The influence of non-native salmonids on circulating hormone concentrations in juvenile Atlantic salmon

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Behavioural endocrinologists have shown that stressors including competition for resources can affect an individual's circulating level of cortisol, whereas agonistic interactions typically affect androgen levels. Conservation biologists have used such data to facilitate the restoration and management of biodiversity by monitoring social interactions and stress among individuals and species. Here, we examined whether competition and agonistic interactions with non-native salmonids is hindering restoration of native Atlantic salmon, Salmo salar, in Lake Ontario. Using semi-natural streams, we examined the effects of competition with non-native brown trout, Salmo trutta, and rainbow trout, Oncorhynchus mykiss, on plasma cortisol and 11-ketotestosterone (11-KT) concentrations in juveniles from three Atlantic salmon strains, as well as the relationship between these hormones and social dominance and growth. Basal hormone levels and the hormonal response to the presence of the trouts varied among the strains. Cortisol increased in the presence of the trouts for two strains and was associated with lowered aggression and food consumption. Conversely, the presence of the non-native species had little influence on overall concentrations of 11-KT in the Atlantic salmon, although unexpectedly, there was a negative relationship between 11-KT and initiated aggression when either one of the trouts was present. Interestingly, elevated 11-KT levels were associated with declines in both food consumption and growth. Overall, our results suggest that competition with non-native salmonids negatively impacts Atlantic salmon. We discuss how our results can improve poststocking success and restoration of Atlantic salmon in Lake Ontario.

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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), a glucocorticoid that mediates the hormonal response to stress (Barton & Iwama 1991; Wendelaar Bonga 1997). Exposure to a stressor can disrupt homeostasis of an organism and elicit compensatory or adaptive physiological and behavioural responses to mobilize energy reserves in an effort to return to an organism's biological equilibrium (reviewed by Wendelaar Bonga 1997). These responses are initiated by the activation of the hypothalamic–pituitary–interrenal axis in fish, or the hypothalamic–pituitary–adrenal axis in birds and mammals. The axes stimulate a cascade effect of several pituitary hormones that target the kidney, controlling the release of cortisol or corticosterone (Iwama 1998; Mommsen et al. 1999; Barton 2002; reviewed by Nelson 2005). Conversely, exposure to chronic stressors, including stress associated with prolonged social confrontations (Fuchs & Schumacher 1990), can trigger the stress response to become maladaptive and dysfunctional when cortisol levels remain elevated (Pickering & Pottinger 1989; DiBattista et al. 2005). When it is not possible to avoid the chronic stressor, individuals may cope by altering biological activities, which can result in reduced aggression or food consumption (Kelsey et al. 2002; Pankhurst et al. 2008). In turn, these responses can lower social status and reduce growth (McCormick et al. 1998; Kelsey et al. 2002), potentially having long-term impacts on population dynamics and viability.

While reduced aggression and social status have been associated with elevated cortisol levels, androgen levels generally have the opposite effect on these characteristics (Dzieweczynski et al. 2006; Parikh et al. 2006; Taves et al. 2009). For example, aggression levels decline in experiments involving the removal of androgens, whereas androgen supplementation increases agonistic

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interactions (e.g. Arnold 1975; Hume & Wynne-Edwards 2005; see also Wingfield et al. 1990). Among conspecifics, dominance in social hierarchies has been linked to heightened aggression and elevated androgen levels (Bouissou 1983; Schoech et al. 1991; Desjardins et al. 2008), but a corresponding link in heterospecific interactions is not as well established. Moreover, most research on androgens and aggression has been on sexually mature individuals (e.g. Oliveira et al. 2009; but see Anestis 2006; Kent et al. 2009), with little known of the relationship in juveniles, especially juvenile fishes. Thus, measuring androgen concentrations and observing aggressive behaviour in juvenile fish will contribute to a void in hormone and behaviour research.

Conservation biologists have applied the study of hormones and behaviour to better understand how non-native species affect native species assemblages (Wingfield et al. 1997; Mooney & Cleland 2001). Such research has shown that competition with non-native species can be a chronic stressor for many native populations (Hamilton et al. 1999; Ruiz et al. 1999; Roberge et al. 2008). Here, we examine how competition with non-native salmonids affects Atlantic salmon, *Salmo salar*. Atlantic salmon were a native top predator in Lake Ontario, but by the end of the 19th century, habitat destruction and overfishing contributed to the species' extirpation (MacCrimmon 1977). Over the past century, the habitat has improved, yet efforts to restore Atlantic salmon to historic tributaries have not thus far resulted in self-sustaining populations (MacCrimmon 1977; Stanfield & Jones 2003; Bowlby et al. 2007). We speculate that these restoration efforts may be hampered in part because of stocking of non-native salmonids in restored historic Atlantic salmon habitat (Crawford 2001; Stewart & Schaner 2002). Two such non-native species are brown trout, *Salmo trutta*, and rainbow trout, *Oncorhynchus mykiss*, both of which show niche overlap with Atlantic salmon and are highly competitive and aggressive towards Atlantic salmon (Gibson 1981; Volpe et al. 2001). Thus, these non-natives may represent a chronic stressor for Atlantic salmon, altering their hormones and behaviour.

Restoration efforts may also be hindered by the strain of Atlantic salmon used for stocking. As the original Lake Ontario strain no longer exists, restoration efforts have focused on a strain originating from the LaHave River, Nova Scotia (e.g. Stanfield & Jones 2003). This strain was chosen in large part because of its availability as broodstock, with little consideration given to the compatibility of the strain with the lake environment (Kerr 2006). Indeed, the LaHave strain is anadromous (i.e. lives in the ocean, returning to freshwater streams to spawn), does not coexist with brown trout or rainbow trout in its native environment, and has been reared in hatcheries for generations (C. Wilson, unpublished data). More recently, the province of Ontario has developed two additional Atlantic salmon broodstocks from other populations (Greig et al., unpublished data). First, the freshwater strain from Lac Saint-Jean, Quebec is potamodromous (i.e. migration occurs within freshwater lakes and streams only; Carter 1974). Although this strain does not coexist with brown trout and rainbow trout, the strain was chosen because of its genetic similarity, geographical proximity, and possible ancestral link to the original Lake Ontario strain (Tessier & Bernatchez 2000; Dimond & Smitka 2005). Second, the Sebago Lake strain (Maine) is also potamodromous (Watts 1999; Boucher 2004), and individuals have been transplanted into Lake Champlain where they appear to have successfully established, despite competition with both brown trout and rainbow trout (Dimond & Smitka 2005). We examine all three strains to assess their relative performance.

We measured cortisol and 11-ketotestosterone (11-KT, the predominant androgen in fishes; Borg 1994; Oliveira et al. 2009) concentrations of juvenile Atlantic salmon in the presence and

absence of juvenile brown trout and rainbow trout in semi-natural stream environments. The juvenile life stage has been suggested to be the critical hurdle for Lake Ontario Atlantic salmon restoration as juvenile mortality is about three times higher than that of adults (Fisheries and Oceans Canada 2009; see also Elliott 1990; Good et al. 2001). We anticipated that if non-native brown trout and rainbow trout, because of their superior competitive ability, are a stressor for Atlantic salmon, then in treatments with the non-natives, cortisol concentrations of Atlantic salmon would be elevated and 11-KT concentrations lowered in response to being defeated in agonistic interactions as compared to treatments with only conspecifics. We also expected that these changes in hormone concentrations would coincide with reduced aggression and reduced dominance in the Atlantic salmon. The Sebago Lake strain, having been successfully stocked in another watershed with brown trout and rainbow trout, was expected to outperform the LaHave strain, which has yet to be established in Lake Ontario. No a priori predictions could be made for the relative performance of the Lac Saint-Jean strain. If the presence of the non-native salmonids elevates cortisol concentrations or lowers 11-KT in Atlantic salmon, then measures must be taken to minimize the interactions of these species if restoration efforts are to successfully establish a self-sustaining population.

METHODS

Study Species

For the current study, 1.5-year-old (yearling) juvenile Atlantic salmon, brown trout and rainbow trout were obtained from Ontario Ministry of Natural Resources (OMNR). Broodstocks were established by OMNR for the purpose of supporting re-establishment efforts of Atlantic salmon in Lake Ontario (Greig et al., unpublished data). LaHave Atlantic salmon ($N = 188$) and brown trout ($N = 180$) were obtained from the OMNR Harwood Fish Culture Station (Harwood, ON, Canada), whereas rainbow trout ($N = 180$) and Atlantic salmon from Lac Saint-Jean ($N = 188$) and Sebago Lake ($N = 188$) were obtained from the OMNR Normandale Fish Culture Station (Normandale, ON). All fish species in the study were representative (same age and culture history) of those currently stocked in Lake Ontario streams; thus, individuals of these species differed in size similar to local natural conditions (see Results, Table 1 for mass and length of the fish). Juvenile Atlantic salmon are not easily sexed morphologically or genetically (P. 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 based on sexually mature adults (C. Wilson, unpublished data);

Table 1
Summary of physical characteristics of juvenile Atlantic salmon, *Salmo salar*, strains, brown trout, *S. trutta*, and rainbow trout, *Oncorhynchus mykiss*

Variable	Atlantic salmon strains			Brown trout	Rainbow trout
	LaHave	Lac Saint-Jean	Sebago Lake		
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]

Values are means ± SD, based on fish at the commencement of the experimental trials. Superscripts denote homogeneous subsets and indicate significant differences between strains or species ($P < 0.05$) using post hoc multiple comparison tests. 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.

thus, there should not be any sex ratio differences among the strains or treatments used here.

All fish were held at the OMNR Codrington Fisheries Research Facility (Codrington, ON) for 1 month in flow-through tanks at a density of about 0.6 fish/litre, under a natural light cycle and fed trout chow (Corey Aquafeeds, Fredericton, NB, Canada) each morning, prior to the start of the experiment.

Experimental Set-up

Twelve semi-natural stream channels were set up at the Codrington facility to perform behavioural trials, each 8 days in length, between May and July 2009. Each flow-through channel consisted of a riffle and a pool, simulating natural substrate and flow conditions of Lake Ontario streams used by salmonids (Gibson 1973; Hearn & Kynard 1986; Kemp et al. 2003). For more details concerning the stream channel construction and set-up see Van Zwol et al. (in press). The three strains of Atlantic salmon (LaHave, Lac Saint-Jean and Sebago Lake) were each exposed to the following four treatments utilizing all 12 of the semi-natural stream channels: (1) Atlantic salmon with conspecifics of the same strain ('Alone' treatment, 12 Atlantic salmon), (2) Atlantic salmon with brown trout ('+1NN' treatment, 6 Atlantic salmon, 6 brown trout), (3) Atlantic salmon with rainbow trout ('+1NN' treatment, 6 Atlantic salmon, 6 rainbow trout) and (4) Atlantic salmon with both brown trout and rainbow trout present ('+2NN' treatment, 4 Atlantic salmon, 4 brown trout, 4 rainbow trout). The three Atlantic salmon strains were considered separately (i.e. strains were not mixed) to evaluate their comparative performance in the presence of non-native salmonids. Six trial blocks were performed with a total of 180 brown trout, 180 rainbow trout and 168 fish from each Atlantic salmon strain. We also examined an additional 20 individuals from each strain for basal hormone measurements. The experiment had seven trial start dates, as one trial block had a pair of start dates because of logistical constraints at the onset of the experiment, with four treatments beginning on one date followed by the remaining eight treatments.

Experimental fish were haphazardly selected with similar catch effort from the different stock tanks and anaesthetized with MS-222. Initial (pretrial) mass and total length were recorded for each fish. Each fish was tagged below the dorsal fin with a coloured 2 cm vinyl anchor tag (Floy Tag & Mfg, Inc., Seattle, WA, U.S.A.) for later recognition of individual aggressive behaviours in the video analysis. A random number generator determined the placement of treatments in the stream channels. Fish density was held constant across the treatments at 10 fish/m^2. This density is at the upper end of those found in natural streams (B. R. Fransen, P. A. Bisson, R. E. Bilby & J. W. Ward, unpublished data, http://www.fs.fed.us/pnw/lwm/aem/docs/bisson/1993_bisson_physical_and_biological_constraints.pdf) and allowed for effective determination of the relative strengths of intraspecific and interspecific competition among ecologically similar species (Fausch 1998).

Behavioural observations began the day after tagging (day 1) and continued 7 days both in the morning (0800–1230 hours) and afternoon (1400–1830 hours). Behaviours were recorded using three high-definition camcorders (Sony HDR-XR200 V) set approximately 1 m above a stream channel, with one camcorder above the pool and two above the riffle. The three camcorders were attached to a portable apparatus that could be easily moved among the channels. Two apparatuses were constructed to record behaviours simultaneously from two channels before moving the apparatuses to the next pair of stream channels. After a camcorder apparatus was placed above a stream channel, fish were given 15 min to acclimate to its presence before recording began. Aggressive and feeding behaviours were recorded during each 30 min session (Van Zwol et al., in press).

For the duration of each trial, fish were fed trout chow (Corey Aquafeeds, Fredericton, NB) and frozen bloodworms (Chironomidae; Hikari Sales U.S.A., Hayward, CA, U.S.A.) in the morning session. The release of 50–100 bloodworms or 1 g of trout chow alternated every minute for the first 10 min of recording (∼2% of biomass in each stream channel) at the top and middle of a stream channel, with food items carried through the channel by the riffle current, simulating natural invertebrate drift. Care was taken to avoid being seen by the fish. Recording order of stream channels within a session was determined using a random number generator.

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 about 30 min. Care was taken to ensure that remaining fish were minimally disturbed during the collection of individual fish within the stream channels (see Supplementary Material for collection order median and range for each strain in each treatment; there was no relationship between hormone concentrations and collection order within a stream channel, data not shown). Netted fish were sacrificed by submersion in a MS-222 solution until operculum movement ceased, followed by a cranial blow. Final mass and total length were used to calculate standard growth rate (%/day) as: $SGR = 100 \times (\ln(\text{final mass}) - \ln(\text{initial mass}))/\text{days fed}$. To obtain blood samples, the caudal fin was severed using a scalpel posterior to the adipose fin at the peduncle. Each fish provided approximately 0.25–0.50 ml blood, which was collected in Microvette CB 500 Li Heparin tubes (Sarstedt, QC, Canada). Plasma was separated by centrifugation (1500 g for 5 min) and stored in 0.5 ml Eppendorf tubes and frozen at −20 °C for later analysis of cortisol and 11-KT concentrations.

To assess baseline hormone concentrations, 20 Atlantic salmon from each strain were netted from the source stock tanks in the middle of the experimental period (1 July 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 served 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/litre, as compared to 0.03 fish/litre 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 to reduce intrawell variation and increase the detection capacity of the assay kit (Van Der Kraak et al. 1989). The diethylether was evaporated in a fume hood overnight and 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, MI, U.S.A.). 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. We analysed 100 blood samples from each Atlantic salmon strain for the cortisol analysis. Twenty individuals of each Atlantic salmon strain were obtained from the stock tanks for basal hormone analysis, and 80 individuals from each strain used in the behavioural trials were selected to represent each of the four treatments across trials: four individuals per stream channel were randomly selected (i.e. 20 individuals from each treatment). In the +1 NN treatment, 40 individuals of each strain were analysed (i.e. 20 individuals from the treatment with brown trout and 20 individuals from the treatment with rainbow trout). Of the 300 blood samples analysed for cortisol, 184 individuals were randomly selected for 11-KT analysis (LaHave, $N = 59$; Lac Saint-Jean, $N = 65$; Sebago Lake, $N = 60$) representing all treatments across trials as well as the basal condition. Additional details of the sample sizes for each treatment can be found in the Supplementary Material. Precision of the assay kits was analysed by determining the intra-assay coefficient of variation (CV), which was 20% ($N = 11$ plates) for cortisol analyses and 17% for 11-KT analyses ($N = 13$ plates). An internal standard included in each assay determined the inter-assay CV was 20% ($N = 8$ plates) for cortisol analyses and 14% ($N = 4$ plates) for 11-KT analyses.

Behavioural Analysis

Video recordings of days 1, 3, 5 and 7 of each trial block were analysed, and approximately 864 h of video were observed for agonistic interactions and feeding behaviours. Videos were watched in real time and, when an action occurred, we recorded the actor, the act and the recipient. Monitored aggressive behaviours included chasing (one acting fish rapidly following recipient fish with both moving in the same direction), charging (one acting fish lunging towards recipient fish or one acting fish lunging towards recipient fish, with recipient moving in a direction other than the acting fish) and nipping (both the actual and attempted biting of recipient fish by the acting fish; for further definition of behaviours, see Keenleyside & Yamamoto 1962). The number of food items consumed was recorded for feeding behaviour. Behavioural scorers were not explicitly told what treatment they were watching, but the three fish species can be differentiated based on their morphology, making it impossible to mask treatment. Three of the four scorers, however, were unaware of the specific hypotheses being tested, and we found no differences in the frequencies of the behavioural measures between those three scorers and the fourth scorer. We summed agonistic behaviours and food consumption of each fish across the 4 days and converted these values to a rate by dividing by the total time of observation. We also calculated a measure of dominance for each individual using David's score, a hierarchical index based on an individual's initiated and received aggressive acts, accounting for repeated interactions among group members (see David 1988; Gammell et al. 2003).

Statistical Analysis

Differences between strains and species in initial mass and total length were analysed using ANOVA. Student's post hoc *t* tests were conducted between pairs of strains or species when the effect of strain or species was found to be statistically significant in the ANOVAs. Initiated and received aggression and food consumption data were normalized using log(x + 1) transformations. The hormonal data were also skewed and thus were log(x + 1) transformed for statistical analyses.

Linear mixed models were used to test for the effects of strain (LaHave, Lac Saint-Jean and Sebago Lake), treatment (basal, alone, +1 NN or +2 NN), and the interaction of these two factors on hormone concentration (cortisol or 11-KT). Initial mass was included as a covariate, while trial block and channel number were entered as nominal, random factors. Random factors that were not significant ($P > 0.05$), as determined by backwards stepwise analysis, were removed from the final model. Linear mixed models used the Satterthwaite approximation for the denominator degrees of freedom (Satterthwaite 1946). If strain or treatment was statistically significant, we used post hoc Student's *t* tests to determine pairwise differences in hormone concentrations, and we used one-way ANOVAs to determine significant interactions between these two factors. We also used one-way ANOVAs to explore trends when marginally nonsignificant results were obtained ($P < 0.10$) for the interaction term.

Linear mixed models were also used to examine the relationships between the hormones and the behaviours or growth measurements of juvenile Atlantic salmon. Basal hormone levels were excluded from these models, as we did not measure agonistic and feeding behaviours of fish from which we obtained basal hormone levels. Plasma cortisol or 11-KT concentrations were included 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 treated as a covariate. Trial block and channel number were again entered as nominal, random factors using the Satterthwaite approximation. Initiated and received aggression, David's score, food consumed and standard growth rate were dependent variables in these models. The individual effects of strain and treatment on behaviour and growth are discussed in detail elsewhere (Van Zwol et al., in press); here, we focus our discussion on the relationships between those behaviours and the circulating hormone concentrations. When the effect of a hormone was statistically significant, we performed linear regression analysis to test for a potential dosage–response relationship. When an interaction was found between one of the fixed factors (strain or treatment) and either hormone on a dependent variable, we used linear regression analysis to determine which strains or treatments had a significant relationship between the hormone and the dependent variable. Finally, we used a Pearson's correlation to determine the relationship between cortisol and 11-KT concentrations. All statistics were performed using JMP 4 (version 4.0.2, SAS Institute Inc., Cary, NC, U.S.A.), SPSS 16.0 (SPSS Inc., Chicago, IL, U.S.A.), or Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA, U.S.A.), and *P* values presented are for two-tailed probabilities ($\alpha \leq 0.05$).

Ethical Note

No adverse effects were noted of either tagging or anaesthetizing fish using nonbuffered MS-222, with fish recovering quickly from both experiences in small flow-through holding tanks prior to release into experimental stream channels. Tagging was only performed once individual fish experienced a total loss of equilibrium and reflexes to stimulation (Iwama et al. 1989). Care was taken to minimize handling stress of fish by reducing the time that each fish was out of the water, captured in a net and returned to the recovery tanks. The experiments performed in this study conformed to animal care guidelines as outlined by the Canadian Council on Animal Care and Ontario Ministry of Natural Resources (OMNR) Aquatic Research and Development Section Animal Use Protocol (ARDS ACC number 79) and were approved by the Animal Use Subcommittee and University Council of Animal Care at University of Western Ontario (Protocol number 2006-062).

RESULTS

Mass and Length Comparisons

The Atlantic salmon strains differed in both their initial mass and total length (ANOVA: mass, $F_{2,297} = 71.5$, $P < 0.001$; total length, $F_{2,297} = 37.8$, $P < 0.001$; Table 1). Fish from Sebago Lake were the heaviest and longest, followed by Lac Saint-Jean fish, while individuals from the LaHave strain were the smallest (Table 1). Among the three species, both mass and length differed (ANOVA: 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 1). Brown trout, however, were heavier than the LaHave and Lac Saint-Jean salmon, but were shorter than both strains (Table 1). Means ± SD for each strain and species in each treatment are presented in the Supplementary Material.

Effect of Strain and Treatment on Plasma Hormones

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: $t_{118} = 0.29$, $P = 0.77$; 11-KT: $t_{60} = 0.42$, $P = 0.67$); hence, we pooled data from these two treatments to form the +1 NN treatment (6 Atlantic salmon, 6 trout of one species) for all subsequent analyses. Hormone concentrations are, however, presented for each treatment and strain in the Supplementary Material.

Atlantic salmon strains varied in both plasma cortisol (linear mixed model: $F_{2,287} = 12.3$, $P < 0.001$) and 11-KT ($F_{2,171} = 6.70$, $P = 0.002$) concentrations (Fig. 1). Lac Saint-Jean salmon had the lowest cortisol concentrations; Sebago Lake individuals were intermediate but not significantly different from Lac Saint-Jean fish, whereas LaHave salmon had the highest cortisol concentrations (Fig. 1a). The LaHave strain also had the highest 11-KT concentrations, but the concentrations were only significantly greater than those observed in Lac Saint-Jean individuals (Fig. 1b).

Treatment significantly affected both plasma cortisol (linear mixed model: $F_{3,287} = 2.82$, $P = 0.04$) and 11-KT ($F_{3,171} = 2.83$, $P = 0.04$) concentrations. Cortisol concentrations were highest in the +2 NN treatment, followed by +1 NN, alone, and finally basal treatments (Fig. 1a). Cortisol concentrations in the +1 NN and +2 NN treatments were significantly higher than basal, whereas 11-KT concentrations in the +1 NN treatment were significantly higher than the basal and +2 NN treatments ($P < 0.05$), but did not differ from the alone treatment ($P > 0.05$).

The pattern of cortisol concentrations being highest in treatments with non-natives appeared to be driven primarily by the Lac Saint-Jean strain as there was a marginally nonsignificant interaction between strain and treatment for cortisol (linear mixed model: $F_{6,287} = 1.96$, $P = 0.07$; Fig. 1a). Basal cortisol concentrations were the lowest for Lac Saint-Jean individuals, followed by concentrations in the alone, +1 NN and +2 NN treatments (ANOVA: $F_{3,95} = 3.19$, $P = 0.03$; Fig. 1a). On the other hand, cortisol concentrations did not vary significantly across treatments for LaHave (ANOVA: $F_{3,95} = 1.10$, $P = 0.35$) and Sebago Lake ($F_{3,95} = 2.18$, $P = 0.10$) salmon (Fig. 1a). Cortisol variation among strains was also apparent in the basal treatment (ANOVA: $F_{2,57} = 10.26$, $P < 0.001$), with LaHave fish having significantly higher concentrations than the other two strains ($P < 0.05$; Fig. 1a).

The interaction between strain and treatment was also significant for 11-KT concentrations (linear mixed model: $F_{6,171} = 2.65$, $P = 0.02$). Specifically, concentrations were lowest for LaHave individuals in the +2 NN treatment compared to the alone and +1 NN treatments (ANOVA: $F_{3,55} = 4.88$, $P = 0.005$; Fig. 1b), while Lac Saint-Jean and Sebago Lake strains showed no differences across treatments (ANOVA: Lac Saint-Jean, $F_{3,61} = 1.64$, $P = 0.19$; Sebago Lake, $F_{3,56} = 0.22$, $P = 0.88$; Fig. 1b). Variation of 11-KT concentrations was apparent among the strains in the basal, +1 NN and +2 NN treatments (ANOVA: basal, $F_{2,30} = 3.96$, $P = 0.03$; +1 NN, $F_{2,64} = 6.00$, $P = 0.004$; +2 NN, $F_{2,41} = 3.79$, $P = 0.03$). Lac Saint-Jean salmon had the lowest basal 11-KT concentrations of the three strains ($P < 0.05$), while the LaHave salmon had the highest concentrations in the +1 NN treatment ($P < 0.05$). In the +2 NN treatment, LaHave salmon had the lowest 11-KT concentrations, but the concentrations were only significantly lower than those observed in Sebago Lake individuals ($P < 0.05$).

For all Atlantic salmon strains, initial mass was positively correlated with plasma cortisol concentrations (linear mixed model: $F_{1,286} = 23.3$, $P < 0.001$) but not with 11-KT concentrations ($F_{1,171} = 0.87$, $P = 0.35$). Finally, plasma cortisol and 11-KT concentrations were not correlated (Pearson correlation: $r_{182} = 0.04$, $P = 0.62$).

Relation between Plasma Hormones and Behaviour

Observations of aggressive behaviours and food consumption totalled 5154 and 18 049 acts, respectively. Linear mixed models examining the relationships of cortisol and 11-KT concentrations with aggressive and feeding behaviours revealed that cortisol concentrations were negatively associated with initiated aggression, such that as cortisol concentrations increased, initiated aggression decreased (Table 2, Fig. 2a). On the other hand, 11-KT concentrations were not associated with initiated aggression (Table 3, Fig. 2b). Neither received aggression nor David's score was related to either hormone (Tables 2, 3). Food consumption and standard growth rate of Atlantic salmon were negatively associated with 11-KT concentrations, while a trend indicated a negative relationship between cortisol and food consumption (Tables 2, 3).

An interaction between plasma cortisol and strain negatively influenced food consumption (Table 2). Specifically, Atlantic salmon from Lac Saint-Jean and Sebago Lake with elevated plasma cortisol levels showed depressed food consumption (linear regression: Lac Saint-Jean, $R^2 = 0.09$, $F_{1,78} = 7.66$, $N = 80$, $P = 0.01$; 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 ($R^2 = 0.01$, $F_{1,78} = 1.12$, $N = 80$, $P = 0.29$; Fig. 3). The negative relationship between 11-KT concentration and food consumption or standard growth rate of Atlantic salmon was consistent across strains as there was no interaction between 11-KT concentration and strain for either variable (Table 3). Meanwhile, an interaction between 11-KT concentration and strain for David's score and a marginally nonsignificant interaction between 11-KT concentration and strain for initiated aggression were largely driven by positive relationships observed exclusively in the LaHave strain (David's score, linear regression: $R^2 = 0.10$, $F_{1,46} = 4.87$, $N = 48$, $P = 0.03$; initiated aggression, $R^2 = 0.06$, $F_{1,46} = 2.81$, $N = 48$, $P = 0.10$; Table 3). Finally, a significant interaction between 11-KT concentration and treatment on initiated aggression (Table 3) revealed a negative trend that only existed in the +1 NN treatment (linear regression: $R^2 = 0.05$, $F_{1,65} = 3.63$, $N = 67$, $P = 0.06$). There were also direct effects of strain, treatment and initial mass on several of the behaviours (Tables 2, 3; see Van Zwol et al., in press, for a full discussion of those effects).

DISCUSSION

Basal circulating hormone concentrations and the sensitivity of hormone concentrations to social interactions and stressors may
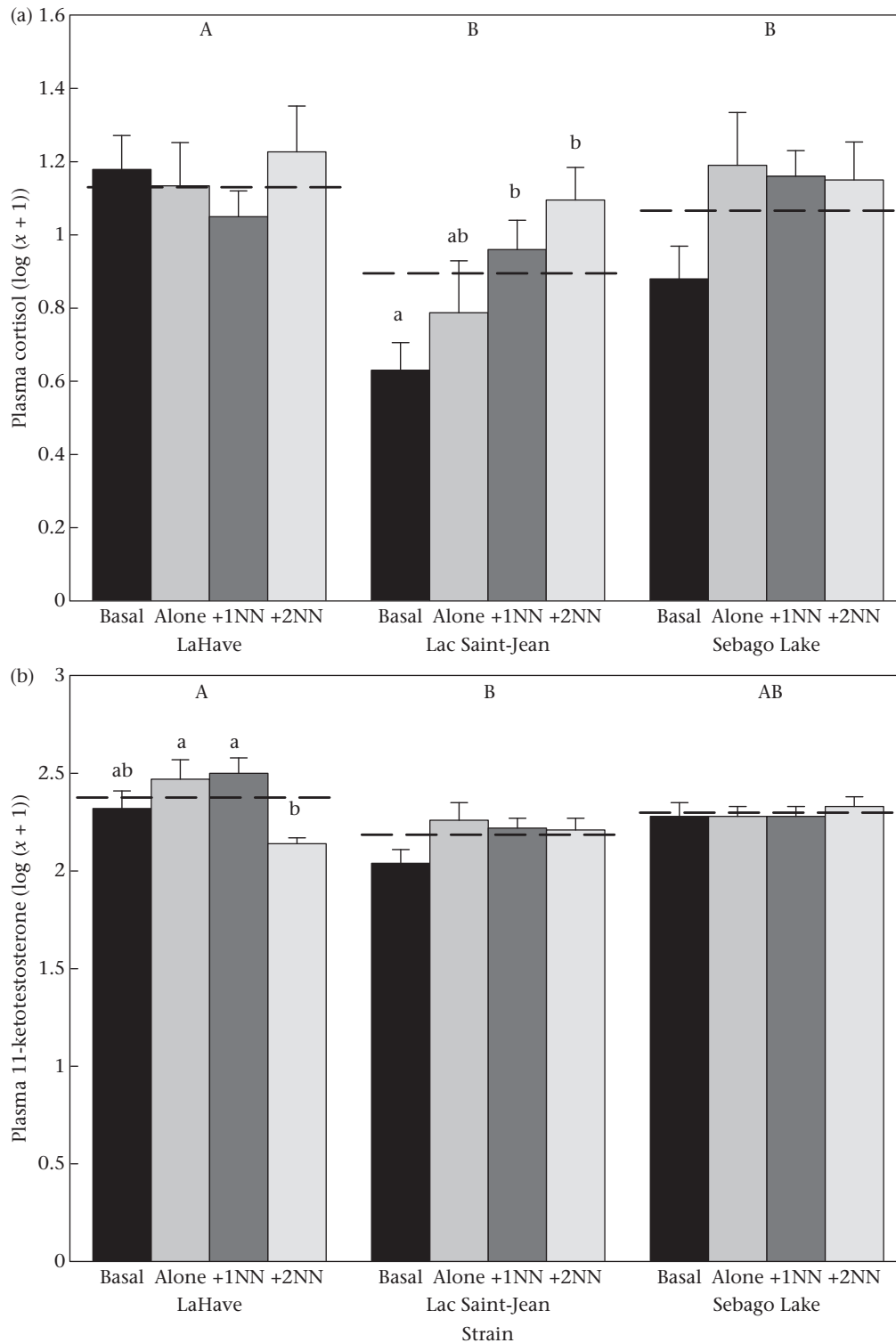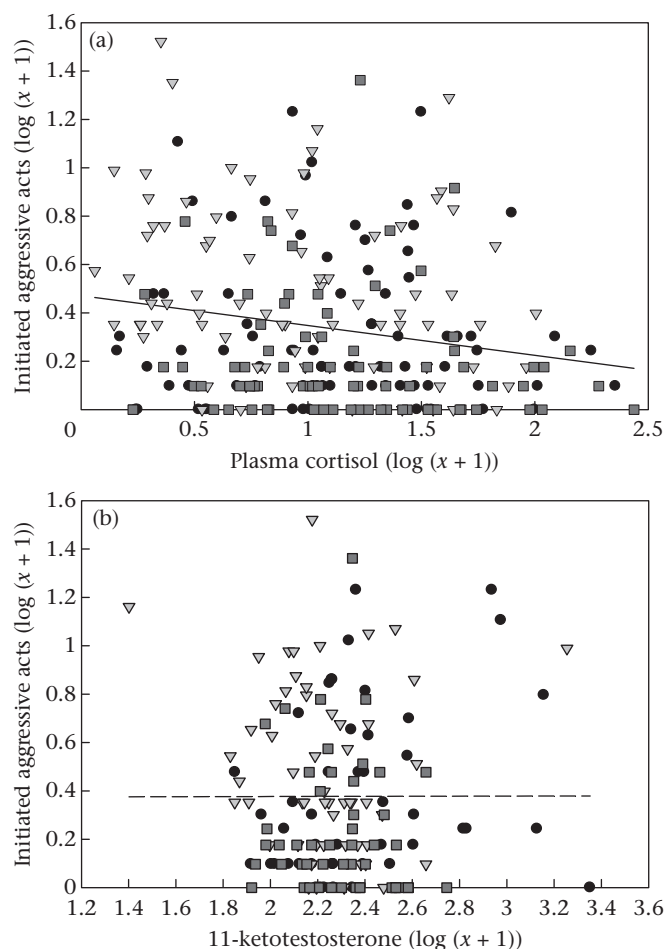
Figure 1. Circulating plasma hormone concentrations for juvenile Atlantic salmon, *Salmo salar*. Data presented are of (a) cortisol and (b) 11-ketotestosterone (11-KT) concentrations for three Atlantic salmon strains (LaHave, Lac Saint-Jean and Sebago Lake) obtained from basal, alone, +1 non-native (+1 NN) and +2 non-natives (+2 NN) treatments. All data were $\log(x+1)$ transformed. Cortisol concentration was measured in ng/ml and 11-KT in pg/ml prior to the transformations. Black dashed lines denote the mean concentration of either cortisol or 11-KT for each strain across all treatments. Different uppercase letters denote homogeneous subsets assessed using post hoc multiple comparisons tests and indicate significant differences between strains ($P < 0.05$); different lowercase letters denote homogeneous subsets assessed using post hoc multiple comparisons tests and indicate significant differences within strains ($P < 0.05$; cortisol: $N = 100$ for each strain; 11-KT: LaHave, $N = 59$; Lac Saint-Jean, $N = 65$; Sebago Lake, $N = 60$). See Supplementary Material for treatment means and standard deviations of each variable for each strain.

Table 2
Summary of linear mixed model results for the plasma cortisol analyses in juvenile Atlantic salmon, *Salmo salar*

Variable	Effects	F	df	P
Initiated aggression/h	Cortisol	4.25	1, 224.6	0.04
	Cortisol*strain	1.31	2, 225.5	0.27
	Cortisol*treatment	0.34	2, 218.9	0.71
	Strain	9.04	2, 226.2	<0.001
	Treatment	2.12	2, 221.9	0.12
	Initial mass	0.05	1, 226.8	0.82
Received aggression/h	Cortisol	0.05	1, 225.2	0.83
	Cortisol*strain	1.85	2, 225.9	0.16
	Cortisol*treatment	0.14	2, 220.4	0.87
	Strain	8.24	2, 225.4	<0.001
	Treatment	1.33	2, 222.9	0.27
	Initial mass	3.45	1, 227.0	0.07
David's score	Cortisol	0.99	1, 225.0	0.32
	Cortisol*strain	1.24	2, 223.4	0.29
	Cortisol*treatment	0.35	2, 219.3	0.70
	Strain	0.21	2, 226.8	0.81
	Treatment	3.81	2, 221.9	0.02
	Initial mass	0.14	1, 220.9	0.71
Food consumed/h	Cortisol	3.22	1, 225.8	0.07
	Cortisol*strain	4.71	2, 226.2	0.01
	Cortisol*treatment	0.22	2, 222.4	0.80
	Strain	1.96	2, 220.6	0.14
	Treatment	0.69	2, 224.4	0.50
	Initial mass	1.63	1, 228.0	0.20
Standard growth rate (%/day)	Cortisol	0.57	1, 221.4	0.45
	Cortisol*strain	0.59	2, 222.7	0.55
	Cortisol*treatment	0.41	2, 217.8	0.67
	Strain	0.72	2, 226.5	0.49
	Treatment	1.58	2, 219.7	0.21
	Initial mass	9.84	1, 224.1	0.002

Analyses examine the relationship of plasma cortisol concentrations with agonistic, foraging and growth characteristics of Atlantic salmon in semi-natural stream behavioural trials. Strain (LaHave, Lac Saint-Jean and Sebago Lake) and treatment (alone, +1 non-native and +2 non-natives) were coded as main factors; plasma cortisol and initial mass were included as covariates. Initiated and received aggression and food consumption were log(x + 1) transformed. N = 300; fractional denominator degrees of freedom were calculated using Satterthwaite's approximation.

vary among populations or strains of a species. Pickering & Pottinger (1989) found significant variation in basal cortisol concentrations of five strains of rainbow trout and three strains of brown trout. Similarly, we found that basal cortisol concentrations of individuals from the LaHave strain were highest among the three Atlantic salmon strains, while basal concentrations of Lac Saint-Jean and Sebago Lake individuals were up to three-fold lower. Variation in 11-KT concentration was evident, with concentrations lowest in the Lac Saint-Jean strain. Pickering & Pottinger (1989) also found that the response to a stressor varied among the strains (also see Pottinger & Moran 1993). Similarly, we found that social interactions with non-native species, a potential stressor, were associated with increases in cortisol concentration in Atlantic salmon. Lac Saint-Jean fish had a greater cortisol response to the presence of non-native salmonids, while concentrations varied little among the treatments for the other two strains. The LaHave strain, however, showed the greatest variation in 11-KT response to the non-native salmonids, with concentrations lowest in the presence of both non-natives. As our strains were each bred using similar protocols and reared in a common hatchery environment, the differences between strains in basal hormone concentration and stress response are likely to have a genetic basis (also see Pottinger & Carrick 2001). Similar results have been found in other taxa including mammals (Kemp & Drinkwater 1989; Malisch et al. 2007). Collectively, these results suggest that population differences in basal hormone concentrations and the response to stressors is partly explained by genetic differences.

Figure 2. Relation between plasma hormone concentrations and initiated aggressive acts for juvenile Atlantic salmon, *Salmo salar*. Data presented are of (a) cortisol (N = 100 for each strain) and (b) 11-ketotestosterone (11-KT) concentrations for three Atlantic salmon strains (LaHave, N = 48, circles; Lac Saint-Jean, N = 54, triangles; Sebago Lake, N = 49, squares), obtained from alone, +1 non-native and +2 non-natives treatments. All data were log(x + 1) transformed. Cortisol concentration was measured in ng/ml and 11-KT in pg/ml prior to the transformation. Solid line: linear regression between initiated aggressive acts/h and plasma cortisol: log(initiated aggressive acts/h + 1) = −0.13 × log((plasma cortisol) + 1) + 0.48; R^2 = 0.04, P = 0.002; dashed line: relationship between initiated aggressive acts/h and 11-KT: log(initiated aggressive acts/h + 1) = 0.002 × log((11-KT) + 1) + 0.37; R^2 < 0.01, P = 0.99. See Supplementary Material for treatment means and standard deviations of each variable for each strain.

Patterns of circulating cortisol concentrations associated with chronic stress may differ among populations. Studies of salmonids have shown that chronic stress can be associated with circulating cortisol concentrations as low as 10 ng/ml, whereas unstressed conditions are associated with concentrations of less than 5 ng/ml (Maule et al. 1987; Pickering & Pottinger 1989). In our study, LaHave salmon had cortisol concentrations that did not vary among treatments and were almost twice the chronic amount even when being held in large groups (i.e. the stock tanks) where hierarchies and other agonistic interactions are not expected to occur (e.g. Kjartansson et al. 1988; Brown et al. 1992). On the other hand, individuals from Lac Saint-Jean, and to a lesser extent from Sebago Lake, showed more expected patterns of stress response, with cortisol concentrations being higher in the treatments with one or both non-native species present as compared to the basal condition, and those elevated concentrations were much higher than those observed in unstressed juvenile salmonids (e.g. Pickering & Pottinger 1989). To better understand the high cortisol

Table 3
Summary of linear mixed model results for the 11-ketotestosterone analyses in juvenile Atlantic salmon, *Salmo salar*

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

Analyses examined the relationship of plasma 11-ketotestosterone concentrations with agonistic, foraging and growth characteristics of Atlantic salmon in semi-natural stream behavioural trials. Strain (LaHave, Lac Saint-Jean and Sebago Lake) and treatment (alone, +1 non-native and +2 non-natives) were coded as main factors; plasma 11-ketotestosterone and initial mass were treated as covariates. Initiated and received aggression and food consumption were $\log(x + 1)$ transformed. $N = 150$; fractional denominator degrees of freedom were calculated using Satterthwaite's approximation.

concentrations in the LaHave strain, a strain reared in a hatchery environment for generations, it would be fruitful to compare cortisol concentrations of wild fish to determine whether the hatchery environment is the source of apparent stress. If the hatchery environment is a chronic stressor for this strain, it may explain why past restoration efforts have had little success: stocking a chronically stressed fish may limit the capability of Atlantic salmon to establish a self-sustaining population in a novel environment (Hutchings 1991; Davis 2006). Alternatively, LaHave individuals may be less sensitive to circulating cortisol (see Carragher et al. 1989; Pickering & Pottinger 1989), have glucocorticoid receptors with a low affinity for the hormone (Maule & Schreck 1991; Maule et al. 1993), or have fewer glucocorticoid receptors (Levine 2005). In such cases, the observed elevated cortisol levels would not actually indicate a state of chronic stress. Nevertheless, the differences among the three strains of salmon that we observed suggest quantitative differences in the functioning of the pituitary–interrenal axis in response to environmental stressors.

Elevated plasma cortisol concentrations have been associated with reductions in food consumption in a number of species (Gregory & Wood 1999; Crockett et al. 2000; Bernier et al. 2004). For example, Gregory & Wood (1999) used cortisol implants to chronically elevate the hormone in juvenile rainbow trout and found that individual appetite and food consumption were reduced. Similarly, Crockett et al. (2000) found that, in a laboratory setting, elevated cortisol in pigtailed macaques, *Macaca nemestrina*, was associated with appetite suppression. We also found a negative relationship between cortisol and food consumption in two of the three strains of Atlantic salmon. While other studies have linked reduced food consumption to reduced searching and capturing of food items (reviewed by Beitinger 1990), we did not differentiate between these behaviours. Regardless, the patterns uncovered in our 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 & 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 lower cortisol levels than less aggressive individuals (Ejike & Schreck 1980; Hannes et al. 1984; Elofsson et al. 2000). Our results confirmed this pattern in juvenile Atlantic salmon: individuals that initiated more aggression had low concentrations of circulating cortisol, whereas individuals that initiated less aggression had higher 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 this relationship is largely unexplored in juvenile fish and we are the first to examine the relationship in juvenile Atlantic salmon. Our results suggest that a direct relationship between 11-KT and initiated aggression does not exist, at least in the context of our study. Observed 11-KT concentrations were similar to those found in juvenile coho salmon, *Oncorhynchus kisutch*, and rainbow trout (Patiño & 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. Although 11-KT is considered to be the primary androgen in fish (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 juvenile fish.

Androgens are also believed to positively aid development and growth (Schwabl 1996), although the benefits associated with heightened levels of the hormones may not always outweigh the costs (Metcalfe & Monaghan 2001). Indeed, some studies have shown that androgen-supplemented individuals actually show reduced or delayed growth (McGivern et al. 1996; Henry & Burke 1999; Sockman & Schwabl 2000). Gannam & Lovell (1991) found that, although feeding low doses of 11-KT to channel catfish, *Ictalurus punctatus*, stimulated growth, higher doses impeded growth. In adult male song sparrows, *Melospiza melodia*, testosterone-implanted males showed lower body mass and less fat than control males, at least during the earliest part of the breeding season (Wingfield 1984). We found that elevated 11-KT concentration in all three Atlantic salmon strains was 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), which may explain the negative relationship in our study. Thus, elevated androgen levels may benefit development in some instances, but may come at the cost of growth.

In conclusion, our research generally supports previous literature showing that circulating levels of glucocorticoid hormones mediate aggressive and feeding behaviour. Our research also suggests that, although 11-KT is associated with feeding behaviours, the hormone is not linked to aggression in juvenile fish.
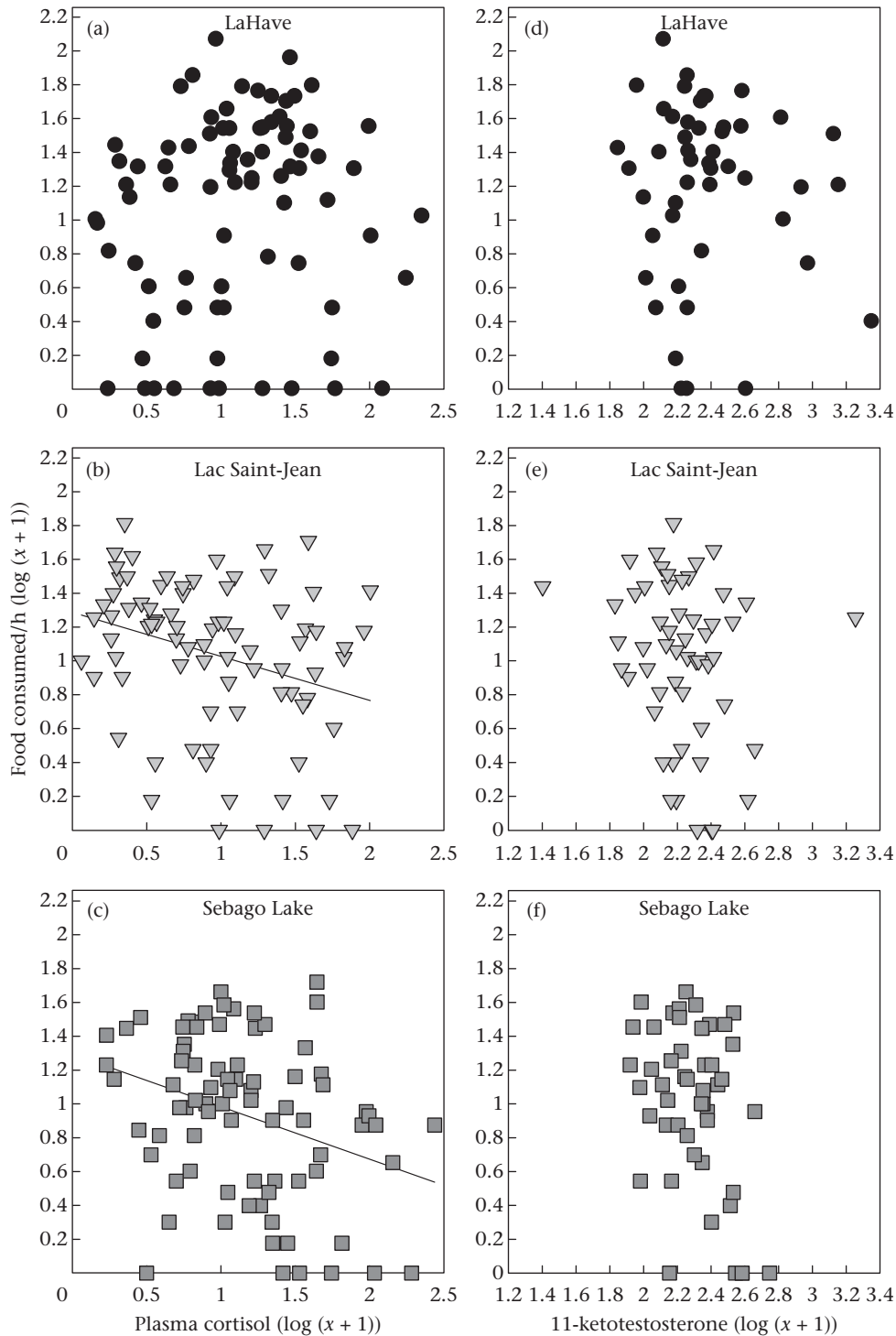
Figure 3. Relation between plasma hormone concentrations and food consumption for juvenile Atlantic salmon, *Salmo salar*. Data presented are cortisol concentrations for (a) LaHave, (b) Lac Saint-Jean and (c) Sebago Lake strains ($N = 80$ for each strain) and 11-ketotestosterone (11-KT) concentrations for (d) LaHave ($N = 48$), (e) Lac Saint-Jean ($N = 54$) and (f) Sebago Lake ($N = 49$) strains. All data were $\log(x + 1)$ transformed. Cortisol concentration was measured in ng/ml and 11-KT in pg/ml prior to the transformations. Solid lines: linear regressions (Lac Saint-Jean: $\log(\text{food consumed} + 1) = -0.26 \times \log((\text{plasma cortisol}) + 1) + 1.29$, $R^2 = 0.09$, $P = 0.007$; Sebago Lake: $\log(\text{food consumed} + 1) = -0.31 \times \log((\text{plasma cortisol}) + 1) + 1.29$, $R^2 = 0.11$, $P = 0.003$). See Supplementary Material for treatment means and standard deviations of each variable for each strain.

Moreover, our results have important implications for restoration of Atlantic salmon in the Great Lakes. We recommend stocking Atlantic salmon in streams without brown trout or rainbow trout, or in reaches of streams that are underutilized by these fish, to reduce the negative impact these fishes have on circulating cortisol levels in Atlantic salmon. The documented differences among the three Atlantic salmon strains in basal cortisol concentrations and stress responses to the presence of non-native species may well affect the poststocking ecological success of the strains. The LaHave strain appears to be chronically stressed and had the lowest 11-KT concentrations when both non-natives were present, suggesting that the Lac Saint-Jean and Sebago Lake strains

may be more suitable candidates for reintroduction in Lake Ontario.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.anbehav.2011.10.015.

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