toth and Brown 1997), which strengthen local economies through increased revenue and job creation for local marinas, restaurants, outfitters, and lodges (Brown and Connelly 2009). In the Great Lakes, angling for non-native salmonids largely contributes to the $400 million recreational sport fishery (see Brown and Connelly 2009; OMNR 2010). Hence, a balance between natural stability of ecosystems and revenue generated from ecosystem services is crucial to maximize the potential benefits from our natural resources.

In Lake Ontario, non-native salmonids were introduced in efforts to exert control of non-native prey and to enhance the recreational fishery (Crawford 2001). Species routinely stocked in the lake include brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), and chinook salmon (*O. tshawytscha*) (Crawford 2001; Mills et al. 2003). These species played a pivotal role in transforming the diminishing fishery of the 1970s and 1980s in Lake Ontario (Mills et al. 2003). However, current efforts are underway to restore a once-native top predator of Lake Ontario, the Atlantic salmon (*S. salar*) (Crawford 2001). Establishing a population of Atlantic salmon would contribute to local ecosystem processes through the maintenance of healthy fish communities by controlling non-native prey fish populations (NYSDC 2009); the provision of food for human and wildlife consumers (EPA 2005); and by serving as bio-indicators of ecosystem health (OMNR 2006). A self-sustaining population would be a step towards restoring the native biodiversity of Lake Ontario (OMNR 2006). Restoring Atlantic salmon in Lake Ontario would equally be advantageous for local socioeconomic growth boosting the recreational fishery (ASF 2008). However, Atlantic salmon and the now established non-native brown trout and rainbow trout share similar habitat (see Hearn and Kynard 1986; Harwood et al. 2002; Heggenes et al. 2002) and evidence suggests that in Lake Ontario, intense competition for resources would exist among the species (Kennedy and Strange 1986; Volpe et al. 2001; Harwood et al. 2002; Stradmeyer et al. 2008). Indeed, there is concern that competition among Atlantic salmon and non-native brown trout and rainbow trout may hinder the current ecological services provided by the non-native species (see Dietrich et al. 2008). Thus, it is essential to examine the potential impact of Atlantic salmon on the current ecosystem processes and services provided by the presence of non-native brown trout and rainbow trout in Lake Ontario.

In this paper, I studied the potential effects of stocking Atlantic salmon in streams with juvenile brown trout and rainbow trout. Using semi-natural stream environments, I examined the agonistic interactions of juvenile Atlantic salmon, brown trout, and rainbow trout and the subsequent hormonal stress response. I measured dominance, feeding behaviours, growth rates, and cortisol, a commonly studied hormone in fish that has been associated with, among other functions, the stress response (Barton and Iwama 1991), to evaluate the competitive impact of Atlantic salmon on each trout species individually and altogether. If brown trout and rainbow trout are stronger competitors than Atlantic salmon as past research suggests, then dominance, feeding behaviours, and growth rates should be higher in the trout species than in the Atlantic salmon and that cortisol concentrations of the trout species should be similar to those of unstressed salmonids. These behaviours and physiological parameters play a major role in determining recruitment and hence, population health (e.g., Walters and Juanes 1993; Bergenius et al. 2002). I discuss the potential impact of Atlantic salmon on the current non-native recreational fishery in light of the competitive interactions among juvenile Atlantic salmon, brown trout, and rainbow trout.

4.2 Methods

Study species

My behavioural and hormonal studies involved 1.5 yr-old Atlantic salmon (*Salmo salar,* N = 348), brown trout (*S. trutta,* N = 200), and rainbow trout (*Oncorhynchus mykiss,* N = 200) reared from brood stocks created by Ontario Ministry of Natural Resources (OMNR). Fish were obtained as outlined in Chapter 2. Similar to local stocking conditions, the yearlings of these species differed in size (see below). Fish were held at the Codrington Fisheries Research Facility (Codrington, Ontario) as detailed in Chapter 2.

Experimental set-up

Semi-natural stream channels were constructed to perform six trial blocks examining behaviour and growth between May and July 2009 at the Codrington hatchery. The channels were designed to provide substrate and flow conditions similar to those used by the trout and Atlantic salmon found in southern Ontario streams (Gibson 1973; Hearn and Kynard 1986). The stream channels were 2.4 m in length and divided into a riffle and a pool. Details concerning the stream channel construction and set up are outlined in Chapter 2.

Trial blocks consisted of three treatments with 12 individuals in each treatment: Atlantic salmon with brown trout (6 salmon, 6 trout), Atlantic salmon with rainbow trout (6 salmon, 6 trout), and Atlantic salmon with both trout species (4 salmon, 4 of each trout species). The selection, tagging, and placement of fish in stream channels are detailed in Chapter 2 in addition to the provision of food items and monitoring and analysis of individual aggressive and feeding behaviours.

On the final day of each trial block (Day 8), fish were collected from the stream channels for final mass and length measurements and terminal blood sampling. Fish capture, anaesthetizing, and treatment and basal blood sampling methods as detailed in Chapter 3.

Enzyme immunoassays

To determine plasma cortisol concentrations, I used a random subset of the individuals from each species (brown trout and rainbow trout, N = 102 for each species; Atlantic salmon, N = 240) to represent all treatments across trials; four individuals per stream channel were randomly generated (i.e., 60 samples of Atlantic salmon in each of the treatments; 40 samples each of brown trout and rainbow trout when just with Atlantic salmon, and 42 samples each of brown trout and rainbow trout when with all three species). Analysis of basal cortisol included 60 Atlantic salmon individuals and 20 individuals each of brown trout and rainbow trout. Assays were run according to the procedure for cortisol outlined in Chapter 3. Precision was analysed by determining the intra-assay coefficient of variation (CV) and was found to be 19% (N = 21). An internal standard was included in the assays in order to determine the inter-assay CV for the trout data, which was 37% (N = 10; standard curve average: $r^2 = 0.98$) and Atlantic salmon data, which was 20% (N = 8; standard curve average: $r^2 = 0.98$, Appendix 2). One outlier was removed from the calculation of inter-assay CV for the trout data, but the data show that the patterns remain the same with or without the data from the outlying plate (data not shown).

Statistical Analysis

Differences in initial mass and total length of the species were analysed using one-way analyses of variance (ANOVA). Post-hoc Student's *t*-tests were used to determine differences between pairs of species. Food consumption and plasma cortisol data were normalised using logarithmic ($x + 1$) transformation for statistical analyses. Behavioural and hormonal data of Atlantic salmon has been discussed in detail in Chapters 2 and 3, so only the results focusing on brown trout and rainbow trout are discussed here.

I used linear mixed models to analyze the influence of species and treatment nested within species as fixed factors on David's score, food consumption, and standard growth rate. Initial mass was treated as a covariate and channel number and trial block were entered as random effects. I used a variance components covariance structure and denominator degrees of freedom were calculated with a Satterthwaite approximation (Satterthwaite 1946). If significance was achieved for a main effect, post-hoc Student's *t*-tests were conducted. Hormonal differences among the three species were also tested using linear mixed models with species and treatment nested within species again as main effects. Basal hormone levels of each species were included in this model as an additional treatment. Initial mass was entered as a covariate and channel number and trial block were entered as random effects. When significance was achieved for either fixed factor, post-hoc Student's *t*-tests were conducted.

Finally, linear regression analyses were used to test the relationships of circulating cortisol levels and David's score, food consumption, and standard growth rate as well the effect of David's score on food consumption and standard growth rate of each trout species. All statistics were calculated using JMP 4 (version 4.0.2, SAS Institute Inc., 2000) or Microsoft Office Excel (Microsoft Corporation, USA) and P-values presented are for two-tailed probabilities ($\alpha \leq 0.05$).

4.3 Results

Mass varied among species ($F_{2,684}$ = 157.5, $P < 0.001$) with both Atlantic salmon (40.5 ± 15.1 g, mean ± S.D.) and brown trout (39.2 ± 14.1 g) being significantly heavier than rainbow trout (20.1 ± 9.3 g) (Atlantic salmon: post-hoc *t*-test, t_{543}= 19.6, $P < 0.001$; brown trout: t_{345}= 16.1, $P < 0.001$); there was no difference in mass between Atlantic salmon and brown trout (post-hoc *t*-test, t_{546} = 0.96; $P = 0.34$). Total length varied among all three species ($F_{2, 684}$ = 225.8, $P < 0.001$); Atlantic salmon (165 ± 22 mm) were the longest (brown trout: post-hoc *t*-test, t_{487}= 8.00, $P < 0.001$; rainbow trout: t_{478}= 22.5, P <0.001), followed by brown trout (151 ± 18 mm) (rainbow trout: post-hoc *t*-test, t_{398}= 14.2, $P < 0.001$), and then rainbow trout (125 ± 18 mm). See Appendix 5 for treatment means of each variable for each species.

David's score, feeding behaviours and growth

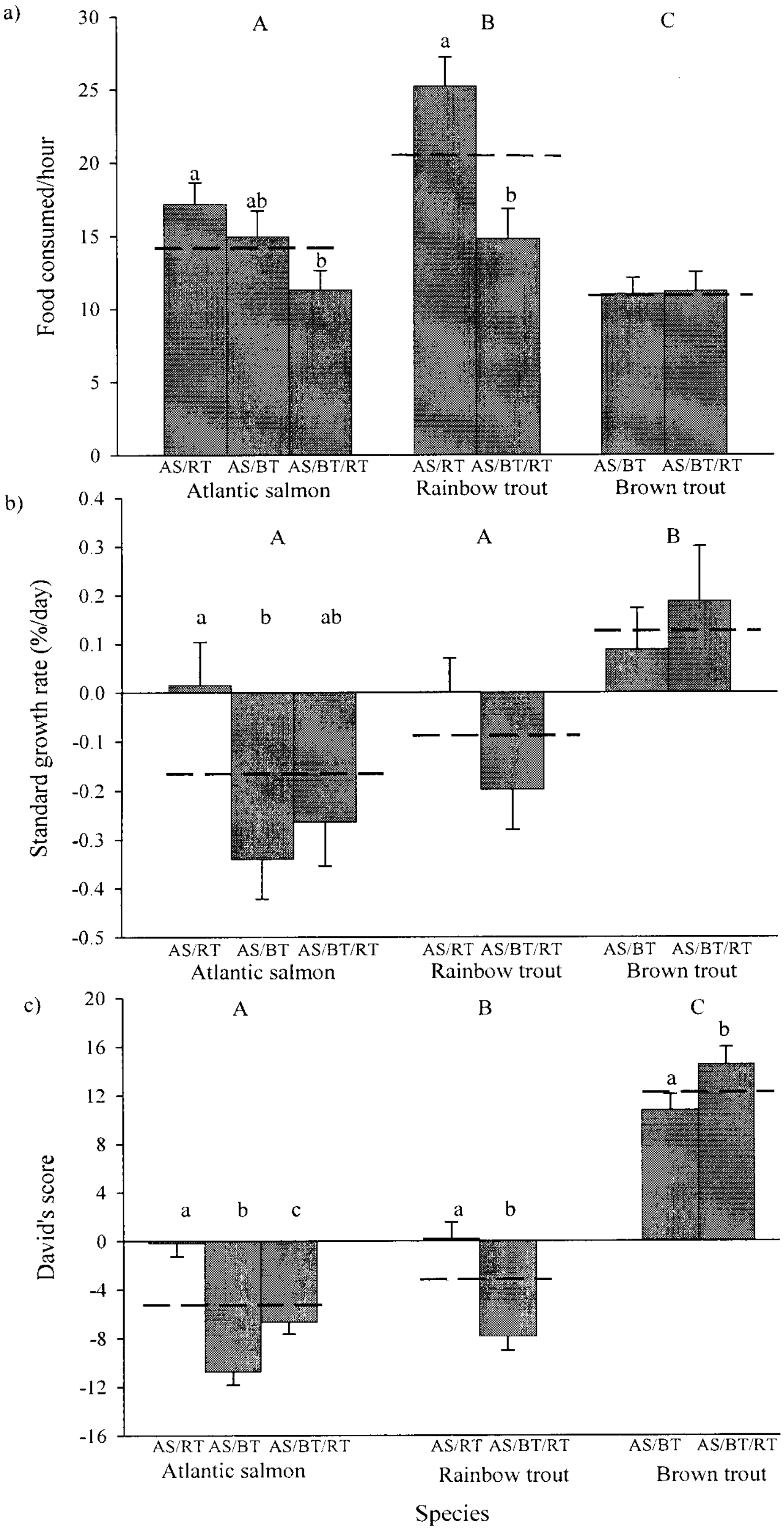
I recorded 19,985 feeding behaviours and 15,881 aggressive behaviours over the experiment. The linear mixed models revealed that the species significantly varied in food consumption, standard growth rate, and David's score (Table 4.1). Brown trout had the highest David's score and standard growth rate, but lowest food consumption compared to both Atlantic salmon and rainbow trout (Figure 4.1). Rainbow trout consumed more food and achieved higher David's scores than Atlantic salmon, but the two species were not found to have different standard growth rates (Figure 4.1).

Table 4.1: Summary of linear mixed model results for the behavioural analyses in juvenile Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), and rainbow trout (*Oncorhynchus mykiss*). The analyses examined agonistic, foraging, and growth characteristics of Atlantic salmon, brown trout, and rainbow trout used in semi-natural stream behavioural trials.

Dependent variable	Independent	Degrees of freedom	F	*P*
David's score	Species	2, 640.0	153.5	< 0.001
	Treatment[Species]	4, 640.0	19.8	< 0.001
	Initial mass	1, 640.0	82.7	< 0.001
Food consumed/hour	Species	2, 634.7	13.8	< 0.001
	Treatment[Species]	4, 555.0	6.65	< 0.001
	Initial mass	1, 637.4	7.88	0.005
Standard growth rate	Species	2, 631.6	11.6	< 0.001
	Treatment[Species]	4, 603.9	2.43	0.047
	Initial mass	1,632.2	4.51	0.03

Note: Species and treatment nested within species were coded as main effects and initial mass was entered as a covariate. Atlantic salmon, $N = 288$; brown trout and rainbow trout each, $N = 180$. Denominator degrees of freedom calculated using Satterthwaite approximation and significance achieved when $P < 0.05$.

Figure 4.1: The influence of treatment exposure on growth and agonistic measurements of juvenile salmonids. Data presented are of (a) food consumed/hour, (b) standard growth rate (%/day), and (c) David's score of Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), and rainbow trout (*Oncorhynchus mykiss*), obtained from the following treatments in semi-natural stream channels: Atlantic salmon with rainbow trout (AS/RT), Atlantic salmon with brown trout (AS/BT), and Atlantic salmon with both brown trout and rainbow trout (AS/BT/RT). Black dashed lines denote mean of each species across treatments. Different uppercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate significant differences among species ($P < 0.05$), while different lowercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate significant differences among treatments within species (Atlantic salmon, $N = 288$; brown trout and rainbow trout, $N = 180$ each).


a)
Food consumed/hour
0
5
10
15
20
25
30
A
B
C
a
ab
b
a
b
AS/RT AS/BT AS/BT/RT
Atlantic salmon
AS/RT AS/BT/RT
Rainbow trout
AS/BT AS/BT/RT
Brown trout
b)
Standard growth rate (%/day)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
A
A
B
a
b
ab
AS/RT AS/BT AS/BT/RT
Atlantic salmon
AS/RT AS/BT/RT
Rainbow trout
AS/BT AS/BT/RT
Brown trout
c)
David's score
20
16
12
8
4
0
-4
-8
-12
-16
A
B
C
a
b
c
a
b
a
b
AS/RT AS/BT AS/BT/RT
Atlantic salmon
AS/RT AS/BT/RT
Rainbow trout
AS/BT AS/BT/RT
Brown trout
Species

David's score, food consumption and standard growth rate were all similarly influenced by treatment (Table 4.1). Individual ANOVAs revealed that food consumption and David's score of rainbow trout were significantly higher in the treatment with just Atlantic salmon than the treatment with both Atlantic salmon and brown trout, and, although standard growth rate exhibited the same pattern, this difference was not significant (Figure 4.1). On the other hand, brown trout displayed no significant differences between treatments with just Atlantic salmon or with both Atlantic salmon and rainbow trout for food consumption and standard growth rate, although standard growth rate was higher in the latter treatment (Figure 4.1). David's score was significantly higher for brown trout in the treatment with both Atlantic salmon and rainbow trout compared to when just with Atlantic salmon (Figure 4.1). For juvenile Atlantic salmon, all three variables were the highest when Atlantic salmon were with just rainbow trout (Figure 4.1). David's score and standard growth rate were both lowest when Atlantic salmon were just with brown trout, while food consumption was lowest when Atlantic salmon were in the treatment with both brown trout and rainbow trout (Figure 4.1). Initial mass was significant for all three dependent variables with a positive relationship between initial mass and David's score and food consumed, but a negative relationship between initial mass and standard growth rate (Table 4.1).

Plasma cortisol and species

There was a positive relationship between collection order and cortisol concentrations for both rainbow trout (Pearson's correlation, $\rho = 0.54$, $N = 102$, $P < 0.001$) and brown trout (Pearson's correlation, $\rho = 0.46$, $N = 98$, $P < 0.001$; note collection order was unknown for four brown trout). Conversely, there was no relationship between collection order and cortisol concentration in Atlantic salmon (see Chapter 3). Thus, for the former two species, we used collection order-corrected cortisol concentrations (hereafter referred to as "corrected cortisol") for any statistical analysis performed on within-species data. The corrected values were obtained by taking the standardized residuals from a linear regression of cortisol concentration onto collection order. For species comparisons, collection order was included as a covariate.

Average cortisol concentrations (including basal levels) did not vary by species ($F_{2,36.7} = 0.93$, $P = 0.40$; Figure 4.2). Treatment, however, had a significant effect ($F_{7,35.3} = 9.09$, $P < 0.001$), with Atlantic salmon having significantly lower basal cortisol than conspecifics in all treatments with non-native trout species (ANOVA: $F_{2,222} = 3.61$, $P < 0.001$, Figure 4.2). Interestingly, basal cortisol levels of both rainbow trout and brown trout were significantly higher than those in the treatment with just Atlantic salmon and the treatment with all three species present (rainbow trout: ANOVA, $F_{2,98} = 17.7$, $P < 0.001$; brown trout: $F_{2,94} = 17.1$, $P < 0.001$, Figure 4.2). Brown trout were also found to have higher cortisol levels in the treatment with just Atlantic salmon than the treatment with all three species present ($P < 0.05$, Figure 4.2). Initial mass and collection order were positively related to plasma cortisol concentrations (initial mass: $F_{1,413.3} = 9.41$, $P = 0.02$; collection order: $F_{1,411.5} = 32.2$, $P < 0.001$; data not shown).

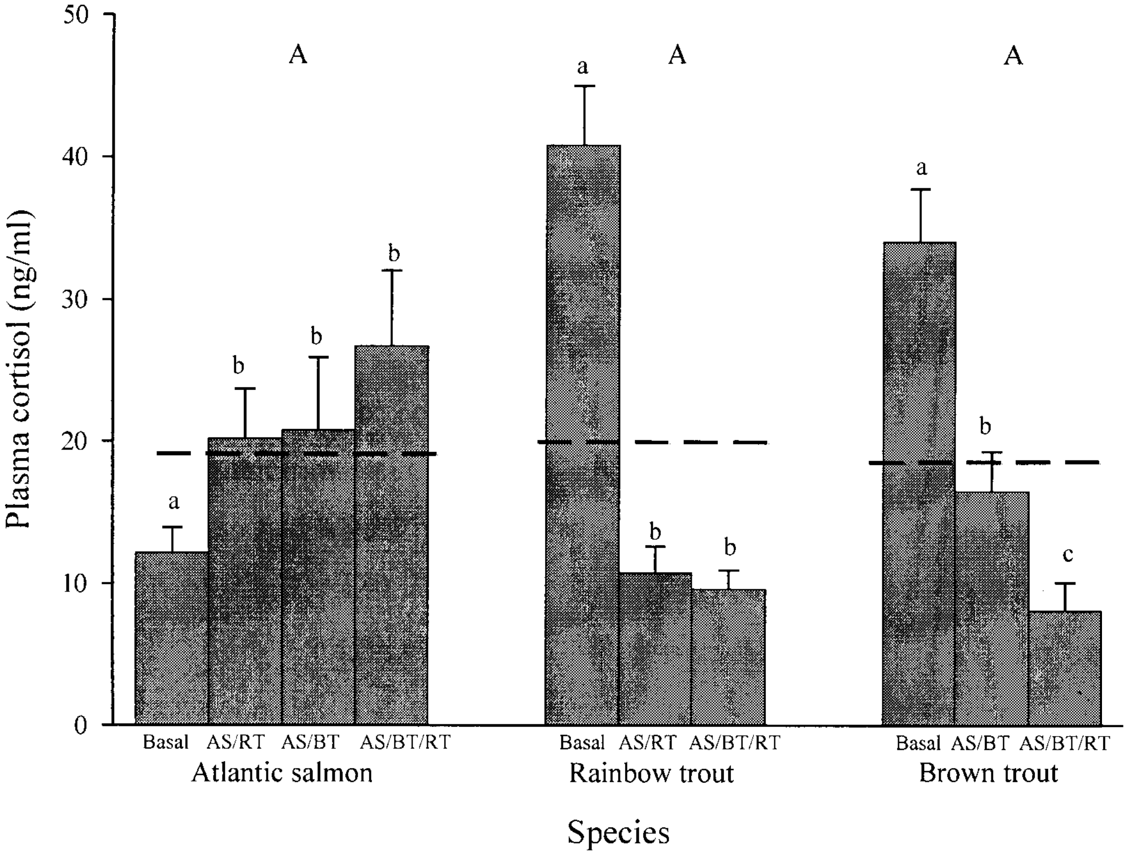


Figure 4.2: The influence of treatment exposure on plasma cortisol concentrations of juvenile salmonids. Data presented are of Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), and rainbow trout (*Oncorhynchus mykiss*), obtained from the following treatments in semi-natural stream channels: Atlantic salmon with rainbow trout (AS/RT), Atlantic salmon with brown trout (AS/BT), and Atlantic salmon with both brown trout and rainbow trout (AS/BT/RT). Basal concentrations were obtained from individuals from the stock tanks. Atlantic salmon cortisol data shown are equivalent to those in Chapter 3 and are used here for comparison. Cortisol concentrations were corrected for collection order and statistical analysis was run on corrected values (see text). Black dashed lines denote mean of each species across treatments. Different uppercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate significant differences among species ($P < 0.05$), while different lowercase letters denote homogeneous subsets assessed using post-hoc multiple comparisons tests and indicate significant differences among treatments within species (Atlantic salmon, $N = 228$; brown trout, $N = 98$; and rainbow trout, $N = 102$).

Behavioural and hormone concentrations

Linear regression analysis revealed a negative trend between corrected cortisol and David's score for brown trout ($R^2 = 0.04$, $\beta = 0.19$, $N = 78$, $P = 0.09$), but not for rainbow trout ($R^2 = 0.004$, $\beta = 0.06$, $N = 82$, $P = 0.59$; data not shown). Standard growth rate and corrected cortisol did not have a significant relationship in either brown trout ($R^2 = 0.02$, $\beta = 0.13$, $N = 78$, $P = 0.25$) or rainbow trout ($R^2 < 0.001$, $\beta = 0.005$, $N = 82$, $P = 0.97$; data not shown). Meanwhile, a positive relationship between David's score and food consumption was evident for both brown trout ($R^2 = 0.11$, $\beta = 0.33$, $N = 180$, $P < 0.001$, Figure 4.3a) and rainbow trout ($R^2 = 0.04$, $\beta = 0.21$, $N = 180$, $P = 0.009$, Figure 4.3b). David's score also had a positive relationship with standard growth rate in brown trout ($R^2 = 0.03$, $\beta = 0.17$, $N = 180$, $P = 0.02$, Figure 4.3c) and to a less extent in rainbow trout ($R^2 = 0.02$, $\beta = 0.14$, $N = 180$, $P = 0.07$, Figure 4.3d).

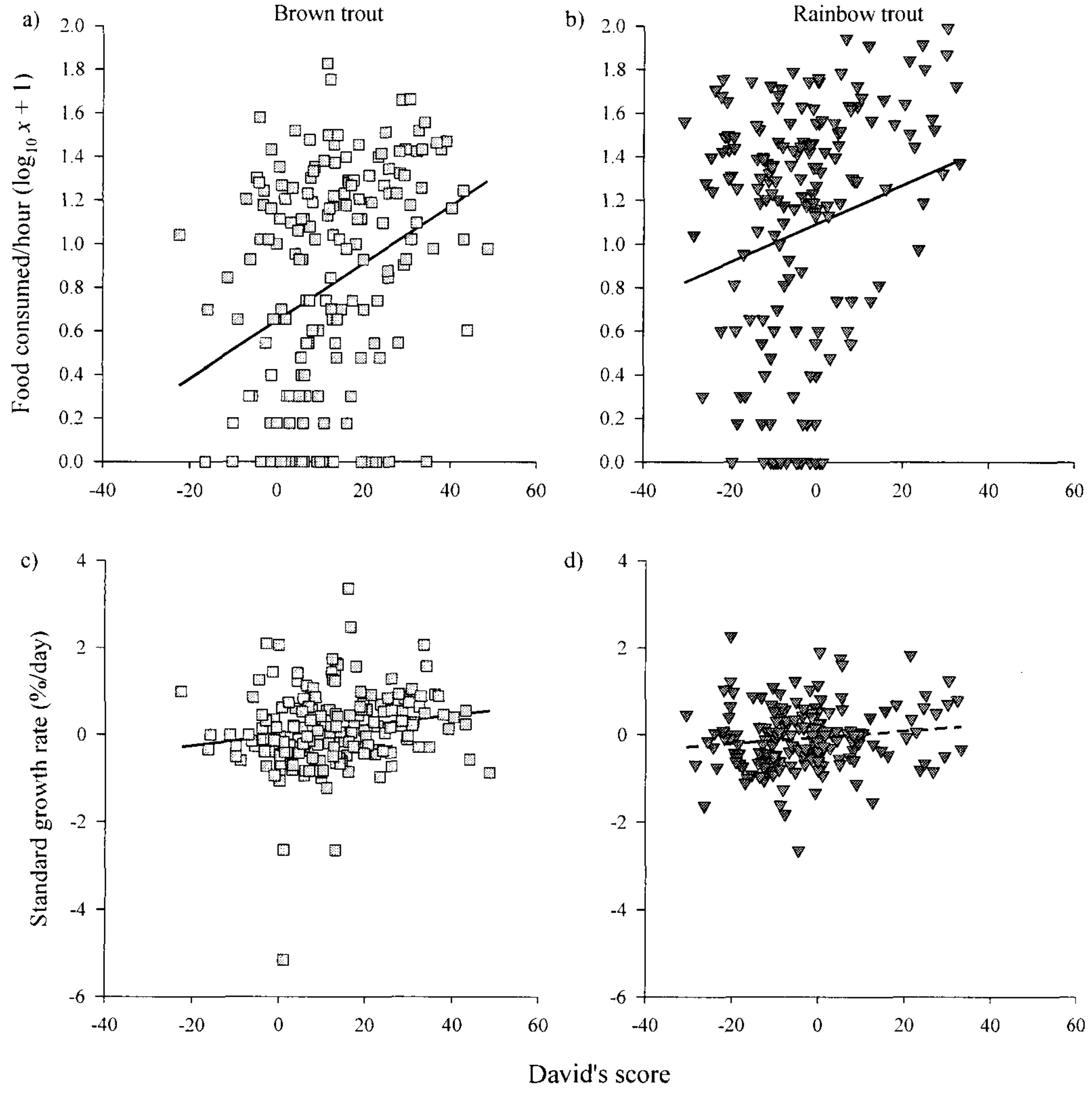


Figure 4.3: The relationship of David's score and food consumption and growth rate of juvenile trout species. Data presented are of (a) food consumed/hour and (b) standard growth rate of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*), obtained from the following treatments in semi-natural stream channels: Atlantic salmon with rainbow trout, Atlantic salmon with brown trout, and Atlantic salmon with both brown trout and rainbow trout. Presented food consumption data of each species are $\log_{10}$ $(x + 1)$ transformed ($N = 180$ for each species). Solid lines denote the results of a significant linear regression (brown trout: $\log_{10}$ (food consumed/hour + 1) = 0.01·David's score + 0.65; and Standard growth rate = 0.01·David's score – 0.02; rainbow trout: $\log_{10}$ (food consumed/hour + 1) = 0.009·David's score + 1.09), whereas a dashed line denotes a trend (rainbow trout: Standard growth rate = 0.007·David's score - 0.057).

4.4 Discussion

Variation in basal cortisol concentrations is common among species (Carragher et al. 1989; Pickering and Pottinger 1989; Barton 2000, 2002). Indeed, a review by Barton (2002) revealed that juvenile fish of 12 different freshwater species had varying basal cortisol concentrations, which ranged from 1.0 ng/ml in brown trout to 11 ng/ml in walleye (*Stizostedion vitreum*). Similarly, I found variation in the basal cortisol of Atlantic salmon and the trout species, with brown trout and rainbow trout levels as much as four-fold higher than those of Atlantic salmon. Basal cortisol concentrations of juvenile salmonids, including brown trout and rainbow trout, typically fall below 5-10 ng/ml, at least when held at densities lower than those in my study (Barton et al. 1987; Pickering and Pottinger 1989; Sloman et al. 2001). The density of my stock tanks (~0.6 fish/L) was similar across species and within the range of common hatchery practices (Pennell and McLean 1996), yet I unexpectedly discovered that basal cortisol concentrations of both brown trout and rainbow trout reflected those typically associated with chronic stress (e.g., Sloman et al. 2001). However, Atlantic salmon basal cortisol concentrations were similar to previous studies and below those associated with chronic stress (e.g., McCormick et al. 1998). The fact that the density of the stock tanks for all three species in my study remained constant for longer than a month prior to my experiment and still cortisol remained high in both trout species, suggests brown trout and rainbow trout may be more sensitive than Atlantic salmon to common hatchery densities and are more at risk for crowding (see Pickering and Pottinger 1989) or are influenced particularly by intraspecific competition (e.g., Volpe et al. 2001). While research shows variation may arise because individuals may be less sensitive to circulating cortisol (see Carragher et al. 1989; Pickering and Pottinger 1989) or have glucocorticoid receptors with a low affinity for the hormones (Maule and Schreck 1991; Maule et al. 1993) or have fewer glucocorticoid receptors (see Levine 2005), the fact that treatment-exposed trout individuals exhibited cortisol concentrations much closer to those of unstressed fish, suggests that basal concentrations were not elevated for these reasons. These data thus have implications for the success of trout stocking programs in Lake Ontario tributaries: post-release establishment may be limited when the juveniles are reared at densities of 0.6 fish/L or higher as prolonged elevation of cortisol is associated with reduced immune response and growth (Barton et al. 1987; Pickering and Pottinger 1989).

Social interactions are known to influence both aggressive behaviours and the cortisol response of individuals within dominance hierarchies (Kelsey et al. 2002; Sloman et al. 2001; Clement et al. 2005; DiBattista et al. 2005). In low densities like those found in my stream channels, dominance hierarchies can form (Brännäs and Alanärä 1993) with aggressive individuals characteristically having lower cortisol levels (Winberg and Lepage 1998; Øverli et al. 1999). Consistent with those findings, I found that Atlantic salmon consistently achieved negative dominance scores and exhibited cortisol concentrations two-fold higher than both treatment-exposed trout species. Additionally, brown trout had much lower cortisol concentrations than Atlantic salmon in the stream channels and were much more dominant suggesting that in the wild, brown trout may continue to be more aggressive than Atlantic salmon. Although brown trout were dominant to rainbow trout, they had similar cortisol levels in the treatment with all three species, and brown trout actually had higher cortisol levels when alone with Atlantic salmon as compared to the levels for rainbow trout when they were alone with Atlantic salmon. Nevertheless, my data suggest that interspecific competition in streams has a greater stress impact on Atlantic salmon than the non-native trout species.

Resource partitioning often occurs within dominance hierarchies, with dominant individuals having preferential access to food and resources and consequently better growth (Nakano 1995; Ryer and Olla 1996; Martin and Moore 1998). Höjesjö et al. (2005) found that lesser competitive Atlantic salmon consumed less food items as they were excluded from foraging areas by more aggressive brown trout; a pattern that has been similarly observed in other taxa (Yamagishi et al. 1974; Fausch 1984; Monaghan and Metcalfe 1985; Gatz et al. 1987). My results further these findings as I found a positive relationship between dominance and food consumption for rainbow trout and brown trout. The relationship, however, did not exist for Atlantic salmon, possibly because even the most dominant Atlantic salmon were excluded from food items from the more dominant trout species. Interestingly, when all three species were present, brown trout, which were typically more dominant than either Atlantic salmon or rainbow trout, and less stressed than Atlantic salmon, consumed about the same amount of food as

Atlantic salmon, but less than rainbow trout. Research suggests that reduced food intake may result from elevated cortisol, although that does not appear to be the case here, as cortisol levels of brown trout are similar to levels observed in unstressed trout (Barton et al. 1987; Pickering and Pottinger 1989; Sloman et al. 2001). Instead, it may be that brown trout spent more time involved in agonistic interactions and less time capturing food (see Cutts et al. 2002). Regardless, brown trout still had the highest growth rates of the three species, possibly indicating that brown trout have better food conversion efficiency (e.g., Abbott and Dill 1989), whereas the other species direct their energy towards avoiding agonistic interactions and less towards growth. Thus, increased food consumption or growth rate are advantages of obtaining high social status in dominance hierarchies.

Many studies examine pair-wise competition between similar species, yet in the wild an organism rarely encounters only a single-competitor environment. Indeed, competition for food and shelter is most intense in environments with three or more ecologically similar species (Inouye et al. 1980; Mittelbach 1988; Bengtsson 1993). Here, I examined the heterospecific interactions of three ecologically similar salmonids and found that the multi-species treatment influenced each species differently. This treatment sharply lowered food consumption, growth rates, and dominance scores of rainbow trout compared to conspecifics that were housed only with Atlantic salmon. Cortisol concentrations, on the other hand, were unaffected, suggesting that multi-species competition was not an additional source of stress. Observations of brown trout revealed that the multi-species treatment did not influence food consumption or growth rates compared to the pair-wise treatment, but the data show that dominance scores are higher in the multi-species treatment and suggest that growth rates are also greater in this treatment. Brown trout were also significantly less stressed (i.e., lower cortisol) in the multi-species treatment compared to the pair-wise treatment. The multi-species treatment did increase cortisol concentrations of Atlantic salmon compared to basal levels, in much the same way exposure to heterospecifics increased cortisol of Indo-Pacific damselfish (*Pomacentrus amboinensis*) (McCormick 2009). Patterns between the pair-wise and multi-species treatments in food consumption, growth rate, David's score, and cortisol in both Atlantic salmon and brown trout suggest these two species exert the greatest influence on each other. Hence, I have shown that each species experiences the complex competitive environments differently. My data further suggests that if restoration of Atlantic salmon is successful, multi-species interactions will have little influence on brown trout whereas rainbow trout survivorship may be adversely affected.

In conclusion, my data suggest that the addition of Atlantic salmon will have varying effects on the current ecological services derived from Lake Ontario. While I have shown that competition exists among Atlantic salmon, brown trout, and rainbow trout, it is apparent that brown trout are the most competitive, suggesting that in stream environments, brown trout, and not Atlantic salmon, will limit the co-existence of all three species. As such, I would recommend that if all three species are to thrive in Lake Ontario tributaries and contribute to services such as recreational fishing, it may be advantageous to limit interactions of the species by stocking Atlantic salmon in reaches of streams without brown trout. Additionally, I suggest preventing access of one or more of these salmonids to various streams through the creation of fishways, which would allow each species to establish territories free from heterospecific competition. Given the importance of reproduction and juvenile survival to overall recruitment, my recommendations should help to maintain a strong and sustainable recreational fishery in the Lake Ontario basin.

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Chapter 5. General Discussion

The main objectives of my thesis were to evaluate the behavioural and hormonal impacts of non-native brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) on juvenile Atlantic salmon (*S. salar*) and compare the competitive performance of three Atlantic salmon strains selected for reintroduction programs in Lake Ontario. Interspecific competition had the strongest influence on Atlantic salmon when held with only brown trout and when held with both trout species; indeed, the greatest declines were noted in Atlantic salmon aggression, food consumption, and growth in these two treatments. Although competition existed between Atlantic salmon and rainbow trout, my results suggest that the latter species had little effect on these behaviours, indicating that rainbow trout may pose a limited threat to Atlantic salmon restoration efforts. Based on the ecological similarities between the two species, the absence of a competitive threat from rainbow trout was unexpected. Such experiments reveal that the ecological underpinnings of interspecific competition may be much more complex than predicted, and that competitive experiments are crucial for expanding our understanding of how species interact with one another. Evaluating Atlantic salmon strain performances revealed that Sebago Lake individuals were the least aggressive, while the Lac Saint-Jean strain was found to be the most aggressive and have the lowest circulating cortisol and 11-ketotestosterone (11-KT) concentrations. Interspecific variation revealed that brown trout were the most dominant of the species and had the highest growth rates while Atlantic salmon had the lowest basal cortisol concentration. Interestingly, rainbow trout consumed the most food particles even though they were not the most dominant nor did they obtain the greatest growth rates. In conclusion, I have elucidated the impacts of brown trout and rainbow trout on Atlantic salmon behaviours and hormones and strain variation using semi-natural streams; both of which, may be contributing to the failure of current Atlantic salmon restoration efforts in Lake Ontario.

General non-native salmonid competition effects on Atlantic salmon

Past research has shown that non-native species can negatively influence native populations through competition for resources and habitat (Dewald and Wilzbach 1992; Edge et al. 1993; Hamilton et al. 1999; Maskell et al. 2006). My study confirmed negative effects of competition among the three species examined. In treatments with non-native species present, there were fewer initiated aggressive acts by Atlantic salmon but more received aggression compared to the Atlantic salmon conspecific treatment. In addition, Atlantic salmon David's scores (a measure of dominance) were found to decline compared to individuals interacting with only conspecifics; decreased aggression combined with increased received aggression in competition is commonly associated with subordinate individuals in a hierarchy (e.g., Abbott et al. 1985). Consequently, subordinate individuals receive less food and shelter as resource distribution is typically unequal (Metcalfe 1986; Ryer and Olla 1996; Figler et al. 1999) resulting in reduced growth and survival (Murton et al. 1971). This thesis demonstrates that the presence of a dominant non-native species can lead to reductions of food consumption and growth among native individuals, such as Atlantic salmon, a similar finding to that of Dewald and Wilzbach (1992) and Edge et al. (1993). Specifically, my thesis reveals that competition with non-native species influences Atlantic salmon aggressive and feeding behaviours, subsequently impacting dominance status and growth rates.

Considerable evidence suggests social interactions can be a chronic stressor for subordinate individuals, which may initiate behavioural changes in attempt to overcome the perceived or actual threat (Wendelaar Bonga 1997; Blanchard et al. 1998; Øverli et al. 1999; Pottinger and Carrick 2001). As predicted, in response to non-native competitors, cortisol concentrations of Atlantic salmon were much higher than basal levels of non-experimental fish. Individuals with elevated cortisol were found to be less aggressive and consume less food; behavioural changes which may have been an attempt to cope with the stress of heterospecific competition, thereby directing energy towards avoidance of competitors (Li and Brocksen 1977; Metcalfe 1986) instead of fighting and feeding. Indeed, Metcalfe (1986) found that stressed rainbow trout altered foraging behaviours in an effort to avoid competition, migrating to faster flowing reaches of the stream despite the decreased food availability in these areas. The confines of the stream channels limited the ability to migrate from competition, so individuals anecdotally observed to hide under river rock may have done so to reduce stressful encounters with aggressive competitors at the expense of food acquisition (Van Zwol, unpublished data). Elevated cortisol was accompanied by reduced aggression and feeding behaviour, suggesting that non-native competition is a chronic stressor for the less competitive Atlantic salmon.

Competition is also known to influence androgens, hormones commonly associated with increased aggression (e.g., Oliveira 2004). In fish, 11-KT is the primary androgen linked to adult aggression (Borg 1994; Oliveira et al. 2009; Taves et al. 2009). Although the relationship between aggression and 11-KT in juvenile fish remains largely unexplored, I expected a similar association to exist in juvenile Atlantic salmon, which would be influenced by non-native competition. Observed 11-KT concentrations of Atlantic salmon were consistent with those found in similar sized and aged juvenile coho salmon (*O. kisutch*) and rainbow trout (Patiño and Schreck 1986; Hou et al. 1999), all of which were much lower than adult concentrations (e.g., Sato et al. 1997; Olsén et al. 1998). However, unexpectedly, I found no relationship between 11-KT and aggression in juvenile Atlantic salmon, suggesting that other circulating androgens such as testosterone may play a greater role in regulating juvenile aggression (e.g., Collis and Borgia 1992). Furthermore, 11-KT may be more involved with sexual development in juvenile fish (Schulz and Goos 1999). However, it appears that competition to an extent influences 11-KT as the lowest Atlantic salmon concentrations were in the multi-species treatment. Nonetheless, these 11-KT levels were only significantly lower than those in the treatment with only one non-native, suggesting that competition with two non-native trout species has a greater effect on Atlantic salmon 11-KT concentrations than just one. Thus, my thesis is the first to my knowledge, to discover a lack of association between aggression and 11-KT in juvenile Atlantic salmon as well as the influence of non-native competition on 11-KT concentrations in juveniles of this species.

On the other hand, androgen levels were negatively associated with food consumption and growth. Although these results contradict some earlier research in juvenile bird development (e.g., Schwabl 1996), where eggs laid later are given greater amounts of androgens from the mother in order for the individuals to better compete with older siblings for food, evidence exists that the costs associated with elevated androgen levels can hinder or delay growth and other traits (McGivern et al. 1996; Henry and Burke 1999; Sockman and Schwabl 2000). Indeed, Gannam and Lovell (1991) found that feeding low doses of 11-KT to channel catfish (*Ictalurus punctatus*) resulted in increased growth, whereas high doses had the opposite effect. It may be that the related metabolic costs and increased energy expenditure of heightened concentrations of androgens may inhibit resources available for growth (Marler et al. 1995; Buchanan et al. 2001). Thus, while in some instances greater concentrations of circulating androgens may be beneficial, I have shown that in juvenile Atlantic salmon, it may come at the cost of growth.

Differences in non-native salmonid competition effects on Atlantic salmon

Rainbow trout

Although their native ranges do not overlap, previous research has shown that Atlantic salmon and rainbow trout have a large degree of niche overlap (Gibson 1981; Hearn and Kynard 1986) with intense competition for prey items and territory expected if exposed to one another. Hearn and Kynard (1986) found that at various life stages, when one species was introduced into the habitat of the other, these two species competed for space within a stream; while Stanfield and Jones (2003) suggest that naturalised populations of rainbow trout in Lake Ontario will interfere with survival of juvenile Atlantic salmon. Hence, I expected competition between the two species to reduce food consumption and growth of the less competitive Atlantic salmon as aggressive rainbow trout would limit access to food. However, similar to a study by Blanchet et al. (2008) on the agonistic interactions of these species, food consumption and growth rate of Atlantic salmon were unaffected by rainbow trout. Although agonistic interactions in the Atlantic salmon and rainbow trout treatment were more common than in the conspecific treatment, received aggression of Atlantic salmon between the treatments did not differ. Indeed, these findings support a study by Volpe et al. (2001), which found rainbow trout aggression to be five-fold higher than Atlantic salmon, with more than half of that aggression directed towards rainbow trout conspecifics rather than Atlantic salmon. Furthermore, these results contradict research that found that in interspecific competition larger fish win more (Kohda 1991); rainbow trout were smaller than Atlantic salmon due to a later date of emergence, yet secured higher dominance scores. Rainbow trout may have ignored Atlantic salmon, finding greater opposition from conspecifics. Thus, aside from density issues, ecological similarity between Atlantic salmon and rainbow trout may not necessarily pose as great a threat to Atlantic salmon restoration efforts as previous research suggests.

Brown trout

Atlantic salmon and brown trout have long lived in sympatry in their native range in Europe (see Harwood et al. 2001, 2002; Armstrong et al. 2003), where brown trout are more dominant and outcompete Atlantic salmon (Harwood et al. 2002; Stradmeyer et al. 2008); however outside this range in North America where brown trout are non-native, it is unknown whether these two species may still be able to coexist. My thesis demonstrates this pattern of dominance by brown trout continues with populations of Atlantic salmon native to North America. Brown trout were consistently more aggressive than Atlantic salmon and were the most dominant, similar to the findings of Scott et al. (2005) and Stradmeyer et al. (2008). In contrast, Atlantic salmon initiated less aggression, yet received more aggression compared to the conspecific treatment. Food consumption and growth rate of Atlantic salmon also declined with the presence of brown trout which, according to Höjesjö et al. (2005), may be the result of brown trout monopolizing feeding areas when with Atlantic salmon, reducing feeding opportunities. In addition, cortisol concentrations of Atlantic salmon when held with brown trout were significantly higher than basal concentrations, further suggesting brown trout create social stress for Atlantic salmon. Thus, competition that exists between brown trout and Atlantic salmon in their native range similarly exists with North American populations of Atlantic salmon at least in the context of my study, with brown trout persisting as a stronger competitor while Atlantic salmon exhibit subordinate behaviours.

Both brown trout and rainbow trout

In the wild, an organism daily encounters any number of species, yet few studies examine the effects of competition in a multi-species environment. Environments with multiple species that are ecologically similar can invoke competition for resources, influencing lesser competitive species (Inouye et al. 1980; Mittelbach 1988; Bengtsson 1993). Indeed, competition with both brown trout and rainbow trout significantly reduced initiated aggression and dominance, while largely increased the aggression Atlantic salmon received; consequently, both food consumption and growth rate of Atlantic salmon declined compared to the conspecific treatment. In addition to behavioural changes, a pattern indicated that competition with both non-native species was the most stressing of the treatments because Atlantic salmon cortisol concentrations were highest in this treatment. Meanwhile, 11-KT concentrations were lowest in this treatment, suggesting that multi-species competition influences androgen levels at least in juvenile Atlantic salmon. Observations of Atlantic salmon when held with only brown trout suggest that the effects on growth and dominance of Atlantic salmon observed in the multi-species treatment are largely driven by interactions with brown trout. Indeed, a study by Avent (2008) postulates that a multi-species environment may more greatly influence a less competitive species (such as Atlantic salmon), rather than a more dominant one (such as brown trout). Thus, Atlantic salmon may be at a competitive disadvantage in multi-species competition for resources in the wild with ecologically similar non-native species, such as brown trout and rainbow trout.

Atlantic salmon strain differences

Behaviour

Across many taxa, behavioural differences are apparent among populations or strains within a species (e.g., Jones 1977; Rex et al. 1996; Moretz et al. 2007) and examining these differences can elucidate phenotypic attributes that can strengthen reintroduction efforts of native species (Curio 1996). I accounted for body size in each analysis to adequately address strain differences since strains differed significantly in size. The first behaviour examined was aggression, which is an important component of successful establishment and persistence of a species (Holway and Suarez 1999). Atlantic salmon strains in the conspecific treatment were different in both initiated and received aggression. Indeed, the LaHave and Lac Saint-Jean strains initiated the most aggression, while Sebago Lake individuals initiated and received the least aggression of the strains. Feeding behaviours also differed in this treatment, with Sebago Lake individuals consuming the least food. These observations elucidate innate strain differences in aggressive and feeding behaviour.

Strains within a species may not only inherently differ in behavioural attributes, but also in response to competition (Saltonstall 2002; Houde et al. 2010). For example, comparisons of populations of coho salmon (*O. kisutch*) revealed that one population was much more aggressive and became dominant in competitive interactions (Rosenau and McPhail 1987). Similarly, I found differences in both aggressive and feeding behaviours among Atlantic salmon strains when competing with non-native trout species. Similar to the conspecific treatment, the Lac Saint-Jean strain initiated the most aggression overall. In addition, this strain appeared to lose the least mass of the three strains, suggesting they are better competitors against brown trout and rainbow trout. Sebago Lake individuals achieved the greatest dominance scores, although still negative, of the strains when held with brown trout and when held with both brown trout and rainbow trout, possibly because this strain largely avoided competition altogether. Although competition avoidance may be considered a successful strategy, in this case measures promoting survivorship, like food consumption and growth (Suthers 1998) suggest that Sebago Lake individuals do not fare well in competition with brown trout and rainbow trout. The discriminant function analysis confirmed strain differences by showing that non-native trout species influence the LaHave and Sebago Lake strains the most, but have the least impact on the Lac Saint-Jean strain. Thus, competition can affect individual strains within a species differently.

Hormones

Hormone concentrations and response to a stressor may also vary among strains (Pickering and Pottinger 1989; Pottinger and Carrick 2001; Barton 2002). All fish from which hormone data were obtained were held under similar conditions and densities, yet variation in hormone levels of Atlantic salmon strains was apparent, suggesting that circulating hormone levels have a genetic basis. Indeed, Lac Saint-Jean individuals were observed to have the lowest overall cortisol concentrations, about half those of either LaHave or Sebago Lake strains; basal cortisol concentrations of the three strains showed the same pattern, but not significantly so. Concentrations of 11-KT were also the lowest in the Lac Saint-Jean strain both overall and for basal concentrations. When examining the strain response to the non-native treatments, 11-KT variation was only apparent for the LaHave strain, with concentrations lowest in the multi-species treatment. Meanwhile, Lac Saint-Jean and Sebago Lake fish exhibited more predicted patterns of stress response: cortisol levels increased with the introduction of heterospecific competitors. On the other hand, cortisol levels of LaHave individuals, remained high, but varied little across treatments with non-native trout. Further investigation of the high basal cortisol in the LaHave strain, a strain bred for generations in a hatchery environment (C. Wilson, Ontario Ministry of Natural Resources, Peterborough, Ontario, personal communication, 2008), may reveal whether this strain is less sensitive to circulating cortisol levels (Carragher et al. 1989; Pickering and Pottinger 1989), have glucocorticoid receptors with decreased affinity for cortisol (Maule and Schreck 1991; Maule et al. 1993), or whether the hatchery environment remains a chronic source of stress. If this environment is a significant source of stress for LaHave individuals, this may be yet another reason why past efforts have had little success; stocking an already stressed fish in a novel environment with the possibility of heightened heterospecific competition may be limiting the capability of Atlantic salmon to establish a self-sustaining population (Hutchings 1991; Davis 2006). Hence, hormonal variation is apparent across strains and may be both genetically based and influenced by social interactions.

In conclusion, behavioural and hormonal differences of Atlantic salmon strains that arise from both intra- and interspecific competition highlight the need to understand the ecology and phenotype of individual strains as these differences have the potential to influence the outcome of restoration efforts. Indeed, differences among the three strains observed here suggest that stocking the Lac Saint-Jean strain, believed to be the closest geographically and genetically of the three strains to the original Lake Ontario population (Dimond and Smitka 2005), will achieve greater restoration success as they are better competitors against brown trout and rainbow trout. Indeed, the fact that the LaHave strain was largely affected by non-native competition may explain the previous failed attempts of restoring Atlantic salmon with this strain.

Non-native salmonid differences in response to competition

Competitive interactions among the three species not only influenced the behaviours and physiology of Atlantic salmon but also that of brown trout and rainbow trout. Comparing behaviours of either trout species in pair-wise treatments with Atlantic salmon to those of the trout species in the multi-species treatment suggests Atlantic salmon hardly directly influenced measures in either trout. Indeed, brown trout maintained highly positive growth rates and dominance scores, while rainbow trout fed more and achieved higher dominance scores when held only with Atlantic salmon. These findings generally support evidence of a positive relationship between dominance and greater growth rates (Yamagishi et al. 1974; Li and Brocksen 1977; Metcalfe 1994), which is thought to be driven by having greater access to food (e.g., Ryer and Olla 1996). All three species together influenced rainbow trout behaviour more strongly than brown trout as the latter species achieved the highest growth rates and David's scores of the entire experiment in this treatment, further confirming the relationship between dominance and growth found previously. Meanwhile, rainbow trout consumed significantly less food and patterns suggest that growth rate and David's score largely declined. These results reveal that competitive interactions also influence non-native trout behaviour and that brown trout are a stronger competitor than rainbow trout.

Although brown trout achieved the highest dominance scores and growth rates, they consumed significantly less food than rainbow trout and Atlantic salmon. Research by Stradmeyer et al. (2008) suggests that brown trout chose to fight for position at the expense of food acquisition as aggression within a novel habitat can be a successful strategy in confirming the dominant position and/or obtaining future preferable feeding territories. Although these benefits for dominant individuals may mean lower food consumption in the short-term, over the long-term, either may provide higher food availability because once territories are established, aggression declines and individuals can focus on feeding (Cutts et al. 2002). Anecdotal evidence from my thesis supports this research: in some stream channels, two brown trout would fight with each other the entire length of a recording session, largely ignoring the others and the food present in the stream channel. The discrepancy between low food consumption yet high growth rates observed in brown trout may be because dominant individuals have a higher food conversion efficiency (Metcalfe 1986, 1994; Abbott and Dill 1989) or they secure energetically profitable areas in a stream, driving subordinates (such as Atlantic salmon or rainbow trout in the multi-species treatment) to energetically demanding areas of faster stream flow and lower foraging opportunities (Höjesjö et al. 2005). In the wild, these differences among species can have long-term implications as faster-growing individuals smolt earlier (Metcalfe 1994) and leave the stream sooner. Thus, greater food acquisition and growth are typically associated with dominant individuals; nonetheless these characteristics may be strongly influenced by competition for dominance and preferable feeding areas.

Among species, it is known that basal cortisol concentrations and individual responses to a stressor vary (Barton 2002). I examined cortisol concentrations and found that basal cortisol concentrations of both trout species resembled concentrations of stressed salmonids in other studies (e.g., Pickering and Pottinger 1989), while cortisol levels of treatment-exposed trout were much closer to basal levels of these species in other studies (e.g., Pickering and Pottinger 1989). Indeed, neither treatment was particularly stressing with respect to cortisol concentrations for rainbow trout or brown trout. These observations may support evidence that for some species, intra- rather than interspecific competition has a stronger influence (see Connell 1983; Volpe et al. 2001; Svanbäck et al. 2008) or it may be that stock tank density, although similar to common hatchery practices (see Pennell and Mclean 1996), was a source of stress. Interestingly, cortisol concentrations of brown trout were higher when held with Atlantic salmon than with all three species, suggesting that competition between Atlantic salmon and brown trout does impact the latter species to an extent. Indeed, fewer individuals of both species were in the multi-species treatment (as compared to the pair-wise treatment) and consequently, brown trout were significantly less stressed. Hence, both intra- and interspecific competition may influence cortisol concentrations among species.

Management implications

My thesis was set up as a common-garden experiment with a density at the upper end of densities found in the wild (Fransen et al. 1993). By keeping density constant however, I was able to determine the relative strengths of intra- and interspecific competition as well as strain differences in this semi-natural environment and at this density (Fausch 1998). From a management perspective, further research would ideally be conducted with juveniles in the natural environment because of stream habitat complexity and lower densities. Regardless, there remain advantages of utilizing semi-natural stream common-garden experiments over wild studies (see Fausch 1998), as these experiments (i) provide the first insight of the results of interactions of these species (ii) enable the evaluation of individual-based measures (iii) control for the migration of fish and (iv) control for environmental influences on competition, as habitat complexity influences competition (Armstrong et al. 2003). Furthermore, in nature it is virtually impossible to find identical stream sites.

Non-native salmonids influenced both the physiology and behaviour of juvenile Atlantic salmon, impacting the three strains differently; hence, I detail a few recommendations that may improve post-stocking success of Atlantic salmon in Lake Ontario tributaries. First, brown trout were a strong competitor. Locating suitable salmon streams without brown trout for stocking may not be practical as brown trout are a part of the ecological community of many streams draining into Lake Ontario (30% occurrence, Stanfield et al. 2006). Thus, I recommend locating and stocking juvenile Atlantic salmon in streams or reaches of a stream that are underutilized by brown trout. In doing so, Atlantic salmon may face less competition and have greater opportunities to obtain suitable feeding territories and shelter.

Second, multi-species competition influenced Atlantic salmon. In Lake Ontario, brown trout and rainbow trout are the main non-native salmonids that Atlantic salmon interact with, but there are other non-native salmonids present that may also interact with Atlantic salmon, such as chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon (Stanfield et al. 2006). Interactions among these species, however, would be infrequent as temporal and spatial niche overlap with Atlantic salmon is limited (Crawford 2001), yet understanding these interactions remains valuable information and should be examined. As such, I recommend stocking Atlantic salmon in streams or in reaches of a stream that are underutilized by or where there are low densities of, established non-native and ecologically similar salmonids. This way, the carrying capacity of the stream will not be compromised and competition in such an environment will have less impact on all species present.

However, in the wild, the competitive effects of a multi-species environment may be off-set by increasing habitat complexity, which can decrease visual contact of competitors. Indeed, in complex habitats juvenile lizards (*Anolis aeneus*) defend smaller territories (Eason and Stamps 1992) and brown trout (Sundbaum and Näslund 1998) and zebrafish (*Danio rerio*) (Basquill and Grant 1998) display reduced aggression and food monopolization. Although I did not examine habitat complexity, it may be a way to mitigate the effects of interactions among individuals of a multi-species environment in Lake Ontario tributaries.

Additionally, the comparative performance of strains can vary. If my results similarly extend to Lake Ontario tributaries, I predict that Lac Saint-Jean individuals will outperform the other strains post-stocking, and that the LaHave strain is less capable of interacting with non-native competitors such as brown trout and rainbow trout.

Behavioural interactions of these ecologically-similar salmonid species largely occur at the juvenile life stage in the stream habitat, which has been suggested to be the critical bottleneck for Lake Ontario Atlantic salmon (Fisheries and Oceans Canada 2009). Indeed, recent research suggests that population dynamics of Atlantic salmon would be very sensitive to mortality during the stream stage, in that juvenile mortality may be three times more that of adults (Fisheries and Oceans Canada 2009). As such, my thesis was focused specifically on this stage of Atlantic salmon life history to determine if restoration efforts can eliminate further possible contributions to mortality as a result of juvenile competition. After two to three years, the juveniles smolt, losing their territorial behaviour and begin to migrate downstream (COSEWIC 2006). Atlantic salmon and rainbow trout migrate to the lake, while the majority of brown trout remain in the stream downstream of their birthplace, then during the spawning season, adult brown trout and Atlantic salmon overlap both temporally and spatially for approximately no more than a month (Fisheries and Oceans Canada 2010a, b, c). I recommend examining adult interactions on the spawning grounds to determine the life-time success of Atlantic salmon in Lake Ontario streams. Such research would complement my thesis, providing a holistic understanding of the impact of these non-native salmonids on the life history of Atlantic salmon.

In conclusion, understanding the influence competition has on both behaviour and hormones of juvenile Atlantic salmon is important not only for restoration efforts in Lake Ontario tributaries but also for determining the feasibility of reintroducing other native species into their former habitats and curbing the current crisis of biodiversity loss. The knowledge obtained from this study can contribute to creating effective management strategies for species preservation. Understanding the interactions of competitive species and their consequences can aid suitable habitat selection and strain selection for conservation efforts. The relationships between hormones and behaviour discussed in this study may further elucidate mechanisms involved with an individual's response to its environment, enabling researchers and managers to better predict how a species will react to both natural and anthropogenic-induced environmental fluctuations. Thus, to achieve success in restoring a native species to its habitat, restoration programs must be based upon ecological knowledge obtained by examining both the behaviour and physiology of the native species and other species with which it will be interacting first in the laboratory setting and following on in the natural setting.

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Appendix 1

Agonistic interactions, growth, and physical measurements of three strains of juvenile Atlantic salmon (*Salmo salar*). Treatments include with conspecifics only (N = 72 for each strain), with rainbow trout (*Oncorhynchus mykiss, N* = 36 for each strain), with brown trout (*S. trutta, N* = 36 for each strain), and with both trout species (N = 24 for each strain). Trials were conducted in semi-natural streams. Physical measurements of both trout species are also included with sample size in parentheses under the Treatment column.

Treatment	**Initial mass (g)**	**Total length (mm)**	**Initiated aggression/ hour**	**Received aggression/ hour**	**David's score**	**Food consumed/ hour**	**Standard growth rate (%/day)**
Alone							
LaHave	30.0 ± 11.0	146 ± 20	4.1 ± 4.7	4.9 ± 3.8	0.0 ± 15.1	23.1 ± 21.5	-0.1 ± 0.9
Lac Saint-Jean	35.3 ± 8.8	160 ± 14	4.5 ± 5.3	5.4 ± 4.1	0.0 ± 15.8	24.5 ± 15.4	0.1 ± 0.6
Sebago Lake	55.2 ± 17.9	183 ± 19	1.3 ± 3.6	1.6 ± 1.9	0.0 ± 6.0	18.1 ± 16.2	0.0 ± 0.7
+Rainbow trout							
LaHave	31.5 ± 13.9	152 ± 24	1.5 ± 2.9	3.8 ± 4.5	-1.4 ± 8.9	17.3 ± 19.2	0.0 ± 1.1
Lac Saint-Jean	37.1 ± 8.9	162 ± 13	4.2 ± 4.2	6.4 ± 4.6	0.6 ± 15.0	17.0 ± 10.8	0.1 ± 1.1
Sebago Lake	48.9 ± 10.9	176 ± 14	1.6 ± 2.0	3.6 ± 3.0	0.2 ± 8.5	17.3 ± 14.0	0.0 ± 0.4
Rainbow trout (108)	21.1 ± 10.2	127 ± 20	-	-	-	-	-
+Brown trout							
LaHave	31.3 ± 11.0	152 ± 23	3.0 ± 5.2	12.2 ± 8.2	-13.8 ± 11.1	25.1 ± 24.4	-0.1 ± 1.1
Lac Saint- Jean	35.9 ± 12.2	159 ± 20	2.5 ± 3.3	9.1 ± 8.2	-13.9 ± 10.9	9.48 ± 9.67	-0.5 ± 0.7
Sebago Lake	53.1 ± 17.5	181 ± 18	1.0 ± 2.3	3.8 ± 4.1	-4.6 ± 8.7	10.3 ± 12.2	-0.4 ± 0.6
Brown trout (108)	39.5 ± 13.2	151 ± 17	-	-	-	-	-
+Brown trout and rainbow trout							
LaHave	32.6 ± 11.7	156 ± 24	0.8 ± 1.4	9.0 ± 4.9	-10.4 ± 7.9	16.7 ± 14.9	-0.2 ± 0.8
Lac Saint-Jean	40.7 ± 8.6	167 ± 13	1.6 ± 2.2	7.0 ± 4.8	-5.9 ± 8.2	9.65 ± 8.16	-0.1 ± 1.0
Sebago Lake	54.2 ± 14.3	180 ± 17	1.2 ± 1.5	5.6 ± 3.0	-3.8 ± 7.7	7.45 ± 9.03	-0.5 ± 0.4
Brown trout (72)	39. 1 ± 14.3	150 ± 18	-	-	-	-	-
Rainbow trout (72)	20.0 ± 8.5	126 ± 19	-	-	-	-	-

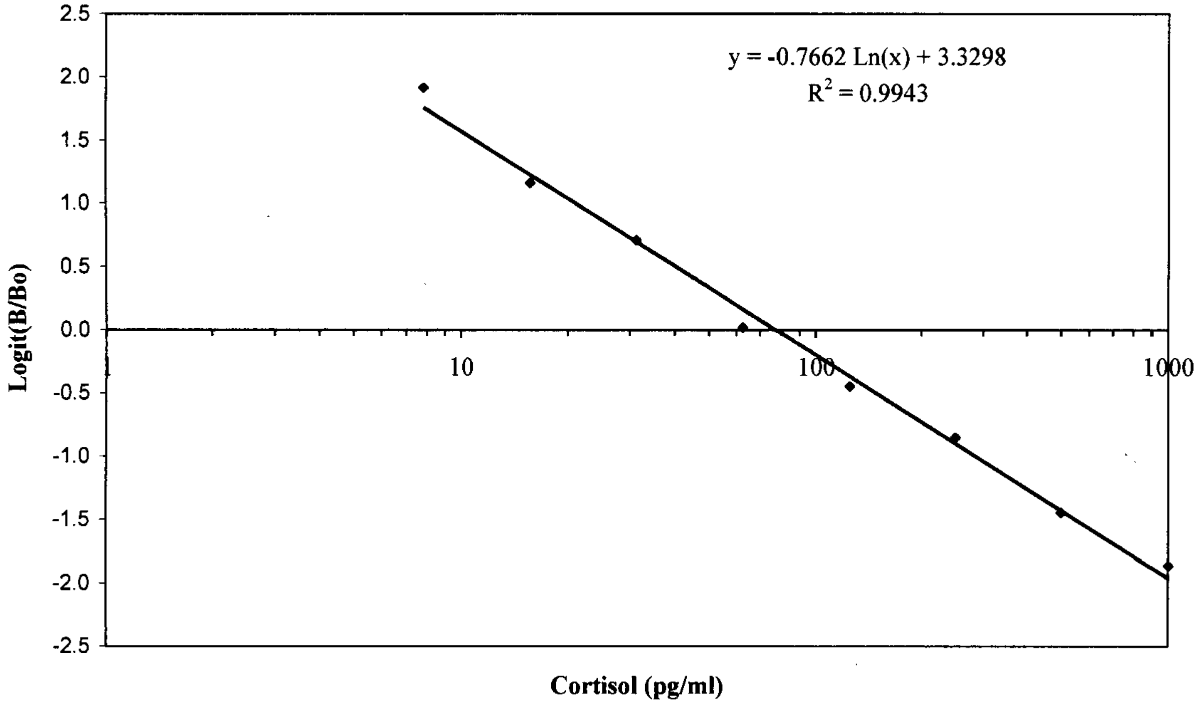
Appendix 2

Aggression, growth and hormone measurements of juvenile Atlantic salmon (*Salmo salar*). Growth and behavioural data (mean ± S.D.) and hormonal data (median, 90% range) shown are of three Atlantic salmon strains when alone, with one non-native trout species (brown trout, *S. trutta,* or rainbow trout, *Oncorhynchus mykiss*), and with two non-natives (brown trout and rainbow trout) in semi-natural streams (*N* provided for each strain and trout species, except for 11-ketotestosterone data, where *N* is in parentheses). Basal hormone levels for each strain and body size measurements of the trout species are also shown.

Treatment	N	Initial mass (g)	Total length (mm)	Initiated aggression/ hour	Received aggression/ hour	David's score	Food consumed/ hour	Standard growth rate (%/day)	Plasma cortisol (ng/ml)	11-ketotestosterone (pg/ml)
Basal										
LaHave	20	29.2 ± 13.2	146 ± 25	-	-	-	-	-	20.1 ± 14.7	261 ± 210 *(11)*
Lac Saint-Jean	20	37.4 ± 7.4	163 ± 10	-	-	-	-	-	5.8 ± 11.9	123 ± 58 *(11)*
Sebago Lake	20	57.1 ± 8.1	188 ± 10	-	-	-	-	-	10.5 ± 11.7	214 ± 125 *(11)*
Alone										
LaHave	20	31.4 ± 8.7	150 ± 18	3.4 ± 4.0	5.2 ± 4.0	-4 ± 16	22.9 ± 21.9	-0.1 ± 0.8	27.0 ± 42.2	393 ± 400 *(11)*
Lac Saint-Jean	20	32.1 ± 8.0	158 ± 14	5.8 ± 7.9	4.6 ± 4.3	5 ± 14	23.5 ± 17.0	0.2 ± 0.7	17.5 ± 30.4	285 ± 457 *(13)*
Sebago Lake	20	55.4 ± 21.7	182 ± 23	1.3± 4.9	1.6 ± 2.1	-1 ± 5	13.3 ± 9.37	0.1 ± 0.7	36.4 ± 44.4	213 ± 114 *(16)*
+1 Non-native (brown trout or rainbow trout)										
LaHave	40	32.5 ± 13.5	152 ± 24	1.7 ± 3.3	7.7 ± 7.5	-8 ± 11	22.5 ± 24.5	-0.2 ± 0.8	18.8 ± 25.5	452 ± 503 *(22)*
Lac Saint-Jean	40	36.8 ± 10.8	160 ± 17	3.3 ± 3.9	7.1 ± 8.1	-6 ± 15	14.3 ± 10.6	-0.2 ± 1.1	16.2 ± 19.7	188 ± 94 *(24)*
Sebago Lake	40	51.9 ± 13.8	179 ± 16	0.8 ± 1.5	3.8 ± 4.0	-4 ± 8	14.5 ± 14.1	-0.2 ± 0.6	24.8 ± 44.7	214 ± 109 *(21)*
Brown trout	108	39.5 ± 13.2	151 ± 17	-	-	-	-	-	-	-
Rainbow trout	108	20.0 ± 8.5	126 ± 19	-	-	-	-	-	-	-
+2 Non-natives (brown trout & rainbow trout)										
LaHave	20	33.7 ± 12.0	158 ± 24	0.6 ± 1.2	9.1 ± 5.4	-11 ± 8	17.2 ± 16.1	-0.2 ± 0.8	33.3 ± 49.7	144 ± 44 *(15)*
Lac Saint-Jean	20	40.0 ± 9.1	166 ± 13	1.7 ± 2.3	7.1 ± 5.2	-5 ± 7	9.58 ± 8.75	-0.1 ± 1.1	16.9 ± 15.9	186 ± 103 *(17)*
Sebago Lake	20	55.5 ± 14.6	183 ± 18	1.4 ± 1.5	5.2 ± 2.7	-3 ± 8	8.68 ± 9.39	-0.5 ± 0.4	25.7 ± 42.1	227 ± 72 *(12)*
Brown trout	72	39.1 ± 14.3	150 ± 18	-	-	-	-	-	-	-
Rainbow trout	72	20.0 ± 8.5	126 ± 19	-	-	-	-	-	-	-

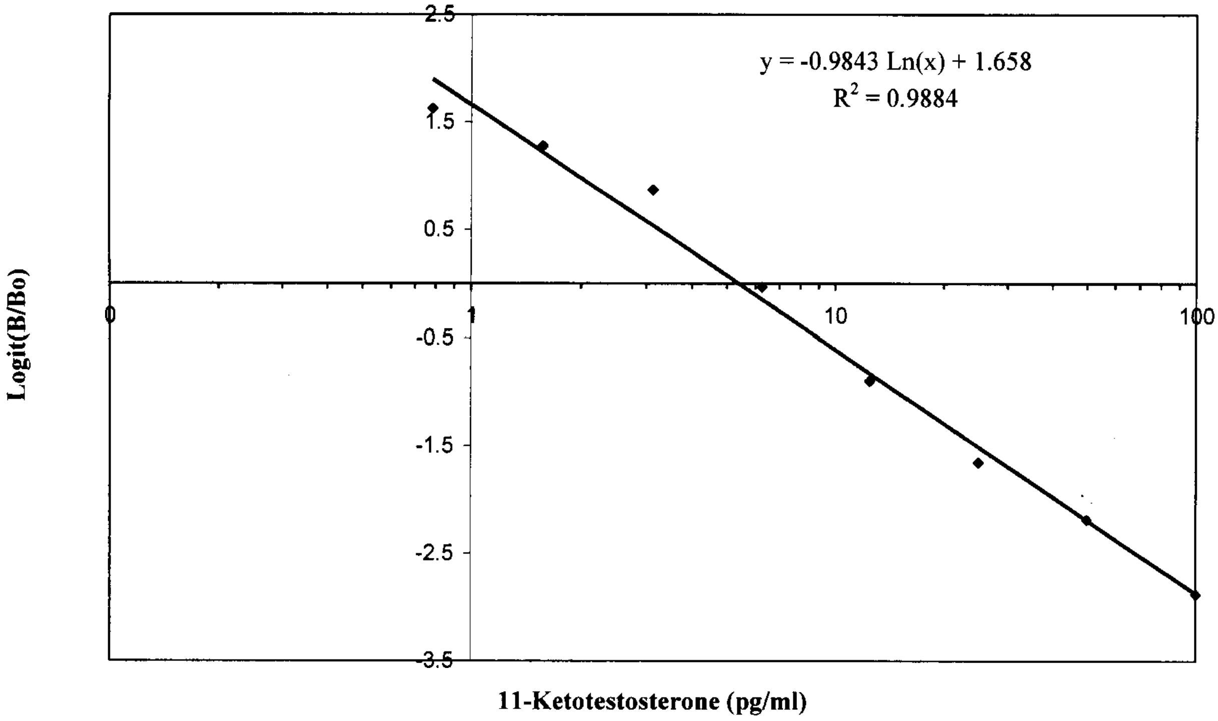
Appendix 3

Representative standard curve of plasma cortisol enzyme immunoassays.



Appendix 4

Representative standard curve of plasma 11-ketotestosterone enzyme immunoassays.



Appendix 5

Summary of physical, agonistic, and growth characteristics of juvenile Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), and rainbow trout (*Oncorhynchus mykiss*) in semi-natural stream channels. Growth and behavioural data (mean ± S.D.) and cortisol data (median, 90% range) are of the three species in treatments including basal (*N* = 60 for Atlantic salmon, *N* = 20 for each trout species), Atlantic salmon and rainbow trout (+Rainbow trout, *N* = 108, each species), Atlantic salmon and brown trout (+Brown trout, *N* = 108, each species), and Atlantic salmon with brown trout and rainbow trout (+Brown trout & rainbow trout, *N* = 72, each species). Plasma cortisol data are provided with sample size in parentheses beside treatment median.

	Treatment			
Variable	**Basal**	**+Rainbow trout**	**+Brown trout**	**+Brown trout & rainbow trout**
Initial mass (g)				
Atlantic salmon	41.2 ± 15.3	39.1 ± 13.5	40.1 ± 16.7	42.5 ± 14.7
Brown trout	38.2 ± 18.3	-	39.5 ± 13.2	39.1 ± 14.3
Rainbow trout	14.8 ± 3.95	21.1 ± 10.2	-	20.0 ± 8.54
Total length (mm)				
Atlantic salmon	165 ± 24	163 ± 20	164 ± 24	168 ± 21
Brown trout	150 ± 22	-	151 ± 17	150 ± 18
Rainbow trout	116 ± 10	127 ± 20	-	126 ± 19
Plasma cortisol (ng/ml)				
Atlantic salmon	12.1 ± 14.0 (60)	19.8 ± 25.4 (60)	20.1 ± 37.2 (60)	25.3 ± 38.7 (60)
Brown trout	34.0 ± 16.6 (20)	-	16.5 ± 17.7 (40)	8.78 ± 12.2 (42)
Rainbow trout	40.8 ± 18.6 (20)	10.75 ± 11.8 (40)	-	9.60 ± 8.72 (42)
David's score				
Atlantic salmon	-	-0.21 ± 11.1	-10.8 ± 11.1	-6.68 ± 8.32
Brown trout	-	-	10.8 ± 13.7	14.5 ± 12.2
Rainbow trout	-	0.21 ± 13.9	-	-7.85 ± 9.93
Food consumption/hour				
Atlantic salmon	-	17.2 ± 14.9	15.0 ± 18.1	11.3 ± 11.7
Brown trout	-	-	11.0 ± 11.7	10.1 ± 11.2
Rainbow trout	-	25.2 ± 20.7	-	15.0 ± 17.3
Standard growth rate (%/day)				
Atlantic salmon	-	0.02 ± 0.92	-0.34 ± 0.85	-0.26 ± 0.77
Brown trout	-	-	0.09 ± 0.88	0.19 ± 0.96
Rainbow trout	-	0.00 ± 0.71	-	-0.20 ± 0.69